

## Characterizing exposure of bystanders and residents to pesticides applied in agricultural fields

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*Published in:*  
Abstract book - SETAC Europe 23rd Annual Meeting

*Publication date:*  
2013

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Ryberg, M., Fantke, P., & Rosenbaum, R. K. (2013). Characterizing exposure of bystanders and residents to pesticides applied in agricultural fields. In Abstract book - SETAC Europe 23rd Annual Meeting (pp. 29)

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**1 Tailored risk assessment of pharmaceuticals – a regulatory point of view** J. Bachmann, German Environment Agency UBA; A. Hein, GKSS Research Centre Geesthacht / Pharmaceuticals, washing and cleansing agents, nanomaterials; J. Schoenfeld, Federal Environment Agency. The European authorization of human and veterinary pharmaceutical products requires the assessment of possible environmental risks before market authorisation. The procedure to assess the environmental risks is defined by several European guidelines and “question & answer” documents by the European Medicines Agency (EMA). Although standardised test and evaluation methods are the basis for comparable assessment of active pharmaceutical ingredients, there is the impression that in regulatory context well established but antiquated methods and approaches are used. Established and validated methods are the basic prerequisite for planning and legal reliability and therefore not every scientific approach can be included in regulatory risk assessment immediately. Providing that knowledge about specific mechanisms of action of the active ingredients of pharmaceuticals is available the standard guidelines allow already a tailored approach. From an agency point of view, candidate groups suitable for tailored approaches are e.g. steroidal hormones resp. their analogs, progestins, anti cancer drugs with anti-androgenic mechanism of action or parasiticides for pasture animal treatments. These are examples of substance groups with specific mechanisms of action. Modified fish life stage tests or fish sexual development tests can be used as adequate methods to evaluate the relevant effects caused by the test substances in the aquatic environment. So far these tailored approaches are a “case-by-case” decision The German Federal Environment Agency develops a decision strategy for tailored environmental risk assessment for a more consistent evaluation and a reliable assessment of potential environmental risks. However, as this is not the standard procedure, applicants are asked to contact the respective authority before the formal procedure of application is started to prevent unnecessary time conflicts and potential refusing of a market authorization. The presentation will deal with the mentioned area of conflict from a regulatory point of view. Examples of tailored approaches and a possible decision strategy will be presented.

**2 Spatial distribution of pharmaceutical residues after environmental discharge: Evaluation of occurrence and degradation in the Gironde estuary, France** y. aminot, UMR CNRS 5805 EPOC-LPTC; P. Pardon, H. Budzinski, University of Bordeaux / UMR CNRS 5805 EPOC-LPTC. In regards to the growing concern on pharmaceuticals in water bodies, assessing the impact of urban discharge on the environment is the key point. Urban effluents are actually considered as the main contributors to the presence of pharmaceuticals in the water compartment in such a way that full scale studies have to characterize both urban effluent and the receiving environment. Once discharged, pharmaceuticals can undergo sorption to suspended matter, biotic or abiotic degradation. These processes can modify bioavailability or toxicity by generating transformation products. The objectives of this work are both to characterize a urban effluent and its receiving environment and to investigate the fate of selected pharmaceuticals after discharge. In this context, the Garonne, a 647 km river located in the South-West of France drains a 56 000 km<sup>2</sup> catchment area and is impacted in its estuarine part by the effluents of Bordeaux city, the sixth most populated urban area in France. After treatment in the two main Waste Water Treatment Plants (WWTP) of Bordeaux city, wastewaters of more than 700 000 population-equivalent reach the Garonne with an average 320 dilution rate (2011 data). The ability of the Garonne riverwater - and especially its Maximum Turbidity Zone (MTZ) - to enhance or inhibit micropollutant degradation has to be investigated for a complete impact assessment. 53 pharmaceuticals including some metabolites were selected for this study. In order to qualify the variability of the pharmaceuticals intakes, a preliminary characterization of the Bordeaux main WWTP effluents has been carried out. The range of degradability in the WWTP was evaluated through the removal rates. In addition, the absence of seasonality was highlighted on the target compounds. Environmental monitoring has then been performed on 6 sites along the estuarine

Garonne river for one year. The fate and persistence in the receiving water body was evaluated through three different methods: a field load calculation, longitudinal attenuation on a low-flow pilot river and on batch experiments at the lab scale. The results obtained in this study clearly demonstrate the different behaviours of the compounds from the most persistent to the most labile ones, likely to generate transformation products.

**3 Bioconcentration and metabolism of pharmaceuticals in marine mussel** C. Boillot, Université Montpellier 1; M. Bueno, University Montpellier 1; M. Bachelot, H. Fenet, Université Montpellier 1; D. Munaron, IFREMER; C. Casellas; E. Gomez, Université de MontpellierCNRS Hydrosociences Montpellier UMR / Sc. Environnement & Sante Publique. The availability of organic contaminants to biota in aquatic environment is assessed firstly through bioaccumulation. Pharmaceuticals accumulate in freshwater organisms as it has been shown for some of them, mainly in fish species, but also in other model species such as invertebrates and algae. Despite those factors are species-dependent, very few is known in nontarget organisms. In marine organisms, pharmaceuticals have been detected with concentration ranging from ng g<sup>-1</sup> d.w. to some thousand ng g<sup>-1</sup> d.w. Other hand, it is generally accepted that substances with octanol-water partition coefficient (log Kow) equal to or higher than 3 have the potential to bioaccumulate in biological tissues; but other factors such as metabolism and the uptake and depuration kinetics must also be taken into consideration. However, studies on pharmaceuticals bioaccumulation in nontarget, marine organisms, determining kinetics for uptake and depuration and taking into consideration metabolites are very scarce. The aim of this work was the determination of bioconcentration, the kinetic constants of uptake and depuration of selected pharmaceutical in *Mytilus galloprovincialis*. The selected molecules were two benzodiazepines (BZD): diazepam (DZP) and tetrazepam (TZP), and the antiepileptic drug carbamazepine (CBZ). Furthermore, two major metabolites of CBZ were investigated in mussels tissues. Two extraction and analytical methods were developed for determine the concentration of selected pharmaceutical and the two major metabolites of CBZ at low concentration in mussel tissues. The first one for BZD, consisted of a microwave-assisted extraction and an analysis on a GCMS. The second, for CBZ and the metabolites, was a pressurized liquid extraction and a LCMSMS analysis. The calculated BCFs of the selected pharmaceuticals were 2.2, 50.7 and 99.3 L kg<sup>-1</sup> d.w for respectively CBZ, DZP and TZP and increased with logKow (2.3, 2.8 and 3.2, respectively). This relation was not linear suggesting involvement of other mechanisms than lipophilicity in bioconcentration. DZP was detected as TZP metabolite. Furthermore, metabolites of CBZ were below the detectable concentrations of 0.7 ng g<sup>-1</sup> d.w. in mussels. According to our results, previous described effects of CBZ in mussel should arrive without major CBZ bioconcentration. This late result deserves further research as mussels metabolism should conduct to other more relevant metabolites than those, humans, investigated in this study.

**4 Ecological risk assessment of veterinary medicines applied in Asian aquaculture** A. Rico, Wageningen University / Aquatic Ecology and Water Quality Management Group; P.J. Van den Brink, Wageningen University and Research Centre. During the last decades, the Asian aquaculture sector has experienced an unprecedented increase and nowadays accounts for about the 90% of the global aquaculture production by volume. Veterinary medicines (i.e., antimicrobials, fungicides, anthelmintics and other parasiticides) have been occasionally used in Asian aquaculture to treat and prevent disease outbreaks and as growth promoters, posing a potential risk for surrounding aquatic ecosystems. Although several studies have qualitatively described the potential environmental consequences of the use of veterinary pharmaceuticals and other chemicals in Asian aquaculture, none of them has been able to quantitatively assess the environmental exposure and their potential ecological risks. The main objectives of the present study were: i) to quantitatively assess the current use and risks of veterinary medicines for several aquaculture scenarios of Asia, ii) to prioritize compounds and scenarios that should be targeted in monitoring

programs, and iii) to identify research needs for performing refined risk assessment studies. In this study, we analysed recently developed databases on veterinary medicines use and aquaculture management practices. These databases were built through interviews performed between 2011 and 2012 in more than 250 and 1600 grow-out farms (for chemical use and management practices, respectively) from Bangladesh, Thailand, Vietnam and China. The collected data was used to build risk assessment scenarios for several aquaculture species (i.e., tilapia, Penaeid shrimps, *Pangasius catfish* and *Macrobrachium* prawn). Risk assessments were performed by using the ERA-AQUA model ([www.era-aqua.wur.nl](http://www.era-aqua.wur.nl)), a newly developed modelling tool designed to evaluate the environmental exposure and risks of veterinary medicines applied in pond aquaculture. The model output was evaluated regarding the percentage of the applied dose that is released through effluent discharges, the concentration dynamics of veterinary medicines in the aquaculture effluents, and the acute and chronic risk quotients for primary producers, invertebrates and fish in surrounding aquatic ecosystems. Preliminary results of this study suggest that the *Pangasius catfish* farms of Vietnam followed by the shrimp farms of China constitute possible hot-spots for environmental pollution due to the intensity of the aquaculture production and considerable discharge of chemical residues into surrounding aquatic ecosystems.

### 5 Reducing uncertainty for PECs in coastal zone: case study on carbamazepine, oxcarbazepine and their metabolites

**F.H. Hélène**, UMR Hydrosiences / UFR des Sciences Pharmaceutiques; A. Lauren, UMR Hydrosiences, Université Montpellier I; V. Alice, m. dominique, F. Annie, IFREMER; M. Olivier, CHU Lapeyronie / Laboratoire de Pharmacologie Médicale et Toxicologie; B. Helene, Université Bordeaux I-UMR EPOC; c. claude, G. elena, UMR Hydrosiences, Université Montpellier I. To assess pharmaceutical contamination in surface waters at a national and regional scale, different geo-referenced watershed models PhATE<sup>TM</sup> and GREAT-ER were used. These models incorporate spatial and temporal characteristics of the receiving environment allowing more accurate and realistic PECs than those calculated according to EMEA guidelines methodology. In coastal zone, models including the diffusion are scarce; however, this interface between the land and the sea should also be affected by drugs contamination. Thus, the aim of the present project is to propose a model to assess pharmaceuticals concentrations in a coastal zone receiving effluents from a WTP through a submarine outfall. For this purpose, pharmaceuticals, carbamazepine (Cbz), oxcarbazepine (OxCz) and their main metabolites were selected. In effluents, PECs calculation took into account in addition to the common wastewater fate, the initial Cbz and OxCz prescriptions, their metabolisms and their excretion rates. The obtained PECs results were similar to the measured concentrations (MECs) in the wastewater effluent. In the coastal zone, PECs were calculated taking into account the diffusion in the coastal zone through the submarine outfall using the hydrodynamic numeric model MARS 3D. PEC values in coastal zone were in agreement with MECs, measured using the passive samplers polar organic chemical integrative sampler implemented around the submarine outfall. The combined approach considering both detailed local consumption of parent compounds and their human metabolisms, in association with the diffusion estimation in coastal water allowed a reliable estimation of the pharmaceutical PEC for parent compounds and metabolites. MARS 3D model allows a refined assessment of concentrations in seawater influenced by specific mechanisms occurring. It can be further applied to others molecules, including elimination half-lives.

### 6 Review of a five year study on pharmaceuticals in the Irish aquatic environment

**B. Quinn**, Irish Centre for Environmental Toxicology ICET / Galway-Mayo Institute of Technology; W. Schmidt, Irish Centre for Environmental Toxicology; D. Sheehan, University College Cork; G. McEneff, Dublin City University; L. Barron, King's College London; B. Kelleher, Dublin City University; B. Paull, University of Tasmania. The presence of pharmaceutical compounds in the aquatic environment is a research area of growing concern, particularly with the current review of annex X of the WFD and the

identification of new potential priority substances including 17 $\beta$ -estradiol (E2), 17 $\beta$ -ethinylestradiol (EE2) and diclofenac. In 2008 the Irish Environmental Protection Agency funded a five year study into the assessment and potential human impact of exposure to novel environmental contaminants (pharmaceuticals) on marine and freshwater bivalves. This multidisciplinary project was divided into the two distinct but inter-related areas involving (a) chemical analysis of various pharmaceutical compounds in municipal effluents and receiving environments and their ability to bioconcentrate / bioaccumulate; (b) biological analysis of the potential effects (both acute and sub-lethal) of these novel pollutants on animals exposed in the aquatic environment and development of new endpoints to measure these effects. The five pharmaceuticals investigated (diclofenac, mefenamic acid, gemfibrozil, trimethoprim and carbamazepine) were all detected in high ng/L low  $\mu$ g/L range in both municipal effluents and the marine receiving environment. Both *Mytilus spp* and *D. polymorpha* showed expression of biomarkers of stress (glutathione transferase) and damage (lipid peroxidation, DNA damage) at environmentally relevant concentrations (1  $\mu$ g/L) of gemfibrozil & diclofenac. Proteomic analysis revealed a significant impact on proteins involved in energy metabolism, oxidative stress response, protein folding and immune responses. An oxidative stress effect was also confirmed by the biomarker response. Steroid levels were measurable in mussels using human immunoassay technology and were significantly effected following exposed to EE2. Human diagnostic techniques showed a significant impact on liver and kidney function from *Mytilus* and fish exposed to diclofenac. From this work it is apparent that pharmaceuticals are entering the Irish aquatic environment from municipal effluent and have the potential to negatively impact on aquatic species.

### 7 Population and seasonal variations in the zebra mussel transcriptome: Physiological changes and environmental inputs

**B. Pina**, IDAEACSIC / Environmental Chemistry; B. Campos, IDAEACSIC / Environmental Toxicology; M. Faria, Unicersidad de Aveiro / Dept. Biología; M. Casado, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); M. Blazquez, CSIC; A. Torreblanca, University of Valencia / Department of Animal Biology; M. Fernandez-San Juan, CSIC; S. Lacorte, IDAEACSIC / Environmental Chemistry; A. Navarro, CSIC; C. Barata, CSIC / Environmetal Chemistry. The zebra mussel *Dreissena polymorpha* is a Caspian Sea bivalve that colonized freshwater bodies worldwide during the XX century. To analyze the impact of seasonal and environmental variations on the physiology and metabolism of this invasive species, we developed a custom microarray using 4057 publicly available DNA sequences from *Dreissena* and other related genera. Seasonal variations of transcriptome profiles were analyzed using half-body samples from a relatively clean site (Riba-Roja, low Ebro River, N.E. Spain), at three different stages of the annual cycle: Pre-spawning (February), spawning (June), and gonad resorption (September). Transcripts from a total of 745 unique sequences showed significant changes among these three groups of samples. Functional characterization of these transcripts based on their closest known homologues showed that genes involved in stress defense (oxidative and infection) were overrepresented in September, whereas genes related to reproductive functions were overrepresented in the spawning and pre-spawning periods. Transcriptomic analyses of gill samples from six populations in the low Ebro showed a clear differentiation between upstream and downstream populations. These differences correlated positively with heavy metals (Cu, Cd, Mn, Ni, Zn) loads, and negatively with indexes of general fitness, like lipid content or tissue condition index. No physiological or reproductive effects were linked to mercury and organochlorine compounds, present at the most downstream Ebro sites due to the activity of a chlor-alkali factory operating in Flix. We concluded that heavy metals were the main stressors for these zebra mussel populations and that this species is resilient to other pollutants proved to be extremely toxic to vertebrates. Results also show that zebra mussel populations having very low genetic differentiation may acclimate to different levels and forms of pollution through modulations in their transcriptomic profile. Our studies may help to identify developmental stages and environmental conditions at

which the organism is more vulnerable for future control strategies.

### **8 Comparative genomics in zinc toxicity: a multi species approach to determine common modes of action** T. De Boer, Vrije

Universiteit / Ecological Sciences; B. Nota, VU University medical center Amsterdam; M. Castro Ferreira, R. Vooijs, H. Welle, J. Legler, N. van Straalen, VU University Amsterdam; M.J. Amorim, Universidade de Aveiro / Department of Biology and CESAM; D. Roelofs, Vrije University Amsterdam. Heavy metal contamination of soil may pose health and hazard risks to ecosystems and human health alike and due to anthropogenic activity these metals have been contaminating the environment since Roman times. We are interested in the effect that zinc, which is both a contaminating heavy metal as well as an essential element, has on gene expression patterns in ecologically important animals. Here we exposed two relevant ecotoxicological test species (*Folsomia candida* and *Enchytraeus crypticus*) to two concentrations of zinc (EC10 and EC50 on reproduction) for four different exposure times (1, 2, 3 and 4 days) in order to investigate the similarities as well as the differences in transcriptomic responses to this heavy metal (comparative genomics). For the single exposure *Enchytraeus* showed, in general, a stronger response in significantly differentially regulated genes at longer exposure times except for the 4 day exposure which showed a smaller response in differentially regulated genes as compared to the 3 day exposure. A possible explanation for this might be that at the 4th day these animals have fully activated their metal excretion system and are able to regulate their internal zinc concentration better. Although the comparative analysis is still work in progress we feel that comparative genomics as a technique might shed light on common responses in multiple species in order to develop sets of genes that may act as biomarkers and fit into pathways that can be used to develop Adverse Outcome Pathways (AOP). These AOP's make it then possible to integrate transcriptomics into environmental risk assessment

### **9 Tracking of novel potential biomarkers in *Porcellionides***

#### ***pruinus* (Isopoda) exposed to nickel: a transcriptomic approach**

N.G. Ferreira, CESAM Universidade de Aveiro / Departamento De Biologia & CESAM; R. Morgado, University of Aveiro / Department of Biology and CESAM; M. Novo, L. Cunha, Cardiff University; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; P. Kille, Cardiff University; S. Loureiro, Universidade de Aveiro / Biology. Isopods represent keystone sentinel detritivorous, widely exploited for ecotoxicological assessment. This study describes development and deployment of an ecotoxicogenomic tool kit that significantly enhances the utility of *Porcellionides pruinosus* as a toxicological model and underpins mechanistic interpretation. It describes the assembly and comprehensive annotation of the transcriptome coupled with global transcript analysis of a dose course of nickel exposure. We further dissect this temporal response to nickel exposure within critical functional pathways using QPCR on specific targeted genes. *P. pruinosus* has proved highly tolerant to metal contaminated environments that may reflect adaptations at various levels of biological organization. Moreover, there is a lack of functional genetic information for isopods, with the majority of data representing phylogenetic mtDNA markers. It was for these two reasons that this species was chosen as a candidate for our current analyses. Furthermore, there is little information as to the mechanistic toxicology underlying the isopods response to nickel exposure. In order to establish a transcriptome representing a mixture of different life-stages from genotyped individuals representing juveniles, adults and pregnant females was pooled out. The resulting material was used to generate 17.5 Million 50bp paired-end sequences using an Illumina Hi-Seq platform. An assembly was performed using various pipelines and a consensus transcriptome was generated representing more than 35K contigs and then annotated by homology to sequences represented in Genbank. Genotyped adults were then exposed for a period of 96h to nickel, in the concentrations of 50mg/kg soil (maximum allowable limit within the Canadian EQG) and 250 mg/kg soil, a realistic concentration found

within the natural soils. Quality validated samples of total whole body RNA were prepared from each exposure. RNAseq was then performed on a pool of three independent individuals from each dose together with controls. More than 15M reads for each sample were mapped to the consensus transcriptome and the Reads Per Kilo-base Million was calculated and used to profile differential gene expression. The study is the first full body descriptive transcriptome for isopods, and shows the global transcriptomics that these organisms undergo when exposed to nickel. Besides these two important features it also presents a target pathway based analysis.

### **10 Looking for biomarkers of metal exposure in complex environment by transcriptome analysis in the aquatic plant *Elodea nuttallii***

C. Cosio, Geneva University. To gain insight into the impact of metal contamination on ecosystems, the potential of various tests on metal accumulating organisms is explored. Previous studies suggest that macrophytes might participate in bioaccumulation and biomagnification of toxic metals in aquatic environment. Rooted macrophytes are well-suited to be used in ecotoxicological tests because they are exposed to both water and sediments. Amongst several macrophytes collected in an Hg contaminated reservoir in Romania, *Elodea nuttallii* showed a high organic and inorganic Hg accumulation and was then further studied in the laboratory. *E. nuttallii* is a rooted macrophyte native to Northern America, but as a neophyte, has spread throughout the world. It is able to accumulate large amounts of metals and is therefore a good candidate for the aim of this study. Recently developed genomics tools have a promising potential to identify early biomarkers of exposure to multiple toxicants. In the present work we used transcriptome analysis (RNA-seq) of *Elodea nuttallii* to identify biomarkers of metal exposure. RNA-seq allowed identification of genes affected by Hg and Cd exposure and also unraveled plant response to the toxic metal: a change in energy/reserve metabolism caused by the inhibition of photosynthesis, and an adaptation of homeostasis networks to control accumulation of Hg. Data were validated by RT-qPCR and selected genes were further tested as biomarkers. Samples exposed in the field and to natural contaminated sediments clustered well with samples exposed to low metal concentrations under laboratory conditions. Moreover, genes responded to low realistic concentrations in a dose-dependent manner. Our data suggest that this plant and/or this approach could be useful to develop new tests for water and sediment quality assessment.

### **11 Proteogenomics, a straightforward approach for protein**

**discovery in unsequenced test organisms in ecotoxicology** J. Trapp, Irstea / Laboratoire d'écotoxicologie; O. Geffard, Irstea; G. Imbert, CEA / Laboratoire de détection et de caractérisation des agents du risque environnemental; J. Gaillard, A. Davin, CEA / Laboratoire de Biochimie des Systemes Perturbés; a. chaumot, Irstea; J. Armengaud, CEA / Laboratoire de Biochimie des Systemes Perturbés. In ecotoxicology, proteomics allows new insights into the molecular mechanisms related to stress response. The interpretation of tandem mass spectra recorded for thousands of peptides generated from the whole proteome proteolysis requires a comprehensive protein sequence database. Unfortunately, for most species used in ecotoxicology, such informations are missing. Thus, many studies experienced difficulties in terms of protein identification. Here, we proposed an approach for quickly identifying specific proteins of the freshwater crustacean *Gammarus fossarum*, an ecotoxicology relevant species, by the alliance of genomic and proteomic (*i.e.* proteogenomic). As for animals only a small portion of the genome encodes proteins, we used next-generation sequencing technologies to sequence mRNA. We characterized the transcriptome of key physiological organs: the female and male reproductive system, the cephalon and the hepatopancreatic *caecum*. For improving structural annotation, this database was used to interpret protein data obtained by high-throughput proteomics on the four tissues. By Illumina sequencing method, we obtained a mRNA sequences dataset comprising 218,574 contigs, with a median read length of 799 bp, of which 127,332 were functionally annotated. A total of 1,033,282 MS/MS spectra was recorded during our shotgun proteomic procedure

and assigned with the RNA-seq derived database, resulting in the identification of 48,633 peptide sequences and 1,624 proteins. Besides the identification of ubiquitous or key metabolic proteins, some well known as biomarkers, the functional annotation of numerous proteins resulted quite scarce. Indeed, 202 proteins could be considered as orphans and 325 proteins as hypothetical proteins, with among them 122 closely related to protein sequences predicted from the *Daphnia pulex* genome annotation. Or, genes uniquely found in the *Daphnia* lineage have been shown to be amongst the most ecoresponsive. Thus, our dataset is of utmost importance for a comprehensive understanding of the molecular mechanisms related to stress response. The approach developed here for identifying specific proteins in test organism in ecotoxicology is proved to be straightforward. Also, in addition to being the first large scale identification of genes and proteins for *G. fossarum*, our results will help to provide useful insights into key physiological functions, especially for neuroendocrine regulation after an in-depth ecophysiological characterization.

**12 Identification of metabolic pathways in *Daphnia magna* exposed to SSRI's using transcriptomic, immunocytochemistry and physiological responses.** B. Campos, IDAEACSIC / Environmental Toxicology; N. Garcia-Reyero, Mississippi State University; L. Escalon, Enginner research and development center, US Army; T. Habib, BTS; H. Dirksen, University of Stockholm; S. Tsakovski, University of Sofia; C. Rivetti, B. Pina, R. Tauler, C. Barata, IDAEA-CSIC.

Assessing the risks of long term exposure to low doses of pharmaceuticals is an unmet reasearch need, particularly for substances that may act as neuronal disruptors in non-target species. Selective Serotonin Reuptake Inhibitors (SSRIs) are used worldwide to treat depression, and act by blocking the reuptake of serotonin in the nerve synapses, thus increasing its concentration in the synaptic cleft and therefore stimulating serotonergic neurons. In this study we explore the hypothesis that SSRIs can affect *D. magna* juvenile developmental rates and offspring production by disrupting serotonin activity, known to regulate carbohydrate and oxidative metabolic pathways. To test this hipotesys, *D. magna* juveniles and adults were exposed to SSRIs (fluoxetine and fluvoxamine) and its transcriptome was analysed using microarrays, then validated by qPCR. Immunocytochemistry assays were also performed on control and exposed adults brains, using an anti-serotonin antibody. Microarray analysis was performed using hierarchical clustering, PLS, GO and Kegg. This allowed to identify over 250 differentially expressed genes in SSRI treated individuals. Many of these genes belong to the metabolism of nucleotides, serotonin and lipids and to the Krebs cycle, while biochemical and respirometry assays indicated that exposure to SSRI increased oxidative metabolism and the catabolism of carbohydrates. These findings are in line with reproduction and survival assays that showed how females exposed to SSRIs increase the number of offspring but have lower survival under anoxia. Immunocytochemistry assays showed for the first time the existence of serotonergic neurons in the brain ganglia of *D. magna* and that serotonin activity is increased in SSRI exposed females. These results support the hypothesis that SSRIs disrupte serotonin activity deregulating carbohydrate and aerobic oxidative metabolism allowing the animals to catalyse carbohydrate reserves more efficiently but at a cost of being more susceptible to anoxic conditions, which can have important consequences in nature. These findings indicated that the use of transcriptomic responses combined with biochemical, physiological and toxicological endpoints in *D. magna* have a great potential to elucidate novel mechanisms of action of emerging pollutants to aquatic biota.

**13 A large-scale field radio-tracking study investigating unintentional acute effects of pesticides on *Perdix perdix* in agricultural landscape** E. Millot, ONCFS; A. Decors, E. Bro, ONCFS (National Game & Hunting Institute). Detrimental effects of pesticides, on wildlife have been little investigated in intensive cereal farmlands at field and landscape scale. We conducted a large-scale radio-tracking study to examine this topic on grey partridges, an emblematic bird of cereal ecosystems that is likely to be highly exposed to

pesticides. During 2010 and 2011, more than 500 wild birds have been tagged on 12 sites (14000 ha) typical of intensive cereal farming landscape of North-Central France. The location of each bird was reported on a mobile GIS twice a day from early March to late August. Carcasses of dead birds were collected less than 12 hours after the death. When possible, a necropsy was carried out and organs were collected for toxicological analyses. At the same time we mapped all habitat features on GIS and made a farmer survey to list every intervention that they carried out on each of their field plots (ie pesticide used, date of application, dose used, etc.). Thus, crossing bird habitat use (ie radio-tracking data) and farming practices data allowed us to determine for every bird its exposure to pesticides in time and space. We focused toxicological analyses on pesticides for which the birds were exposed during the last ten days before their deaths. Such analyses were made for 98 dead birds. 282 analyses screened more than 15 chemical families such as pyrethroides, triazoles and strobilurins (see table 1). In addition we compared the exposure of pesticides in space and time of dead vs. surviving birds. We will detail these results at the SETAC meeting and discuss the impact on grey partridge survival rate, through direct (ie lethal intoxication ) vs. indirect (ie sublethal intoxication that could make birds to be more sensitive to other mortality causes such as predation) effects. Chemical group Number of analysis % of cases higher than the detection limit pyrethroids 97 24% triazoles 87 0% Strobilurins 39 0% others 58 9% *Table 1 : chemical groups searched, number of analysis and % of cases for which the we detected a concentration higher than the detection limit* \n Our field dataset (farming practices, complex pesticide exposure patterns) could be used in a further step in mesocosm studies to test in controlled conditions the impact of various realistic exposure scenarios as well as in individual-based or life-cycle models

**14 Trace elements in seabirds from French Southern Lands** C.V. Zecchin Cipro, Université de La Rochelle / LIENSs; Y. Cherel, Centre d'Etudes Biologiques de Chizé, UPR 1934 du Centre National de la Recherche Scientifique; P. Bocher, F. Caurant, Université de La Rochelle / LIENSs; P. Miramand, Université de La Rochelle; P. Bustamante, Université de La Rochelle / LIENSs. The French Southern Lands are breeding site for more than 40 seabird species, many of them long lived and situated at the top of the food web. These seabirds are therefore prone to bioaccumulation and biomagnification of contaminants, both organic and inorganic ones. Among the latter, cadmium (Cd) and mercury (Hg), are non-essential elements, potentially toxic. Marine life can be exposed to these elements from anthropogenic and natural sources, and exposure is governed by various factors, including foraging location and trophic position. Sample collection (n=134, 26 species, n=6 for 10 of them) occurred from October/1998 to March/2000 at Kerguelen, Amsterdam and Crozet Archipelagos, Southern Indian Ocean. All birds used in this study died accidentally either killed by station lights/skuas or as a result of bycatch in longline vessels in these areas. Liver, muscle and kidney tissues were analysed for Cd, Cu, Se, Hg and Zn. Results were majorly, but not exclusively, driven by the different diets of the species: zooplankton-eating birds showed, in a general way, lower Hg concentrations when compared to fish/squid-eating ones and a Se:Hg molar ratio always superior to 1. Hg is mainly due to myctophids in diet, because its biomethylation in the water column renders Hg bioavailable to mesopelagic organisms. Cd, by its turn, is more related to cephalopod consumption but also to limpets'. Nevertheless, zooplankton-feeders may also present elevated Cd levels as a result of preying on the pelagic amphipod *Themisto gaudichaudii*, which has exceptionally high Cd levels in the region. Correlation analyses showed significant inter and intra-tissue results, notably between Se and Hg (due to Hg detoxification process) and within these, liver ones emphasised its importance as long term storage organ. In an analogous manner, Cd and Zn coaccumulation is seen in the kidney. Renal Cd and hepatic Hg presented a stratification that stresses differences not only in ecological niches, but in detoxification mechanisms as well. The present work brings up detailed data that is unpublished for the area or absolutely scarce to many of the sampled species, most of them of high conservation concern. The work also

indirectly confirms ecological data, specially due to the results linked to feeding ecology and Hg detoxification due to moulting schemes, even though some questions about the subject remain, particularly the ones in regard to the non-breeding period of these birds.

**15 Species Sensitivity Distribution (SSD) and SPEAR – Validation of predictions by empirical data with pesticide mixtures** S. Jesenska, Masaryk University / Faculty of Science, RECETOX; L. Blaha, Masaryk University, Faculty of Science, RECETOX / Research Centre for Toxic Compounds in the Environment (RECETOX); M. Liess, UFZ - Helmholtz Centre for Environmental Research / Dept. system ecotoxicology; R.B. Schaefer, M.A. Beketov, UFZ - Helmholtz Centre for Environmental Research. Species sensitivity distribution (SSD) is statistical method broadly used in the ecotoxicological risk assessment. Originally it has been used for prospective risk assessment of single substances but nowadays it is becoming more important also in the retrospective risk assessment of mixtures SSD. Validation of SSD is thus of high importance. *SPEAR*<sup>pesticide</sup> is bioindicator index, which is based on biological traits responsive to the effects of pesticides and post-contamination recovery. It was shown to be specific for pesticide stress in streams and rivers and its relevance and specificity has been proved by a number of studies. The aim of this study is to compare the outcomes of both methods, and to validate the use of SSD method in retrospective risk assessment of pesticide mixtures using empirical *SPEAR* data and concentrations of 25 pesticides from Germany, France and Finland. The thresholds used for the comparison were  $SPEAR = 45\%$  (LOEC level) and  $msPAF = 0.05$  (corresponds to  $HC5$ )<sup>pesticides</sup>. First results shows statistically significant correlations (Pearson's R,  $p < 0.01$ ) between  $msPAF$  values and *SPEAR*<sup>pesticide</sup>. Comparisons of the thresholds established for the SSD and *SPEAR* approaches show that the SSD based on the acute toxicity data underestimate the actual effects. The use of chronic data, based on the frequently applied acute-to chronic ratio of 10, improved the agreement between the two methods. However, the *SPEAR*<sup>pesticides</sup> index is still more sensitive and a higher acute-to chronic ratio would be required to match the thresholds from both methods. According to results, the present study suggests that field data should be used more frequently for validation of the toxicological predictive methods.

**16 Pesticides reduce biodiversity – Losses in stream invertebrate taxa in Europe and Australia** M.A. Beketov, UFZ - Helmholtz Centre for Environmental Research / Department of System Ecotoxicology; B.J. Kefford, University of Technology Sydney (UTS) / Centre for Environmental Sustainability, School of the Environment; R.B. Schafer, University of Koblenz-Landau / Quantitative Landscape Ecology, Institute for Environmental Sciences; M. Liess, UFZ Center for Environmental Research / Department of System Ecotoxicology. The biodiversity crisis is one of the greatest challenges facing humanity, but our understanding of the roles of biodiversity loss drivers remains limited. Thus, despite the general concern and immense amount of studies, it remains unknown whether, to what degree, and at what concentrations modern agricultural pesticides cause regional-scale species losses. We analyzed the effects of pesticides on the taxa richness of stream invertebrates in Europe and Australia by applying rarefaction and richness estimators. Analyzing the sets with quantified pesticide concentrations, we show that pesticides produced statistically significant effects on both the regional species and family richness for both Europe and Australia, with losses in taxa up to 42% of the recorded taxonomic pools. Furthermore, the effects in Europe were detected at concentrations that current legislation considers environmentally protective. These results show that currently used pesticides do have pronounced effects on the regional biodiversity, and development of more protective standards and mitigation measures, as well as new large-scale-oriented approaches in the ecological risk assessment of contaminants is urgently needed. For details see the upcoming publication: Beketov M.A., Kefford B.J., Schäfer R.B., Liess M. Pesticides reduce biodiversity – Losses in stream invertebrate taxa in Europe and Australia. Submitted.

**17 Multichemical pollutants' exposure on Mediterranean (Iberian) rivers: occurrence, risk and effects on the aquatic ecosystem** M. Kuzmanovic; A. Ginebreda, M. Petrovic, IDAEA-CSIC; I. Munoz, UB; s. sabater, ICRA; D. Barcelo, IIQABCSIC. The increasing contamination of aquatic systems with numerous man-made and natural chemicals is one of the key environmental problems. Although most of these chemicals are present at low concentrations, many of them may pose ecotoxicological concerns especially when occurring as components of chemical mixtures. In the EU there are more than 100000 registered chemicals listed by EINECS of which many are in daily use. One approach for identifying potentially dangerous compounds is screening of the environment for a large set of chemicals together with an assessment of the potential toxicity of the observed concentrations, which can be done by use of measured or predicted effect concentrations for standard test species. Moreover, combined biological and chemical-analytical approaches provide an important progress in identification of those toxicants that are relevant for site-specific risk and towards an estimation of the portion of an effect that can be explained by analyzed chemicals. In this study we assessed risk for almost 200 compounds of possible concern and estimated their relationships with 7 relevant biological descriptors along 77 sites located in 4 Iberian river basins (Llobregat, Ebro, Jucar and Guadalquivir). Comprehensive data set was gained from the ongoing multidisciplinary SCARCE project. There were 3 objectives of this study. First, to identify pollutants which are relevant to each specific site in terms of their occurrence and hazard to the environment. Second, estimate the ecotoxicological risk associated to the simultaneous occurrence of many pollutants, considering both their sum and complexity and to include the statistical distribution of data as tool for interpretation of results. And third, to estimate the relationship between chemical and ecological status, correlating the ecotoxicological risk with some representative biological variables.

**18 The influence of suspended particles on the effects of pulse exposure of pyrethroids on aquatic biota** J.J. Rasmussen, National Environmental Research Institute / Department of Bioscience; B. Kronvang, Aarhus University; B. Strobel, H. Hansen, Copenhagen University. The use of synthetic pyrethroids has raised much concern in the preceding decades because of their high toxicity to especially non-target freshwater macroinvertebrates. The sorption of strongly lipophilic insecticides, such as pyrethroids, to sediments has however been shown to reduce their bioavailability (and therefore toxicity) to sediment dwelling macroinvertebrates by up to several orders of magnitudes. The reduced ecotoxicity of particle associated pyrethroids to sediment dwelling macroinvertebrates has promoted a general belief among managers and stakeholders that the increased concentrations of suspended particles during storm water runoff probably mitigate some of the effects of pyrethroids in aquatic ecosystems. We studied the pulse exposure effects of lambda-cyhalothrin (LC) on locomotor behaviour and mortality of the freshwater amphipod *Gammarus pulex*. Locomotor behaviour was monitored during exposure and on each of 7 postexposure days, and mortality was registered prior to each video tracking sequence. The exposure scenarios were conducted in the presence of humic acid or montmorillonite or without microparticles. Three concentrations microparticles (5 - 125 mg/L) and five concentrations of LC (0.032 - 3.2 µg/L) were used in a full cross factorial design. The addition of humic acid particles to the LC treatments slightly reduced the acute behavioural stress and reduced the fraction of individuals with unnaturally low locomotor activity during the postexposure period. The obtained LC50 concentrations for combined LC and humic acid treatments were increased two to fourfold compared to the pure LC treatments indicating a relatively low mitigating effect probably due to reduced bioavailability. Unexpectedly, the addition of the clay mineral montmorillonite produced the opposite and slightly synergistic effect prompting an increased behavioural stress response during exposure and an increased fraction of individuals with unnaturally low locomotor activity during the postexposure period. The obtained LC50 concentrations in the combined LC and montmorillonite treatments were decreased by a factor two compared to the pure LC treatments indicating that the presence of montmorillonite increases the

bioavailability of LC to *G. pulex*. These findings imply that the presence of suspended particles during pyrethroid exposure in the field may not mitigate the effects on aquatic biota.

### 19 Direct and indirect effects of fungicides on a leaf-shredding

**invertebrate** J.P. Zubrod, N. Koksharova, P. Baudy, M. Kanschak, K. Englert, R. Schulz, Institute for Environmental Sciences, University of Koblenz-Landau; M. Bundschuh, Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences. Decomposer-detritivore-systems are considered responsible for the leaf litter breakdown in streams – a fundamental ecosystem function. Microbial decomposers (especially fungi) are of great importance in this process as they alter the leaves (i.e. conditioning) resulting in a higher food quality for detritivorous macroinvertebrates (i.e. shredders). Consequently, shredders may be affected two-fold by fungicides, i.e. by direct, toxic and indirect, food-quality related (due to an alteration of the leaf-associated microbial community) effects. The present study investigates both pathways using the model shredder *Gammarus fossarum* and several organic (azoxystrobin, carbendazim, cyprodinil, quinoxifen and tebuconazole) and inorganic (three copper-based substances and sulphur) fungicides. All organic fungicides resulted in direct, toxic effects on gammarids' feeding activity, while sulphur was ineffective. Furthermore, indirect effects of fungicides were assessed using food-choice experiments. Gammarids preferred leaves conditioned (i.e. microbially colonized and altered) in the presence of inorganic fungicides over the control. However, they preferred control leaves over leaves conditioned in the presence of organic fungicides. This difference may be triggered by a broad-spectrum activity of inorganic fungicides, affecting also leaf-associated bacteria. The resulting lower competitive pressure may have promoted fungal growth potentially resulting in a higher nutritional value of leaf material. On the contrary, quality of leaves exposed to organic fungicides may be reduced due to their intended specificity to fungi. The underlying mechanisms for the observed indirect effects on *Gammarus* will be further elucidated by analyses of the leaf-associated microbial communities. Nonetheless, it is obvious that organic and inorganic fungicides may affect decomposer-detritivore-systems and thus leaf litter breakdown in different ways, which should be considered in environmental risk assessment of fungicides.

### 20 Effects of food starvation on digestive activities in *Gammarus*

**fossarum** L. Charron, UFR Sciences Exactes et Naturelles; O. Geffard, A. Chaumot, R. Coulaud, Irstea, UR MALY, Laboratoire d'écotoxicologie; A. Geffard, Université de Reims Champagne-Ardenne / Sciences Exactes et Naturelles; O. Dedourge-Geffard, Université de Reims Champagne-Ardenne / UFR Sciences Exactes et Naturelles. Among freshwater species intensively used in ecotoxicology, *Gammarus fossarum* is a relevant test species. Many sub-individual and individual responses are studied in gammarids in order to monitor and predict effects of chemical water quality on aquatic life. In this framework, energetic metabolism variations appear to be a good predictive tool to detect physiological disturbance of organisms linked to ecosystem quality. Among biological processes involving energy uptake, digestive enzymes play a key role. Some papers clearly demonstrated that the activity of these enzymes may be disturbed by pollutants. Consequently digestive enzymes can be proposed as relevant indicators of health organisms. In this way, the monitoring of digestive responses was recently successfully applied with caged organisms for the diagnosis of aquatic system. However, confounding factors may highly influence activity levels of these enzymes, consequently the impact of these parameters should be known and taken into account for an accurate interpretation of activity levels in term of toxicity. The use of control organisms for experiments allows preventing the impact of these biotic factors. However, some other factors as the amount of available food or the food eaten by organisms cannot be easily controlled. The present study aimed to show the influence of food availability on digestive enzyme activities (amylase and trypsin) on both gender of the freshwater amphipod *Gammarus fossarum*, throughout their reproductive cycle. Organisms were exposed to 3 levels of food

available. First data showed a decrease of amylase activity correlated with the food availability. In contrast, for trypsin no effect of starvation was observed on enzyme activity for the both gender. In female, an interaction effect between moult cycle and level food available was also observed on digestive enzyme activities. So male appears as a better biological matrix for using digestive enzymes in biomonitoring survey. Moreover results indicated in starved female a delay in reproductive cycle compared to *ad libitum* fed organisms which demonstrated the diet stress effect on physiological responses. The digestive capacity inhibition may induce changes in energy allocation. These results concerning digestive capacities (both gender) and reproductive cycle (female) will be discussed in regard to energy reserve measurements and reproductive outcomes (fertility and fecundity).

### 21 Modeling physiological and chemical factors controlling lead (Pb) bioaccumulation in *Gammarus pulex*: Application from

**laboratory to field studies** N. urien, J.D. Lebrun, O. Geffard, Irstea; E. Uher, HBAN; C. Gourlay-France, Irstea. Bioaccumulation is a good indicator of the metal exposure of aquatic organisms and also enables to integrate the effect of water chemistry on metal bioavailability, fraction expected to be toxic for biota. However, the link between contamination of waters and those in organisms is complex. Indeed, bioaccumulation depends on both the physiological abilities of organism to regulate contaminant, the metal considered and chemical factors of water, which can change metal uptake. The development of bioaccumulation model as a tool for quantifying metal bioavailability constitutes promising approaches for understanding and predicting metal impacts. Pb is considered within the EU Water Framework Directive as dangerous substance because it is both non-biodegradable in aquatic media and non-essential for organisms. However, few data are available about Pb bioaccumulation in aquatic organisms. The present study aims at modeling the waterborne bioaccumulation of Pb and the effects of chemical characteristics of water on this process in a ubiquitous amphipod in Europe, *Gammarus pulex*. Then predictive quality of the model will be assessed using environmental dataset. In mesocosms, gammarids were exposed to several dissolved Pb concentrations (1 to 100 µg.L<sup>-1</sup>) so as to establish toxicokinetics and assess their capacity to regulate metal over time. Results show a significant accumulation of Pb for each tested concentration. Thus, accumulation (ku) and elimination (ke) rate constants were determined. Then, gammarids were exposed to 10 µg.L<sup>-1</sup> Pb in mesocosms with various concentrations of Na<sup>+</sup>, Mg<sup>2+</sup> or Ca<sup>2+</sup>. Results show no significant effects of Na<sup>+</sup> or Mg<sup>2+</sup> on Pb uptake. However, a protective effect of Ca<sup>2+</sup> on Pb bioaccumulation was observed. This protective effect was taken into account in the bioaccumulation model, as the apparent accumulation rate constant (ku') expressed as a function of Ca<sup>2+</sup>. Pb bioaccumulation measured in native gammarids collected from 24 sites of Seine watershed, all different in terms of contamination and water chemistry, were compared to those predicted. Results show that forecasts better match measured data when the model includes Ca<sup>2+</sup>. So, these results prove the significance of considering water chemistry for assessing metal bioavailability and to improve the predictions of the bioaccumulation model. As a perspective, prediction quality will be assessed by using field datasets especially from native and transplanted gammarids.

### 22 Toxicokinetic-toxicodynamic modeling explains differences in sensitivity among invertebrate species

A. Nyman, K. Schirmer, R. Ashauer, Eawag. Species respond to chemical stress in different ways and interspecies variation in sensitivity causes large uncertainty in chemical risk assessment. But why do species differ in their sensitivity to toxicants? Generally it is assumed that closely related species have similar ways to deal with toxicants mostly due to their similarities in morphology and physiology. Calibrating toxicokinetic-toxicodynamic (TKTD) models is one way to investigate this question - toxicokinetics describe the chemical uptake, biotransformation, distribution and elimination while toxicodynamics describe the processes causing the toxicity. By comparing differences in TKTD model parameters, we can estimate whether the differences in sensitivity are caused by toxicokinetics (bioaccumulation, activation) or toxicodynamics

(presence of sites of toxic action). In this study, we investigated three aquatic invertebrate species: *Gammarus pulex*, *Gammarus fossarum* and *Lymnaea stagnalis*. The two Gammaridae species are related (family level) but the fresh water snail *L. stagnalis* represents a different phylum amongst animals. We used three pesticides: diazinon, imidacloprid and propiconazole. To study toxicokinetics (TK), we performed metabolite screening experiments, toxicokinetic experiments (measuring internal and external chemical concentrations over time) and chemical distribution analyses using quantitative whole body autoradiography (QWBA). Toxicodynamic (TD) tests included acute and pulsed toxicity experiments. Data collected were used to calibrate the General Unified Threshold model of Survival (GUTS). Our results show that even the closely related species, *G. pulex* and *G. fossarum*, differ in their sensitivity to diazinon: the 4-day LC50 value was roughly 5 times higher for *G. fossarum*. This can be explained by toxicokinetics because the simulated concentration of the metabolite diazoxon, which causes the toxic effects, in LC50 exposure concentrations is the same for both species. *Lymnaea stagnalis* was more than 200 times less sensitive to diazinon than *G. fossarum*. It did not activate diazinon to diazoxon as much as Gammaridae but this does not fully explain the differences in sensitivity among the species: in the snail internal diazoxon concentrations in the LC50 exposure exceeds those of Gammaridae species. Instead, the differences are explained by chemical distribution in the organisms as well as likely differences in biochemical and physiological sensitivity to this neurotoxin.

**23 Using the simplified DEB model to analyse ecotoxicity data - a case study with the pond snail *Lymnaea stagnalis*** E. Zimmer, Vrije Universiteit Amsterdam; V. Ducrot, INRA / Ecotoxicology and Quality of Aquatic Ecosystems; A. Agatz, University of York; T. Jager, Vrije Universiteit / Dept. of Theoretical Biology. Dynamic Energy Budget (DEB) models offer the possibility to link effects on several endpoints simultaneously. The model captures how the energy that is taken up from food is translated into the main processes of life, such as growth, reproduction, maturation, and maintenance. Toxic effects are incorporated as changes in the parameter values that capture those fluxes. Using the full DEB model can provide a consistent explanation of the effects on all endpoints simultaneously, however, it is very data intense. In this study, we discuss the benefits and limitations of using a simplified DEB model which is less data intense. The model was applied to analyse life-cycle trait values in the pond snail *Lymnaea stagnalis* exposed to pulses of diquat, a non-selective herbicide. A hormetic effect pattern was found in juvenile feeding and time to sexual maturity. Although all snails ate the same amount of lettuce according to their size, they grew differently. The snails exposed to 5 µg/L grew faster and reached maturity earlier than the control, while the snails exposed to 10 µg/L grew slower and reached maturity later than the control. We observed a dose-dependent degradation of the lettuce that was used as food source for the snails. To investigate the link between these findings, we used the simplified DEB model and analysed the growth and reproduction data simultaneously. We found that the observed effect patterns can be explained by effects on assimilation only, with assimilation enhanced by low doses of diquat, and decreased by higher exposure. Higher assimilation lead to faster growth, with differences in growth explaining the shift in time to sexual maturity. By applying the model to the toxicity data, the importance of the interaction between the lettuce and the herbicide was revealed. Effects of food stress vs toxic stress were disentangled even though assimilation efficiency was not measured during the test. When using a food source that is similar to the target of the toxicant (e.g., a plant and a herbicide), one should be careful when interpreting the test results. To this end, DEB models are an excellent tool to investigate the dynamic interactions between toxic effects and food quality and quantity on multiple endpoints.

**24 Accounting for inter-individual differences between animals improves the evaluation of toxic effects** A. Barsi, INRA / UMR ESE; T. Jager, Vrije Universiteit / Dept. of Theoretical Biology; L.L. Lagadic, INRA / UMR INRAAgriculture Oest Ecology and

Ecosystem Health; V. Ducrot, INRA / Ecotoxicology and Quality of Aquatic Ecosystems. When analyzing toxicological test data it is assumed that individual animals in the test are not unique but equal regarding their physiology and life-cycle traits. When modelling toxic effects, a single model curve is often fitted to the toxicity data. The same set of physiological and toxicological model parameters applies to each individual. However, the use of such an approach may introduce a bias in data analysis because even genetically identical animals differ in their physiology and thus in their responses towards toxicants. In our study, we used a simplified Dynamic Energy Budget (DEB) model and a novel statistical approach to investigate how accounting for inter-individual variability in toxicity test leads to more accurate estimation of metabolic and toxicity parameters, and thus to improved evaluation of toxic effects. We combined experimental and modelling approaches. We designed and performed partial life-cycle toxicity tests on juveniles and adults of the pond snail *Lymnaea stagnalis*. Snails were exposed in isolation to a range of acetone concentrations during 56 days. Survival and clutch production were followed every day, while shell lengths were measured once a week. Obtained results indicated greater toxicity of acetone on juveniles than on adults. Toxic effects were further modelled by using simplified DEB model where we accounted for inter-individual differences between animals. Firstly, we applied a single set of model parameters to fit the model to the obtained data on control and exposed snails. In the second step we fitted model to the experimental data for each individual in the control group. A distribution of model parameters (allocation fraction to the soma and specific assimilation rate) that drive growth and reproduction processes of control snails was obtained. These parameters were negatively correlated and showed considerable differences between individuals. The third step will be to use the parameter distribution obtained from control snails to constrain fits for growth and reproduction of each individual in the treatment. Starting toxicity parameters will be those obtained in the first step. In this way, we expect to achieve better estimations of physiological and toxicity parameters (e.g. narrower confidence intervals) for the exposed snails, which will be great improvement of the evaluation of toxicity test data. Keywords: Dynamic Energy Budget – Toxic effects – *Lymnaea stagnalis*

**25 Time-resolved target and non target screening with LC-MS/MS and LC-HRMS techniques as a tool for water quality monitoring.** M. Schluesener, T. Ternes, Federal Institute of Hydrology. The increasing worldwide contamination of water bodies with thousands of industrial and natural chemical compounds is one of the key environmental problems facing humanity. Although most of these compounds are present at low concentrations, many of them raise considerable toxicological concerns, particularly when present as components of complex mixtures [1]. More as 100,000 chemicals are registered in the European Union, on which 30,000 up to 70,000 are daily used (EINECS, European Inventory of Existing Commercial Chemical Substances). Significant sources of the entry of chemical substances to the water bodies are closely coupled with anthropogenic activities e.g. run-off of chemicals from agriculturally used areas and urban areas, the introduction of municipal and industrial waste water to receiving waters. Furthermore, by accidents or even through spills and upset condition in technical systems a unintentional discharge of chemicals into the water bodies can occur. To identify the chemicals in water bodies, a reliable water quality monitoring strategy has to be established. Therefore, two contrary approaches can be discussed: (1) target analysis and (2) non-target screening. The target analysis is based on the knowledge of the pollutant and an established analytical method. The chemical substance can be identified and quantified with a high precision in the environment, the generated data is reliable and comparable to datasets from other laboratories. However, unknown substances are not determined. Accidents or spills can not be detected with this procedure if the substance is not included in the target list. The second approach is non-target screening. This technique is based on high resolution mass spectrometry (HRMS) coupled with liquid chromatography, together with a mass-spectrometrical databases a powerful tool for the identification of substances in the environment. In this study we present target screening data of some pharmaceuticals



from seven years of monitoring with 28 days composite samples at the Rhine near Koblenz and discuss the temporal trends. This data is compared to a non-target approach on one day composite samples. There, data is processed using principal component analysis (PCA) combined with a mass-spectrometry database search to identify unknown substances in the river Rhine.

**26 Universal Solid Phase Extraction method suitable for laboratory and field analysis prior to toxicity assessment** V. Kokkali; B. Bajema, A. Berg, R. Bosch, W. van Delft, Vitens. 1.Introduction The improved quality of surface water has lead to the integration of a pre-treatment step for concentrating the samples prior to toxicity assessment [1].This study aims to develop a universal Solid Phase Extraction (SPE) method usable in the laboratory and on field attached to toxicity biomonitoring.This method should ensure that the selected 257 compounds give comparable recoveries when received from both the SPE advanced laboratory method and the SPE field method.3 experiments were performed.The first one involved online SPE using Symbiosis™ (Spark,The Netherlands) tandem to Q-TOF liquid chromatography-high resolution-mass spectrometry analysis (LC-HR-MS) (Agilent,The Netherlands), the second one dealt with offline SPE using Symbiosis™ and the final one involved the application of the SPE unit of TOXcontrol (microLAN, The Netherlands).The extracts were analysed using Q-TOF LC-HR-MS. 2.Materials and methods 10ml (two experiments) or 1L (third experiment) of drinking water samples were spiked with 257 compounds and concentrated using Symbiosis or the SPE unit of TOX-control with Oasis® HLB cartridge material. The eluting solvent was 80% acetonitrile for the online experiment and 100% methanol for the offline ones. 3 runs were performed for each experiment and the extracts were analysed with Q-TOF LC-HR-MS in triplicates. 3.Results and discussion The recoveries of 237 polar compounds measured with Q-TOF LC-HR-MS–positive mode were comparable for offline SPE using Symbiosis and the SPE unit of TOXcontrol although the sample volume was 10ml and 1L (Fig 1). Furthermore, the average recovery of all the tested compounds was higher for the online experiment (84%) than for the other two whose procedure was more prone to human error. In particular, the average recovery ranged at the same level for both offline experiments with either Symbiosis (70%) or SPE unit of TOXcontrol (68%). 4.Conclusions In conclusion, the obtained results demonstrated that the SPE unit of TOXcontrol can be applied for concentrating high volumes of samples spiked with a wide range of polar compounds with the same efficiency as advanced instruments. Consequently, the SPE offline method can be used in the field prior to toxicity assessment for producing high quality alarms. 5.References [1] Penders E.J.M.2011. Development of aquatic biomonitoring models for surface waters used for drinking water supply. Ph.D. Thesis,Wageningen University,The Netherlands.

**27 Comprehensive Target and Suspect Screening for Insecticides/Fungicides in Surface Waters by Liquid Chromatography High Resolution Mass Spectrometry.** Hollender, Eawag / Dept Environmental Chemistry; C. Moschet, Eawag / Uchem; A. Piazzoli, ETH; H. Singer, Eawag. The use of pesticides in agriculture inevitably leads to losses of residues into surface waters. Many studies investigated the occurrence of either herbicides or legacy insecticides (organochlorines, organophosphates) in surface waters. On the other hand, fungicides (e.g., anilino pyrimidines, azoles) and modern insecticides (e.g., diacyl hydrazines, neonicotinoids) have rarely been measured in rivers although they can show ecotoxicological effects in the low ng/L range. Here, we present a combined target and suspect approach for surface water screening to measure all organic synthetic insecticides and fungicides registered in Switzerland. The method consists of an offline solid phase extraction (SPE) using a mixed-bed multilayer cartridge (enables the enrichment of a broad substance spectra) followed by liquid chromatography with electrospray ionisation and high-resolution mass spectrometry (LC-ESI-HRMS/MS; Thermo Fischer QExactive). Twenty insecticides, 23 fungicides and 8 metabolites were selected for a quantitative target method based on consumption amounts, physical-chemical properties and predicted fate

behaviour. For the target method, normalized collision energies were optimized and characteristic MS<sup>2</sup> fragments were derived. Absolute and relative recoveries of most target analytes lay between 80-120% and LOQs were below 2.5 ng/L for 75% of analytes. The exact masses (m/z [M+H]<sup>+</sup>, [M+Na]<sup>+</sup>, [M-H]<sup>-</sup>) of the remaining insecticides, fungicides and selected metabolites were screened in environmental samples. The peaks were studied further if a meaningful peak shape as well as isotopic pattern was visible, and if the peak was missing in the blank sample. A targeted MS<sup>2</sup> spectra was compared with library spectra (Thermo Fisher Library Manager 2.0). If possible a standard was purchased for unequivocal confirmation. With this combined target and suspect approach, 84% of all registered insecticides and fungicides were covered (remaining substances were pyrethroids/dithiocarbamates which are not ionisable with ESI). Field measurements in 5 agriculturally influenced rivers showed low insecticide and fungicide concentrations (90% of all detections below 20 ng/L and 50 ng/L, respectively). In addition to the target approach, 3 insecticides/-metabolites and 15 fungicides were found with the suspect screening. A non-selective enrichment step together with high-resolution mass spectrometry allowed the screening of nearly all registered insecticides and fungicides.

**28 Transformation of non-steroid anti-inflammatory drugs during sewage treatment** E. Larsson, Lund University; S. al-Hamimi, Lund University / Centre for Analysis and Synthesis; J. Jonsson, Lund University / Department of Chemistry. Non-steroid anti-inflammatory drugs (NSAIDs) are frequently occurring in water both entering and exiting sewage treatment plants (STPs) as well as in recipients due to the consumption and later urinary/bile extraction by humans. However, a major part of these compounds are excreted from the human body and thus entering the STPs as their phase I or phase II metabolites. Several studies also indicate that the major removal mechanism for the parent compounds during sewage treatment is biological transformation. Thus, significant amounts of transformation products might also be formed during the treatment and released into the recipient. However, few studies have so far been performed regarding the presence, formation and release of specific transformation products, mainly due to the analytical challenges associated with such studies. In this work we have developed a hollow fiber liquid phase microextraction method combined with LC-MS/MS analysis to simultaneously extract the NSAIDs ketoprofen, naproxen, diclofenac and ibuprofen together with six of their known degradation products (*o*-desmethoxynaproxen, 1-hydroxyibuprofen, 2-hydroxyibuprofen, carboxyibuprofen, 4'-hydroxydiclofenac and N-(2,6-dichlorophenyl)anthranilic acid) from sewage water. Enrichment is obtained in 5 h and the method provides excellent clean-up resulting in extracts which can be directly injected onto LC-MS/MS. So far the method has been applied on influent water from a Swedish STP and showed the presence of *o*-desmethoxynaproxen, carboxyibuprofen, 1-hydroxyibuprofen and 2-hydroxyibuprofen in amounts at the same level or exceeding those of their parent compounds. Analysis of water exiting the primary and biological treatment as well as effluent water will also be analyzed and the final aim is to calculate a mass balance for all compounds and quantify their presence, formation and release during sewage treatment which is planned to be finished in early 2013.

**29 HPLC-QqLIT-MS2 multiresidue trace analysis of sulfonamide antibiotics and their metabolites in soils and sewage sludge** S. Diaz-Cruz, IDAEACSIC / Environmental Chemistry; M. García-Galan, CSIC / Environmental Chemistry; D. Barcelo, IQABCSIC. Several studies have demonstrated the widespread presence of sulfonamides (SAs) in the environment and identified their entrance pathways [1]. The occurrence of their main metabolites, the acetylated conjugates, has become evident recently, especially in environmental waters [2]. These metabolites, once excreted, can deconjugate and revert back to the original parent drug [3]. Therefore, the inclusion of these metabolites in environmental occurrence surveys for SAs is key to obtain more complete and reliable information on the actual levels, avoiding potential underestimations. The lack of sensitive enough methodologies for the sulfonamide analysis in solid matrices and the need to consider

the presence of their metabolites promoted this study. The present work describes the development, validation and a practical application of an automated analytical method based on pressurized liquid extraction (PLE) followed by solid-phase extraction-liquid chromatography–tandem mass spectrometry (SPE-LC–MS/MS) for the simultaneous determination of 22 SAs and acetylated metabolites in sewage sludge and soil samples. Matrix effects were evaluated, and different internal standards were used for quantification. The recovery efficiencies were found to be 60%–130% for almost all SAs in both matrices and at two spike levels. The MLODs achieved were in the range 0.01–4.19 ng g<sup>-1</sup>. The method was applied to evaluate the occurrence of the target SAs in sewage sludge and soil samples taken in wastewater treatment plants and agricultural areas. Results confirmed the wide presence of SAs in both matrices. Maximum concentrations corresponded to sulfamethazine (139.2 ng g<sup>-1</sup> and 8.53 ng g<sup>-1</sup> for sewage sludge and soils respectively). The acetylated metabolites were detected in sewage sludge and in soils. To the author's knowledge, it is the first time that acetylated metabolites of SAs have been identified in sewage sludge. Acknowledgements We acknowledge the Spanish Ministry of Economy and Competitiveness (SCARCE project, Consolider Ingenio 2010 CSD2009-00065). References S.C. Kim, K. Carlson, *Environ Sci Technol* 41 (2007) 50. M.J. García-Galán, T. Garrido, J. Fraile, A. Ginebreda, M.S. Díaz-Cruz, D. Barceló, *Anal Bioanal Chem* 399 (2010) 795. M.J. García-Galán, T. Frömel, J. Müller, M. Peschka, T. Knepper, S. Díaz-Cruz, D. Barceló, *Anal Bioanal Chem* 402 (2012) 2885.

**30 Environmental fate of nitramines and nitrosamines released as degradation products from post combustion CO<sub>2</sub>-capture plants**  
L. Sorensen, SINTEF Materials and Chemistry / Marine Environmental Technology; O. Brakstad, K. Zahlén, E. da Silva, A. Booth, SINTEF Materials and Chemistry. Recently there has been increased awareness of the need to understand and control emissions from post-combustion CO<sub>2</sub> capture (PCCC) plants. Among the large number of possible and identified degradation products that may form in amine based PCCC plants, nitramines (R<sub>2</sub>NNO) and nitrosamines (R<sub>2</sub>NNO) are of particular concern, due to their carcinogenic potency. In this study, the hydrolytic and photolytic degradation rates of a suite of four nitramines and five nitrosamines are reported in freshwater. In addition, the potential of these compound groups to degrade by microbial action under oxic and anoxic conditions in freshwater and lake sediment is also reported. As nitrosamines and nitramines will be released in very low concentrations from a PCCC plant during normal operation, a major focus of the project has been to develop suitable analytical methods for working with these contaminants at environmentally relevant trace levels. For this purpose, chemical analysis was performed by LC-MS/MS QqQ using deuterated internal standards for quantification. Of the nine studied compounds, seven showed no hydrolytic degradation at any pH. Hydrolytic stability of a range of nitramines and nitrosamines has also previously been observed. In the current study, two compounds derived from the amine piperazine (1-nitropiperazine and nitrosopiperazine) showed degradation, but only at pH 7. Results from a rate determining study of these compounds at three different temperatures will be presented. Preliminary tests have shown that nitrosamines in surface waters will degrade rapidly (within days) when exposed to natural sunlight, whilst nitramines are photostable. Results from an experimental study aimed at determining the degradation rates of the four nitrosamines NDELA, NDMA, NMOR and NPz exposed to artificial sunlight will be presented. Previous studies have indicated that the biodegradation of nitrosamines in freshwater is enhanced greatly by the presence of a hydroxyl group, such as for n-nitrosodiethanolamine (NDELA). It is expected that the same trend will be observed for the nitramines. Two freshwater biodegradable compounds, NDELA and the nitramine 2-nitroaminoethanol (MEA-NO), will be tested alongside two less biodegradable compounds, n-nitrosodimethylamine (NDMA) and n-dimethylnitramine (DMNA), to determine whether or not microbial degradation in the water column and the lake sediment are comparable for these compounds.

### 31 Abandoned uranium mine-induced effects in caged roach: a

**multiparametric approach for the evaluation of in situ metal toxicity**  
B. Gagnaire; A. Bado-Nilles, Université de Reims/INERIS; S. Betoulle, Université de Reims; R. Amara, E. Kerambrun, Université du Littoral Côte d'Opale; X. Denier, C. Minier, Université du Havre; I. Cavalie, C. Adam-Guillermin, IRSN; A. Le Guernic, IRSN/INERIS; W. Sanchez, INERIS. In the frame of the characterization of environmental risk assessment linked to abandoned uranium mines in the Limousin region (France) and in the context of the DEVIL program (Development and validation of fish biomarkers for the implementation of environmental legislation) led by INERIS (National institute for industry environment and risks), a study has been conducted on the effects of uranium mining releases on roach health status. The first part of the work was the search of potential sites for the study. Based on information from IRSN (National institute for radioprotection and nuclear safety) and from the owner of old mines, two private pounds were identified: one presenting uranium contamination, and one upstream the mining zone presenting no uranium in water. Roaches were therefore caged during one month on these two pounds. Biological sampling have been performed at the beginning of the experiment, and 2 and 4 weeks after, in order to measure several parameters: immune parameters followed by flow cytometry (phagocytosis, ROS production, apoptosis, lysosomes), genotoxicity (comet assay), oxidative stress (glutathione, lipoperoxidation), biotransformation (EROD, GST, ABC transporter), neurotoxicity (AChE), endocrine disruption (vitellogenin) and physiological parameters (ADN/ARN ratio, stomach contents, otoliths, condition index, sex ratio). Physico-chemical parameters of water and sediments (cations, anions, metals, radioactive elements) were also followed and bioaccumulation of metals in several organs (muscle, liver, kidney, gonad and gill) was measured. The results showed on the uranium contaminated site a water contamination of iron, aluminium, barium and manganese. Aluminium and iron were also present in the water of the uranium-free site. The sediments from the uranium contaminated site showed high levels of radioactive elements coming from the disintegration chain of uranium. Preliminary biological results indicated a stimulation of immune parameters, an increase of oxidative stress and a decrease of AChE in fish coming from the uranium contaminated site compared to the uranium-free site. The whole results, using integrated index, will allow the establishment of roach health status in the context of pluri-metallic mining release pollution. These data will strengthen the research for the improvement of methods and tools needed for the environmental risk assessment associated to radioactive substances in the environment.

**32 Evolution of aquatic ecology at the abandoned Silvermines site, Co. Tipperary, Ireland.** S. Barnes, Golder Associates UK Ltd; K. Harrington, B. Balding, A. Murray, Golder Associates Ireland / Town Centre House, Dublin Road. Abstract The Silvermines area of North Tipperary in Ireland has experienced mining activity for over a thousand years, with lead, zinc, copper, barite and sulphur being extracted from a series of Carboniferous limestones. The last mine to operate in the area was closed in 1993. A legacy of contamination and derelict surface structures remain. Following the death of cattle in the vicinity in 1999, a governmental Inter-Agency Group was established to conduct an investigation. A 'Silvermines Rehabilitation Project' resulted, and included proposed development aimed at containing mine wastes derived from problematic sites across the area and to ultimately help reduce emissions to the water environment. The Project involved an evaluation of aquatic ecology spanning the termination of mining activity. Biological water quality data since the 1970's from the Kilmastulla and Yellow Rivers area of the Shannon catchment have been sourced and supplemented by update survey, and spot stream water chemistry data. Historical mining activities have rendered an extremely varied habitat and water quality distribution within a relatively small study area of approximately 13 km<sup>2</sup>. Q-values from the Environmental Protection Agency (EPA) Q-rating (biotic indices) methodology ranged between 1 and 5 during the mining period. Q-values have been fairly consistent at individual locations between consecutive observations; but there is limited evidence that significantly impacted waters persist at distance downstream. In the 20 years since mine closure, locations in

proximity or down stream to mining activities have often shown some natural improvement in River Quality Classification, but slight to moderate pollution persists. Upstream samples typically have high diversity and a frequency of Group A and B taxa including pollution sensitive mayflies and stoneflies. Impacts have severely reduced overall macroinvertebrate diversity and eliminate sensitive fauna. A moderate to strong inverse correlation ( $r$ ) exists between Q-values and the spot zinc concentrations (-0.73) determined, which reflect an inverse correlation of -0.64 between zinc concentration and the total number of taxa. A habitat enhancement programme is vital to rehabilitate the study area. It is essential remedial works, not yet implemented, do not result in future release of mine tailings and/or excessive sediment loads.

**33 Mine Dewatering into a Tropical Ecosystem** J. Woodworth, GHD; A. Sawicki, Vista Gold Australia; A. Brandis, Envirotech Monitoring. Vista Gold Australia Pty Ltd (Vista Gold) proposes to re-establish and operate the Mt Todd Gold Mine, located 55 kilometres north of Katherine and 250 kilometres south of Darwin in the Northern Territory, Australia. The site was previously mined for gold in the 1990s with mining operations ceasing in the early 2000s. Mining infrastructure such as open pit, tailings dam, waste rock dump, retention ponds and remains of processing facilities remain on-site. The site has many surface water bodies (retention ponds or RPs), some of which contain water with high metal and low pH levels that have the potential to overflow during the wet season. The site needs to be dewatered prior to mining commencing. The poor water quality on site combined with the tropical wet and dry seasons required a site specific ecotoxicological program integrated with environmental monitoring to allow the site to be dewatered and provide maximum protection to the receiving ecosystem. Currently Vista Gold is treating 10.5 GL in the Batman Pit (RP 3) to reduce the high cadmium, copper, lead, nickel and zinc concentrations to allow increased discharge from the site during the 2012/2013 and following wet seasons. Ecotoxicological analysis of the treated water has enabled the correct volume to be calculated for discharge to meet the 80% species protection values (ANZECC 2000) at a specified point on the Edith River as stated in the Waste Discharge Licence for the site. Trigger values at the 80% species protection level have been applied for a two year period only to allow site dewatering and prevent catastrophic uncontrolled discharge from the site. Community concern has been shown regarding the use of the 80% values as the discharge from the mine site enters the Edith River which is a tributary of the Daly River, a world renowned barramundi fishing location. Aquatic organisms caught in the Edith River also provide a traditional source of food for the indigenous population. Ecotoxicity testing and monitoring programs have helped to alleviate the community concerns to some extent as no significant impacts have been detected in macroinvertebrate and sediment quality studies. This presentation will discuss the application and results from ecotoxicological analysis using tropical species bioassays and the integration of a macroinvertebrate monitoring program designed to ensure that the downstream ecosystem is not adversely impacted by the mine dewatering.

**34 The environmental legacy from centuries of metals mining in south central Scotland** I.W. Oliver, Keele University / Physical Geographical Sciences; F. Naysmith, Scottish Environment Protection Agency / Operations; A.M. Moorhouse, L.M. Wyatt, I.A. Watson, the Coal Authority; J. Redshaw. Mining for lead (Pb) and other metals in the Leadhills area of S. central Scotland dates back to the 1200s. The long mining history has left its mark and an environmental legacy. Elevated levels of Pb have been noted in the soils of the area. Fish monitoring in the 1980s in one stream, the Glengonnar Water, found trout with blackened tails (an indicator of chronic Pb poisoning) while analysis of fish tissues revealed elevated Pb concentrations. Monitoring of the Glengonnar Water under the Water Framework Directive<sup>3</sup> has also revealed it to persistently fail environmental quality standards (EQS) set for cadmium (Cd), zinc (Zn) and Pb in water. The local environment is thus affected by its mining legacy and this influences public perceptions. To inform decisions on potential future remediation work, the Coal Authority, with support from SEPA, investigated the

extent of contamination in the soils, sediments and water of the Glengonnar catchment to pinpoint the locations where metal inputs occur. A preliminary examination of another stream, the Wanlock Water, in a neighbouring catchment that had not previously been assessed for metal contamination was also conducted. Mine drainage and stream water assessments revealed that while Zn and Cd were entering Glengonnar Water as dissolved metals directly from mine drainage points, Pb was entering via diffuse inputs (i.e. run-off bearing particulate Pb from soils and surface depositions). Pb concentrations reached 4.8% (w/w) in soil and 10% (w/w) in stream sediments. Whilst having no statutory standing in the UK, sediment quality guidelines proposed elsewhere<sup>4</sup> suggest that metal concentrations are present here at potential effect levels and thus ecological impacts beyond those previously noted for fish may have occurred. Water build-up behind blockages within old mine adits were also identified as posing a potential flood risk. For the Wanlock, preliminary analyses indicate that it too is likely to exceed EQS for Pb and Cd in water. The work highlights the mining legacy in the area and the future work required to develop remediation options. 1 Towers et al. 2006 Scotland's soil resource- current state and threats. 2 ClydeRiverPurification Board 1984 Concentrations of trace metals in water and brown trout from Glengonnar Water. 3 Directive 2000/60/EC of the European Parliament: The Water Framework Directive. 4 Council of Ministers of the Environment (Canada) 2002 Canadian Sediment Quality Guidelines

**35 Venus Mine Site, Yukon, Canada: Acid Mine Drainage Assessment and Mitigation** M. Dodd, Royal Roads University / School of Environment & Sustainability; W. Liebau, Aboriginal Affairs and Northern Development Canada / Waste Management. Venus Mine is a former silver mine located on the at the base of Montana Mountain along the South Klondike Highway, 20 km south of Carcross, Yukon, Canada. The mine produced approximately 84,000 tonnes of ore over its lifetime generating about 50,000 tonnes of waste rock which was piled along the edge of the mountain near adits and shafts. The results of acid base accounting test in this study indicated that the waste rock contained patches that were oxidized and had acid pH while the remaining un-oxidized materials had the potential to become net acid generating. Arsenic, Cd, Pb, and zinc in effluents from adits and waste rock piles were very high. The elevated levels were attributed to metal leaching from the waste rock since laboratory leach test showed elevated levels in the leachate. The migration of As, Sb, Cd, Pb, Ag and Zn towards Tagish Lake was occurring however metal concentrations in the lake were comparable to background levels due to attenuation by natural on-site vegetation. Despite the indication of potential net acid generation of the waste rock the effluents were alkaline (pH 7.99 – 8.71) while SO<sub>2</sub> and Fe concentrations were relatively low. It therefore appeared any acid generation within the waste rock was occurring at a rate that was not exceeding the available neutralization potential. Remedial options to prevent further oxidation and ensure the neutralization potential of the waste rocks were not exceeded were investigated. The use of biosolids amendments was unsuccessful since that yielded increased metal leachate concentrations. Underwater storage and covering the waste rock in place were also considered undesirable due to the steepness of the slopes. The use of vegetative cover was deemed the most feasible based on field observations and laboratory data. Natural vegetation at the Venus Mine site could be used to minimize the effects of acid mine drainage and associated elevated metal concentrations in effluents from the adits and waste rock. This could be accomplished by diverting the effluents and any surface run-off into an identified vegetated area on-site. Drainage ditches could also be constructed to intercept and divert water from waste rock piles in addition to the use of vegetative cover. Ongoing studies include investigations into metals uptake into the on-site vegetation and laboratory bench scale studies on soil amendments to improve neutralization and re-vegetation.

**36 When is clean “clean enough”? Integrating ecological function with remediation goals for the historic Britannia Mine** B.G. Wernick, Golder Associates Ltd. The former Britannia Mine operated near Vancouver, Canada from 1904 to 1974, and at its peak was the largest

copper mine in the British Commonwealth. During its 70 years of operation it generated more than 40 million tonnes of tailings, much of which was deposited in the adjacent marine environment of Howe Sound and used as fill along the Britannia Beach shoreline. The acid-generating tailings and upland former mine workings leached dissolved copper and zinc into Britannia Creek (which drains to Howe Sound) as well as directly into Howe Sound until recently when the provincial government began an ambitious remediation program intended to intercept, collect and treat water-borne metals discharging to the environment. Monitoring of water quality and intertidal ecology is providing information confirming that environmental conditions are improving along a majority of the shoreline and that residual impacts appear to be localized around areas of historical infrastructure. A remediation program or risk manager for a contaminated site needs to know "how clean is clean enough?" The objective of the initial remediation activities was to address severe, acutely lethal conditions that posed a mortality hazard to out-migrating juvenile salmon and this objective has been achieved. As site characterization continues and additional remediation is undertaken, realistic remediation goals need to be defined. In the case of the Britannia Mine, the return of the shoreline to pre-development conditions is likely not possible as the long-term operations of the mine resulted in significant filling and replacement of the natural shoreline with engineered surfaces (e.g., rip rap). Moreover, the construction of a transportation corridor adjacent to the shoreline will limit the use of intrusive remediation techniques. "Resource conservation objectives" (RCOs) have thus been articulated to help direct remediation efforts. The RCOs consist of a description of important shoreline ecological functions and/or features as well as acceptable levels of those features that are based on: 1) the results of the monitoring program which provide an understanding of the ecological conditions of near-field and reference areas, including natural variability; 2) the broader mandate of environmental agencies to maintain and/or restore a healthy productive ecosystem; and 3) the local community's desire for environmental improvement but not at all costs.

**37 Use of environmental science and scientific results by ECHA: present and future** L.V. Tarazona, C. AJAO, D. APE, R. CESNAITIS, B. DILHAC, D. KNIGHT, H. PIHA, H. SCHIMMELPFENNIG, ECHA. The European Chemicals Agency (ECHA) is the driving force among regulatory authorities in implementing the EU's groundbreaking chemicals legislation. The use of the best available scientific knowledge, within the limits of the regulatory context, is an essential element in all REACH processes. This is partially addressed through the ECHA Scientific Committees and complemented with internal horizontal and targeted scientific activities. As the Agency is entering into the consolidation phase and has new regulatory roles in the field of Biocides and PIC regulations, its strategy for addressing the scientific challenges is being adapted. This contribution will present a general overview on the current ECHA practices for monitoring and examining the regulatory implications of knowledge progress in environmental sciences, and for ensuring that updated states of the science regarding environmental fate and ecotoxicity of chemicals can be applied for substantiating ECHA decisions, opinions, recommendations and guidance, giving concrete examples on particularly relevant topics. In addition, we will present our approach to ensure that ECHA can play an increased and significant role of the scientific community in Europe and world-wide. With this we aim at a wider contribution of ECHA in the identification of scientific needs for environmental hazard and risk assessment, and at channelling ECHA contributions to the challenge of transforming research results into regulatory scientific knowledge, ready for supporting decision-making processes. ECHA Topical Scientific Workshops are a key element of this development, and the first one, already in 2013, will address the risk assessment for sediment.

**38 Missing links in EU chemical risk management of consumer products** L. Molander, Stockholm University / Department of Applied Environmental Science (ITM); M. Breitholtz, S. Bejgarn, A. Sobek, C. Ruden, Stockholm University / Department of Applied

Environmental Science (ITM). The aim of this study is to investigate the interlinks between EU legislations concerning chemicals in consumer articles and cosmetic products with regard to how they share and integrate information useful for assessing and reducing negative environmental impacts. This contribution consists of two case studies, including (1) an analysis of the extent to which the regulation of hazardous substances in articles under REACH is coherent with the restrictions under the Water Framework Directive (WFD), and (2) an investigation of whether publicly available data on PBT properties of sunscreen UV-filters would lead to hazard classifications under the CLP regulation, as they are now exempted from CLP. The coherence analysis shows that the majority (22 of 24) of the substances or groups of substances that are prioritized for phase-out under the WFD are allowed to be used in most articles according to REACH. The incoherencies in which substances are prioritized and restricted between REACH and the WFD constitute a possible obstacle for reaching environmental goals as set out by the WFD. For 13 of 26 UV-filters a classification as hazardous to the aquatic environment could be made based on publicly available data, or was already made by industry. Two sunscreen products were also evaluated and both could be classified as hazardous to the aquatic environment according to the CLP criteria for mixtures. The aquatic hazard classifications of UV-filters in sunscreens and sunscreen products derived from publicly available data currently fall between different regulatory frameworks. Such missing links hamper important information from being disseminated. In conclusion, information that is made available, e.g. via the REACH registration database, should be utilized to a greater extent in order to increase coherence between different regulatory frameworks. This would more effectively reduce environmental risks and enable a better management of these risks.

**39 UVCB : Which data entries for unknown substances ?** P. Garrigues, University of Bordeaux / Institute for Molecular Sciences; F. Lafaye, University of Lyon / ENTPE/UMR CNRS EVS; N. Leca, University of Bordeaux / Institute for Molecular Sciences. The categorization of UVCB substance has been introduced into the REACH regulation. UVCB is a substance of unknown or variable composition, complex reaction products or biological materials. Since characterization of UVCB is difficult due to its complex composition, the production of data could be waived or be replaced by more specific analytical techniques. The lack of some data or the production of data under specific techniques will lead to generate sets of data that may not really fit with the analytical data obtained with well defined substances. The presentation will be focused on the characterisation and the identifiers of UVCB, required for the registration of chemicals, the first step of REACH procedure. Some difficulties appear in this first phase and others will certainly be stressed in the future in the evaluation phase. Regarding analytical data, spectroscopic methods must be produced when providing information on the composition of UVCB substance (UV/Vis, Infra-red, nuclear magnetic resonance or mass spectrum). Some of these techniques appear as non appropriate for characterization of some UVCB substances for several reasons. Most of the spectroscopic techniques are well suited for analysing individual chemical products or very simple complex mixtures. When several dozens of constituents are present, the resulting spectra will provide average broad-banded spectra without any clear identification. This is particularly true for UV/Vis spectra. In that presentation, several case studies will be presented on identification of constituents some complex UVCB (natural products, oil refinery products). The results will be discussed toward the requirement of the REACH regulation annex 6.

**40 Have Sediment Quality Guidelines Improved Since the SETAC 2002 Pellston Workshop?** R.J. Wenning, ENVIRON International Corporation; K.M. Leung, The University of Hong Kong; G.E. Batley, CSIRO Land and Water / Centre for Environmental Contaminants Research; T.S. Bridges, US Army Corps of Engineers / USERDC-WES-EM-D; S. Hall, ENVIRON International Corp; G. Merrington, Environment Agency. Historically, the significance of contaminant concentrations in sediments has been assessed by making comparisons

between measured concentrations and numerical sediment quality guidelines (SQGs). Based on such comparisons, the potential risks, or hazard of sediment-bound contaminants can be estimated and decisions made to reduce perceived threats to aquatic life. The derivation and scientific validity of different approaches used to derive SQGs was reviewed in 2002 at a SETAC Pellston Workshop (Wenning et al. 2005). At that time, limitations were identified, specific research activities recommended, and cautions issued on the misuse of SQGs. Here we consider the progress made to advance scientific knowledge about the relationship between sediment chemistry and impacts on aquatic ecosystems. Advances have occurred, but translation into improved ecological risk assessments has been less rapid and remains problematic. In ecotoxicology, a limitation was the suite of bioassays; however, the number of bioassays has expanded beyond amphipods to include copepods, benthic algae, bivalves, polychaetes and snails. Ecogenomics has revolutionized ecological assessments allowing thousands of taxa to be compared to the traditional macrofauna used in standard ecological surveys. The recognition that major drivers of sediment contaminant bioavailability are grain size and organic carbon content led to development of the sediment biotic ligand model and new approaches to deriving SQGs that incorporate these factors. Weight of evidence approaches have been embraced in California, Australia and elsewhere, lessening reliance on expert judgment. Improved numerical guidelines are now available for several key contaminants. Problematic, however, remains lack of clarity about contaminated sediments referred to as 'transition zone' conditions not easily identified as either "contaminated" or "clean". The challenge remains determining the role contaminated sediment conditions play in supporting either a 'healthy' (but possibly altered), or 'degraded' ecosystem. Although understanding of the ecotoxicology of contaminants has improved, research on organism and species responses to contaminant exposure is less advanced. In fact, non-chemical stressors have complicated interpretations about causal relationships between contaminant concentrations in sediments and biological responses.

**41 Chesar – ECHA's Chemical Safety Assessment tool** c. team, h. magaud, A. Ahrens, r. bernasconi, e. nogueiro, r. gilioli, z. gabor, s. frattini, ECHA. The Chemical Safety Assessment and Reporting tool, Chesar, has been developed by the European Chemicals Agency (ECHA), aiming at supporting companies in carrying out their Chemical Safety Assessment (CSA), preparing their Chemical Safety Report (CSR) and finally generating the Exposure Scenarios to be annexed to the Safety Data Sheet for communication to downstream users. Chesar provides a structured workflow: it guides the users to assess a substance starting from the hazard information and conclusion available in a IUCLID 5 dataset, through the description of the uses of the substance and the definition of the existing operational conditions and risk management measures (OC/RMM). It calculates or accommodates the results of the environment and human health exposure estimations for quantitative assessments. Exposure scenarios are generated and risk characterisation (quantitative and/or qualitative) is performed. The Chesar library enables the re-use of pre-defined CSA elements like use maps, conditions of use and SpERCs (specific environmental release categories) that can be exchanged easily among users. Chesar increases the efficiency in conducting the CSAs (re-use of CSA elements, communication with IUCLID) and provides consistency between CSAs and the information communicated downstream. **Presentation preference:** poster/presentation **Session** Risk assessment, regulation, policy and public perception **Keywords:** CSA, CSR, exposure scenario, (Co-)authors: Hélène Magaud – ECHA, Andreas Ahrens – ECHA, Roberta Bernasconi – ECHA, Stefano Frattini – ECHA, Zsolt Gabor – ECHA - Roberto Gilioli - ECHA, Eugénia Nogueiro – ECHA.

**42 MOSAIC\_SSD: A new web-tool to include censored data in SSD** G. Kon Kam King, Université Claude Bernard Lyon / Laboratoire de Biométrie et Biologie Evolutive; P. Veber, Université Claude Bernard Lyon 1 / Laboratoire de Biométrie et Biologie Evolutive; S. Charles, University Lyon / Laboratory of Biometry and Evolutionary Biology; M. Delignette-Muller, VetAgro Sup. The *species sensitivity distribution*

(SSD) is a key tool to assess the ecotoxicological threat of pollutants to biodiversity. SSD is widely used by environmental regulatory bodies to define *safe levels* for toxicants. SSD is a representation of the sensitivity of a community to a pollutant as a probability distribution. Toxicity experiments provide *critical effect concentrations* (CECs) for single species, which are used to characterize this distribution. Very often, CECs cannot be estimated precisely, because the real values lie outside the range of the tested concentrations, or the uncertainty is very large. Then only a lower or a higher bound for the real CEC values is known, or the interval including them. Such data are called *censored data*. The general practice is to discard left/right censored data and to consider the centre of the interval censored data as a pointwise value. However, this severely undermines the reliability of any conclusion drawn from the SSD analysis. Therefore, we present a new statistical web-tool to properly include censored data in SSD using maximum likelihood methods. MOSAIC\_SSD is based on a general R package, *fitdistrplus*, which can be used to fit an SSD, encompassing all the functionalities of existing software such as CADDIS SSD Generator, ETX or BurliOZ [5]. Package *fitdistrplus* allows fitting any user-defined distribution through a variety of methods and computes confidence intervals using bootstrap. The possibility to include not only left/right, but also interval censored data using a frequentist framework is not available in any of the aforementioned software, and is generally not put into practice in the literature. We will demonstrate how discarding censored data leads to a biased estimation of the SSD, and illustrate the versatility of MOSAIC\_SSD. By lowering the barrier to using sophisticated statistical tools, our web-tool offers a convenient and user-friendly interface to perform a statistically sound SSD estimation without worrying about technicalities. It is easily accessible within the familiar framework of a webpage, providing a turnkey alternative for ecotoxicologists. Moreover, the *fitdistrplus* R-package allows for many more refinements, such as fitting through moment matching or minimum distance estimation, as well as comparing goodness-of-fit statistics for non-censored data. Together, MOSAIC\_SSD and *fitdistrplus* should satisfy the needs of both casual and advanced users in ecotoxicology.

**43 A novel approach to allocation of by-products in background systems and databases** B. Weidema, The ecoinvent centre G. Wernet, ecoinvent Centre. The problem of allocation is a long-standing issue in Life Cycle Assessment (LCA). The question of recycling represents an especially difficult question, as physical or economic allocation properties can be difficult to determine in such cases. The ecoinvent database has so far used 3 distinct approaches to deal with by-products, recyclable materials and wastes. Recyclable materials were simply handled using the cut-off approach. This presentation describes a new approach considered for the next version of the ecoinvent database, called *Allocation at the point of Substitution (ApoS)*, that removes the cut-off approach and handles all by-products consistently. In ApoS, any allocation is deferred to a point after any necessary treatments of by-products, e.g. in recycling. A process system can be combined to create a larger system that only stops when by-product treatment has resulted in a marketable product. This does not remove the need for allocation completely, but it does avoid the need for allocation factors within treatment systems. In ApoS, any by-product requiring treatment is moved to be a negative input in the producer and a negative output in the treatment activity, comparable to a treatment service input. The by-product of the treatment is merged into the producer to result in a system where the treatment activity is no longer multi-functional. Thus, allocation within the treatment activity between the function of a waste removal service and a useful by-product is avoided and the distinction between individual activities is maintained, a feature critical for regionalized impact assessment. The resulting datasets are further adapted to consolidate the resulting datasets. Using ApoS, treatment by-products are available to be included in other product systems carrying the share of the burdens from the previous life cycles. Such assessments were not possible in previous versions of the ecoinvent database due to the cut-off approach. The presentation shows results of such comparisons. ApoS is a novel approach to determining the impacts of by-

products on a database-wide level, and allows the comparison of treatment products with comparable virgin materials. Therefore, it can offer a critical new perspective on process systems.

#### **44 Methodology applied to the background analysis of energy data to be considered for the European Reference Life Cycle Database (ELCD)**

**M. Recchioni**, EC JRC IES; D. Garrain, CIEMAT / Energy Dpt. - Energy Systems Analysis Unit; S. Fazio, EC JRC - IES; C. De la rua, CIEMAT; F. Mathieux, EC JRC - IES; Y. Lechon, CIEMAT / Energy Dpt. - Energy Systems Analysis Unit. In the Integrated Product Policy Communication of 2003, the European Commission recognised LCA as “the best framework for assessing the potential environmental impacts of products”. Since then, life cycle approaches were further strengthened in EU policies through the Sustainable Production and Consumption/Sustainable Industry Policy Action Plan Communications that encompasses various policies. Within this context there is an urgent “need to improve data availability and quality worldwide by internationally cooperating on LCA data and methods”. To address this situation, the European Reference Life-Cycle Database (ELCD) has been developed by the EC’s Joint Research Centre (DG JRC). Within the ELCD, several energy-related data are provided, being energy a major input for almost all the environmental analyses of products or processes. The present paper will provide the description of the methodology that will be used for the analysis of the quality of energy data for European markets that are available in 3<sup>rd</sup> party life cycle databases and from authoritative sources that are, or could be, used in the context of the ELCD. The methodology has been developed by a joint project with the cooperation of the EC JRC and the Energy Systems Analysis (ASE) Unit of CIEMAT (Public Research Centre for Energy, Environment and Technology). The methodology was developed and tested on the most relevant energy datasets for the EU context; however it can be widely applied for the quality evaluation of energy data, even if provided by other sources. Basing on the above mentioned principles, some 3<sup>rd</sup> party life cycle databases have been selected, to be compared with ELCD database. The proposed approach has been based on the quality indicators developed within the ILCD handbook that have been further developed to facilitate their use in the analysis of energy systems. The quality indicators have been refined in order to identify key aspects that are involved in both quality and methodological aspects for energy related LCI datasets. The quality of each dataset can be estimated for each indicator and then, compared across the different databases/source. Results will point out the findings and/or recommendations in order to improve the data quality as regards the established criteria. A template including quality parameters, rating, and suggestions for improving (e.g. sources or studies to be included) will be compiled for each analysed dataset.

#### **45 Global Sensitivity Analysis: a tool to analyse LCA variability of energy systems**

**P. Padey**, EDF Mines ParisTech; D. Le Boulch; R. Girard, Mines ParisTech; **L. Blanc**, Mines ParisTech / Centre for Energy and Processes. Policy makers are nowadays debating about the future electricity mixes that should be deployed. The environmental impacts of electricity generation systems is one of the central issue for this debate. They have been widely assessed over the past decades, in particular with the LCA approach. Several literature reviews have shown the large variability associated with these results. It leads sometimes policy makers to consider LCA as an inconclusive method. Improving the understanding of the LCA results variability origins is a key issue to extend the use of LCA as a decision support tool. One approach to address variability are sensitivity analysis (SA). However, when dealing with environmental impact assessment, most SAs remain at a local level or evaluate the variation of the input parameters one factor at a time. These approaches only partially reflect the LCA results variability; indeed, it does not consider the full range of input parameters interval and their probability distribution. To overcome these limitations, Global Sensitivity Analysis (GSA) approach has been developed in statistics. It enables apportioning the results variability of a model to its different input parameter variability, by varying all of them simultaneously according to their probability distributions. This link between result

variability and parameter variability is quantitatively evaluated by the calculation of the so called Sobol indices. While it has been applied in only a few analyses in the field of environmental impact assessment, this statistical tool is yet to be embedded in LCA methodology. Thereby, this paper aims at proposing a method to implement GSA in the LCA field to address the results variability issue related to energy pathways. We applied the proposed methodology for assessing the carbon footprint variability for two renewable systems: the photovoltaic (PV) electricity and the wind power electricity pathway in Europe. Geographical parameters (irradiation and wind profile) are found to be the parameters inducing the highest sensitivity while technical parameters (PV efficiencies for example) and methodological parameters (life time of the system for example) are of the same order of importance. Such ranking is of interest for decision makers to understand the relation between the carbon footprint and its sources of variability. Applying the GSA approach in the LCA field enables a better understanding of the environmental impact variability.

#### **46 How to fill data gaps in life cycle inventories? A case study on CO<sub>2</sub> emissions from coal electricity**

**Z. Steinmann**, Radboud University Nijmegen / Department of Environmental Science; M. Hauck, Radboud University Nijmegen; A. Schipper, Radboud University; M.A. Huijbregts, Department of Environmental Science; A. Venkatesh, R. Karupiah, ExxonMobil Research and Engineering Company. Life cycle assessment (LCA) is increasingly being used for decision-making purposes. Approximations and assumptions are often made if appropriate data are not readily available. These proxies may introduce error or uncertainty into the results, leading decision makers to implement suboptimal or deleterious actions. Electricity constitutes one of the largest sources of environmental impacts for many products and processes. Approximations of power generation data inventories using ‘typical’ values from other contexts (for example, assuming Indian power plants have impacts similar to U.S. power plants) may significantly influence an assessment of environmental impacts over a product’s life cycle. We employed a multiple linear regression and a locally weighted linear regression approach to estimate the greenhouse gas emissions associated with power generation in the absence of reported data. Both approaches are built on reported emissions in selected nations and technological information reported in the World Electric Power Plants (WEPP) database. Emissions data were collected independently from governmental databases and reports and other public literature for 446 coal power plants, primarily in the U.S., Australia, India, the EU and South Africa; approximately 90% of these plants (401) were used for model training, and the remainder (45) were used for model validation. WEPP reports data on over 3000 coal power plant characteristics across the world, including steam pressure, type of fuel, age and capacity of plant; however, no data on efficiencies, net generation or emissions are included. Our framework hypothesizes that emissions from coal power plants can be explained by a subset of factors reported in the WEPP database as well as the GDP per capita of the resident nations of those plants. We then applied these models to all coal power plants in the WEPP database to predict CO<sub>2</sub> emissions per kWh of electricity they generate. We propose that this<sup>2</sup> framework may be employed to assess “missing” data in LCAs of other products and processes.

#### **47 Exploring parameter uncertainty and seasonal variability for human health intake fractions in life cycle assessment.**

**R. Manneh**, Chemical Engineering; M. Margni, Ecole Polytechnique de Montreal / Department of Mathematical and Industrial Engineering; **R.K. Rosenbaum**, Technical University of Denmark / Management Engineering; L. Deschenes, Ecole Polytechnique de Montreal / Genie Chimique. The intake fraction (iF) is used to calculate the potential human health burden of exposure to chemical emissions. It combines environmental persistence, direct and indirect exposure via bioaccumulation in the food chain. It takes the emission instead of the receptor perspective and is therefore a very useful tool in life cycle and comparative chemical screening assessments. However, it yields uncertainty from parameter uncertainty, spatial variability and model and scenario uncertainty. The objectives of this study are to i) carry an

importance analysis to determine the parameters that contribute the most to iF uncertainty and identify where to focus research efforts and ii) perform a seasonal scenario analysis on the most important parameters to determine the relevance of seasonal differentiation when assessing iFs. The organic chemicals included in Canada's national toxic pollutant inventory and a set of 32 organic chemicals that represent the variability of physico-chemical properties were selected to calculate the contribution to iF uncertainty for emissions to air and water using the Canadian nonspatial version of the IMPACT 2002 fate and exposure model. The model includes 11 physico-chemical properties and 105 landscape and exposure parameters. Using importance and cluster analyses, it was determined that for emissions to air, the parameters that were the most important were rainfall rate, the chemical half-life in air and water, soil area and the octanol/water partition coefficient ( $K_{ow}$ ). For emissions to water, the important parameters were Henry's constant, the half-life in air and water and the  $K_{ow}$ . The important parameters were then varied to define two scenarios: the summer and winter seasons. iFs for the set of 32 organic chemicals were calculated for both seasons for the Canadian and global context. For Canadian air emissions, results indicated that iFs for winter emissions could be up to one to two orders of magnitude higher than summer iFs (and model default average temperature, 25°C). For Canadian water emissions, results showed that iFs for both summer and winter conditions were, in general, closer to each other with outliers within one order of magnitude to iFs calculated at 25°C. Results also indicated that seasonal variability was of lesser importance when assessing iFs within a global context. Because the ranking between chemicals was maintained, it is concluded that seasonal variability is not relevant within a comparative context.

#### 48 Towards an improved accuracy of Input / Output environmental assessments using a general equilibrium economic model

A. Some, CIRAIQ, Ecole Polytechnique de Montreal; T. Dandres, CIRAIQ; C. Gaudreault, R. Samson, CIRAIQ, Ecole Polytechnique de Montreal. Input/Output tables describe monetary trades between sectors among a whole economy. Since the 90's some of them have been included a satellite matrix that links emitted substances to the economic sectors. I/O analyses are therefore able to model life cycle emissions of a major change occurring in a large scale system. These I/O analyses however have been used in case studies that aim only at a few economic sectors. The structure of the I/O tables yet relies on the interdependence among sectors. Studying a major economic change while changing only the demand for a few sectors may therefore not consider every affected sector and ultimately underestimate the impacts. GTAP[1] is a computable general equilibrium model that relies on national I/O tables to assess the variations due to a major change in the economy. It therefore seems interesting to couple economic data from GTAP with emissions from an I/O database to obtain a proper LCA for the whole economy of a given region. Recent developments in GTAP have also enabled to model direct and indirect land use changes and the related greenhouse gas emissions using IPCC conversion factors. In this study, GTAP model has been run for two scenarios: (1) a baseline scenario modeling rises in population and gross domestic product (GDP) by 2020 assuming a business as usual situation; (2) a biofuel scenario, assuming the same evolution of population and GDP but also including the policy of the USA and of the UE regarding biofuels by 2020. Economic production variations given by GTAP have then been coupled with the emissions of an I/O database. The impact assessment has been done using the Impact 2002+ method. Land use change impacts have been calculated coupling GTAP land use data and IPCC factors. The results show that both scenarios have almost the same economic impacts with the exception of agriculture where the biofuel scenario causes more land use change. Therefore environmental impacts are rather the same excepted for greenhouse gas emissions where the baseline scenario emits more than the biofuel one even when land use change is included. Using GTAP to calculate the economic data that are then coupled directly with environmental I/O tables enables a more complete LCA compared to classical I/O analysis. The inclusion of the emissions due to land use change considers the indirects impacts in the final results and therefore improves the completeness of the method. <br clear="all" />

[1] Global Trade Analysis Project

**49 Challenges of modelling the environmental exposure of nano-pesticides** S. Beulke, FERA / Food and Environmental Safety Programme; S. Monteiro, FERA; M. Kah, University of Vienna; K. Tiede, FERA; T. Hofmann, University of Vienna. The knowledge of the mechanisms controlling pesticide behaviour is extensive. Models incorporating these mechanisms can match the environmental exposure of conventional pesticides reasonably well. Such models are now well established tools to estimate the potential for pesticides to leach to groundwater and surface water within the regulatory framework. Standard experiments are performed to derive the model input parameters. These studies are usually only undertaken with the active substance and not with the formulated product. It is assumed that the intact formulation exists in the environment only for a short time and there is no effect on the long-term behaviour of the active. But this view has recently been challenged. Novel formulations of pesticides are continuously developed to enhance efficacy and optimise delivery to the target. Nano-pesticides have increased in importance as the potential of nano-materials in various disciplines has been recognised. Nano-pesticides could potentially pose new risks to humans or the environment and it is unclear whether the current regulatory framework is adequate for the evaluation of these products. A literature review was carried out [1] to identify possible impacts of the properties on nano-pesticides on environmental fate, and evaluate the suitability of current experimental protocols and exposure assessment procedures within the EU regulatory context. This was followed by experimental work and modelling. The sorption, degradation and leaching behaviour of free and nano-formulated active ingredients was investigated. The leaching of the nano-formulated pesticide was simulated using conventional input parameters and modelling tools and the results were compared with the experimental data. Alternative modelling techniques were explored. [1] Kah M, Tiede K, Beulke S, Hofmann T. 2012. Nano-pesticides: state of knowledge, environmental fate and exposure modeling. Critical Reviews of Environmental Science and Technology. DOI:10.1080/10643389.2012.671750.

**50 Volatilization of pesticides from the bare soil surface- modelling of the humidity effect** M. Schneider, Analytical Environmental Chemistry; K. Goss, Eawag / AUC. Volatilization of pesticides from soils under conditions dryer than the wilting point can be significantly influenced by sorption to hydrated mineral surfaces. This sorption process strongly depends on the hydration status of the mineral surfaces and their available surface area. Indeed established pesticide fate models considering volatilization do include the humidity effect by assuming an increase of sorption under conditions dryer than the wilting point. However they do not explicitly consider the hydrated mineral surfaces as an independent sorption compartment and therefore cannot correctly cover the moisture effect on volatilization. For this work we integrated our knowledge on the sorption of organic compounds to the mineral surfaces and its dependence on the humidity state of the soil into a simple volatilization model. The model considers availability and contribution of the mineral surfaces as a function of their hydration status, described via the equilibrium relative humidity in the pore space of the soil. The resulting model was tested with experimental volatilization data for the herbicide triallate from a bare soil surface in a wind tunnel experiment under various well defined humidity conditions. A sensitivity study was performed to estimate the influence of the different input parameters. The model captures the general trend of the volatilization under different humidity scenarios and shows the required degree of sensitivity to humidity changes in the soil. The results reveal that it is essential to have high quality input data for  $K_{min/air}$  the available specific surface area SSA, the penetration depth of the applied pesticide solution and the humidity conditions in the soil. The model approach presented here in combination with an improved description of the humidity conditions under dry conditions can be integrated into existing volatilization models that already work well for humid conditions but still lack the mechanistically based description of the volatilization process under dry conditions. However the rather accurate



information on the humidity state of the soil that were extracted here from the well defined wind tunnel experiments might be very challenging to gain from field situations.

### 51 Science Behind the Plant Uptake Factor in Leaching Models

W. Schmitt, Bayer CropScience AG / Environmental Modelling; R. Sur, Bayer CropScience LP / Environmental Safety; G. Goerlitz, Bayer CropScience AG / Environmental Safety. Leaching models used to predict groundwater concentrations of pesticides and their metabolites consider uptake into plants as one dissipation process. For this the focus is on the disappearance of material from soil. Previous experimental investigations of uptake and distribution of chemicals in plants, on the other hand, primarily focused on the emergence of the substances in the plants because they were basically performed for understanding the emergence of plant residues or for estimating bioavailability in case of pesticides. For this reason quantitative measures are established describing absorption of substances by roots and the transport into shoots, i.e. root concentration factor (RCF) and transpiration stream concentration factor (TSCF). However, the plant uptake factor (PUF) implemented in leaching models was differently named and it was never specified how to measure or estimate its value. Unfortunately in some cases it was also called TSCF. That use of non-zero PUF values is increasingly challenged by regulatory agencies may be caused by uncertainties raised simply by missing or misleading definitions of terms used in model descriptions, guidances and publications. Since the transport by the transpiration stream through the plant is a chromatographic process, the concentration in this stream depends on location and time. While the temporal behavior is described in the literature, the spatial aspect was widely ignored so far. We applied a mechanistic biokinetic model for plants that describes uptake and distribution on the basis of physicochemical properties, i.e.  $\log K_{ow}$  and molecular weight, for simulating the fate of the substances. Thus it was possible to systematically investigate the dependence of the different measures characterizing transport processes in the plant-soil system on different factors. One important result is that the TSCF and the PUF show opposing time dependence and merge only after considerable time, which depends on the lipophilicity of the substance. Moreover the simulations support the experimental finding that plant uptake factors are close to one, almost independent of the compound properties. It is concluded that the TSCF does not represent the PUF required for leaching calculations. Moreover, simulation results confirm recent experimental findings that the default value for the PUF of 0.5, mentioned in the current guidance, is conservative for a very high percentage of substances.

### 52 Risk Envelope Approach: Applicable for PEC groundwater calculations?

D. Nickisch, Efat & Modelling; N. Seiterle-Winn, . The idea of the risk envelope approach as presented in SANCO/11244/2011 indicates that in the environmental fate area the supported uses of a product can be grouped taking into account certain criteria (e.g. crop, application rate). So the assessment can be targeted at the worst case group for a specific assessment covering all other supported uses. Estimation of the predicted environmental concentration in groundwater is driven by a variety of factors e.g. application rate, degradation rate or leaching behavior. Leaching into soil of a substance is a very complex process dependent on various factors related to soil type and weather conditions that are taken into account within the models. Thus options for a reasonable grouping based on the above mentioned parameters may be limited. Therefore, we tested an envelope approach for parent substances using the model FOCUS PEARL in two different ways: a) based on substance parameters and b) based on crop Substance based calculations: We assumed that degradation of a substance and its sorption behavior in combination with the application rate are the key drivers of leaching. We calculated several combinations of  $DT_{50}$  (10 – 100 d),  $K_{oc}$  (50 – 500 L/kg) and application rates (10 – 1000 g/ha) for the FOCUS scenario Kremsmünster. Crop based calculations: For the four FOCUS substances (A-D) we calculated all scenarios and crops as well as a bare soil scenario with the same application dates used for the single crops. The application rate was set to

1 x 1000 g/ha neglecting crop interception. Results showed that application to bare soil does not represent the worst case and that no worst case crop could be defined. Only trends could be shown, e.g. that crops like winter wheat are more conservative than others which can probably be traced back rather to application timing than to the crop scenario itself. Nevertheless, substances with a  $K_{oc} > 500$  L/kg were below 0.1 µg/L for every calculated combination. Based on our results it is questionable if a worst case crop or location can be defined. However, it seems reasonable that the influence of the  $K_{oc}$  on the leaching process is strong enough to define a  $K_{oc}$  criterion so that a time consuming risk assessments for substances above this trigger is not necessary. This assumption is supported by a literature review of EFSA conclusions from 2005 – 2012.

### 53 GERDA: Risk assessment for pesticide inputs into surface waters via surface runoff, erosion and drainage in Germany

M. Bach, University of Giessen; D. Grossmann, German Federal Environment Agency (UBA); D. Guerniche, RLP Agrosience; U. Hommen, Fraunhofer IME; M. Kaier, German Federal Environment Agency (UBA); M. Klein, Fraunhofer IME; R. Kubiak, RLP Agrosience; A. Mueller, German Federal Environment Agency (UBA); T.G. Preuss, RWTH Aachen University / Institute for Environmental Research; S. Reichenberger, Footways SAS / FOOTWAYS S.A.S.; K. Thomas, M. Trapp, Agrosience RLP. The regulation (EC) No 1107/2009 increases the need for harmonized rules and conditions for the authorisation of plant protection products (PPP). The German national registration procedure currently uses the model EXPOSIT 3.0 beta to evaluate the surface water exposure and risk from PPP input with runoff, erosion, and drainage. The German Federal Environment Agency (UBA) has launched a project to harmonise the German national exposure and risk assessment procedure for surface waters with the FOCUS surface water scenario approach. The new approach shall comprise the following elements. The models MACRO and PRZM are regarded as sufficiently accurate for the estimation of pesticide inputs into surface waters by runoff, erosion and drainage on the field scale. Since the inter-annual variability of the weather is by far greater than the variance between different climate scenarios it is essential to simulate at least 20-year long-term weather records. PRZM and MACRO simulation runs calculated long-term PEC<sub>sw</sub> time series for numerous soil/climate/crop/substance-combinations in Germany. For each of these time series ecotoxicologically relevant exposure endpoints were derived from Monte Carlo analysis with a Toxicokinetic/Toxicodynamic (TKTD) model. The PEC<sub>sw</sub> time series were grouped and ranked according to the calculated exposure endpoints to create area-weighted cumulative distribution functions for Germany. This approach defines a set of national soil/climate-combinations, which assure the worst-case scenario requirement for a substance with given properties. With the parameterisation for these scenarios PRZM and MARCO simulate the runoff, erosion, and drainage losses of the respective substance from the field. In tier 1 subsequently the model STEPS-1234 is used to calculate PEC time series in the receiving water body. A higher tier approach should respect elementary properties of stream systems such as losses from all fields hydrologically connected to the receiving stream within a catchment as well as hydrodynamic and geomorphological dispersion. Further-more soil conservation measures should be implemented as appropriate risk mitigation options. The features and pre-defined national soil-climate scenarios will be implemented in a German runoff, erosion and drainage risk approach for PPP. The new approach shall harmonize the German authorisation with the established FOCUS procedure but allows to consider the specific agro-environmental conditions in Germany.

### 54 Soil/climate scenarios for modelling pesticide inputs into surface waters via surface runoff, erosion and drainage in Germany

S. Reichenberger, Footways SAS / FOOTWAYS S.A.S.; M. Bach, University Gießen / Institute of Landscape Ecology and Resources Management; D. Grossmann, German Federal Environment Agency; D. Guerniche, RLP Agrosience / Institute of Agroecology; U. Hommen, Fraunhofer Institute of Molecular Biology and Applied Ecology; M.



Kaiser, German Federal Environment Agency; M. Klein, Fraunhofer Institute of Molecular Biology and Applied Ecology; R. Kubiak, RLP Agrosience / Institute of Agroecology; A. Mueller, German Federal Environment Agency; T. Preuss, RWTH Aachen University / Institute for Environmental Research; K. Thomas, M. Trapp, RLP Agrosience / Institute of Agroecology. The new EU regulation 1107/2009 requires a harmonization of the various national pesticide exposure and risk assessment approaches between EU member states. The German Federal Environment Agency (UBA) therefore launched a project (FKZ 3711 63 427) to harmonize the German national exposure and risk assessment procedure for surface waters with the FOCUS approach used at the EU level. Within the project the new national exposure assessment tool GERDA (German Erosion, Runoff and Drainage Assessment) is being developed, which will take into account the full range of agro-pedo-climatic conditions in Germany. The agricultural area of Germany (arable and permanent crops) has been classified into 12 climatic zones. Moreover, the German 1:1000000 soil map has been translated into FOOTPRINT Soil Types (FST). Climate zones and soil map were intersected and the areas of the resulting 1153 climate/FST combinations determined. In the next step, soil/climate combinations representing a realistic worst cases for Germany were identified. For this aim, 93312 long-term drainage simulations were performed with MACRO 5.2 for all 324 occurring combinations of climate and drained FSTs in Germany. Similarly, 415080 surface runoff and erosion simulations were performed with PRZM for all 1153 occurring FST/climate combinations. From these results long-term hourly time series of Predicted Environmental Concentrations in surface water (PEC<sub>sw</sub>) were generated using the model STEP-3, for a standard surface water body. A Toxicokinetic/Toxicodynamic (TKTD) model was employed to estimate generic combinations of exposure endpoints which can be used to define realistic worst case exposure profiles for several compound/species combinations for all relevant species in aquatic risk assessment. The PEC<sub>sw</sub> time series were grouped and ranked according to the values of the selected exposure endpoints, and area-weighted cumulative distribution functions (CDF) were generated. The obtained CDFs allow, based on the substance properties, crop and application pattern to be modelled, the identification of a set of soil/climate combinations with a defined worst-case-ness for the surface water risk assessment. This constitutes a major improvement in comparison to FOCUS, where representativity and worst-case-ness are only vaguely known.

**55 World café interactive session** R. Owen, . This session will start with a world café interactive session, in which also video clips of short vox pops will be showed, where people will describe their own thoughts on responsible innovation. This will be complemented with te results of a short questionnaire on Responsible Innovation

**56 Adaptive Governance for Responsible Innovation** J. Petts, . Emerging technologies create the possibility of novel materials, new life forms and opportunity not offered in nature; indeed deliberately intervening in the 'natural'. But opportunity is accompanied not only by data and model uncertainty, but often by ambiguity as well as sheer ignorance. Further, innovation is a complex nonlinear and collective process enacted over varying but often very long timescales, involving multiple actors and at both local and global scales. Against this background, calls for a new and, importantly, adaptive framework for the governance of innovation have been emerging. The new governance framework implies a move away from a reliance on the top-down risk-based, regulatory approach to one that attempts to set the parameters of a system within which people and institutions behave. This paper will consider the components of this framework addressing important issues of responsibility and accountability and particularly the relative roles of soft law and cooperative approaches not least in the early stages of research and innovation. The discussion will consider the fundamental principles of effective governance and the necessary tools to ensure anticipation, reflection, openness and responsiveness. It will speak directly to the implications for science and scientists.

**57 A Framework for Responsible Innovation** R. Owen, . I will

describe a framework for responsible innovation developed for the UK Research Councils. I will first briefly describe the foundations and rationale for responsible innovation and important commitments emerging at an EU policy level. I will argue that the departure point and first challenge for responsible innovation is not 'what are the risks?' – important though this is – but 'what kind of future do we want science and innovation to bring into the world?' – and how this can be defined in a democratic, equitable and sustainable way. This will require reflection primarily on the very visions, purposes and underlying motivations for innovation and the values these are anchored in. Acknowledging the place and limits of both market choice and regulatory governance underpinned by risk characterisation, I will then argue that responsible innovation must also meet the challenge of how to proceed (innovate, conduct science) responsibly under conditions of ignorance and uncertainty. I will develop a framework around integrated dimensions of *anticipation, reflection, deliberation* and (importantly) *responsiveness* (personal, institutional, political) that attempts to address these challenges before highlighting practical experiments in the areas of environmental nanoscience and geoengineering. In doing so I will emphasise the collective nature of responsibility and the need to enlarge, reframe and even redefine role responsibilities, including those of the SETAC community itself.

**58 Industrial discharges of pharmaceuticals induce adverse effects in wild fish** O. Cardoso, INERIS / Unité d'écotoxicologie in vitro et in vivo; O. Palluel, E. Chadili, INERIS; S. Betoulle, Univ Reims; W. Sremski, ONEMA; B. Piccini, J. Porcher, W. Sanchez, INERIS. Recent evidences reveal high concentrations of a large number of active pharmaceutical effluents (APIs) in effluents from pharmaceutical manufactures and in receiving aquatic ecosystems. Laboratory experiments reveal adverse effects in aquatic organisms exposed to these industrial effluents. However, no data is available on adverse effects induced by industrial pharmaceutical discharges in wild aquatic organisms. The aim of the present work was to assess wild fish health disturbance downstream from industrial effluents. For this purpose, a set of biochemical and histological biomarkers was measured in fish sampled upstream and downstream from several API manufactures selected among more than 200 industrial sites involved in French pharmaceutical production. Firstly, between 2008 and 2012, gudgeons were electrofished in four sites located upstream and downstream from the discharge of pharmaceutical factory involved in steroid synthesis. In all sampled fish, a set of biomarkers including endocrine disruption endpoint (vitellogenin and gonad histology) was measured. Sex-ratio and fish assemblage were analyzed to identify potential population disturbance. Strong signs of endocrine disruption including vitellogenin induction, intersex and male-biased sex-ratio were observed at several kilometres downstream from the industrial effluent. Secondly, to characterise the occurrence of adverse effects induced by API industrial discharges in wild fish, a monitoring campaign was performed around 4 pharmaceutical factories including a manufacture of veterinary pharmaceuticals. First observations confirm that effluents from API manufactures induce adverse effects in wild fish from several locations. Results presented in this study confirm that effluents from pharmaceutical manufactures contain active compounds able to disturb the health of wild fish and populations. This conclusion argues for an increase of knowledge related to the occurrence of these effects around the world and for the deployment of specific monitoring approaches around pharmaceutical factories *This work was supported by the French Ministry for Ecology and Sustainable Development (MEDDE-Program 181)*.

**59 Enantiomerism of medicinal products – a new paradigm in environmental risk assessment** B. Kasprzyk-Hordern, University of Bath / Chemical and Biological Sciences; J.P. Bagnall, University of Bath; D.R. Baker, University of Huddersfield; S.E. Evans, University of Bath. Chiral pharmacologically active compounds (PACs) are environmental pollutants. They enter the environment mainly through insufficiently treated sewage, waste effluents from manufacturing processes, runoff and sludge. They are bioactive, ubiquitous and

persistent with synergistic properties. Surprisingly, the environmental fate and effects of PACs are assessed without taking into consideration their enantiomeric forms (this is despite existing knowledge on enantiomer dependant toxicity of PACs to humans, taking thalidomide as a flagship example). Such an approach leads to an underestimation of toxicity of PACs, incorrect environmental risk assessment, and direct risk to the environment and human health, as PACs are likely to be present in the environment in their non-racemic forms (in the case of single enantiomer PACs racemisation *in-vivo* can also take place). This presentation aims to discuss: The phenomenon of chirality in the context of fate and effect of chiral pharmacologically active compounds (PACs) in the environment Monitoring of several wastewater treatment plants and receiving waters in the UK to study stereoselective biodegradation of PACs Stereoselective biodegradation of PACs in river microcosms Implications of enantiomer-dependant fate of PACs for procedures applied in environmental risk assessment

#### **60 How does sewage effluent exposure affect the pharmacokinetics of non-steroidal anti-inflammatory drugs (NSAIDs) in fish?**

L. Gunnarsson, Göteborg University / Department of Infectious Diseases, Institute of Biomedicine; J. Fick, Umea University / Department of Chemistry; A. Grans, M. Axelsson, University of Gothenburg; J.D. Larsson, The Sahlgrenska Academy at the University of Gothe / Department of Infectious Diseases, Institute of Biomedicine. We have previously performed a meta-analysis comparing bioconcentration factors (BCFs) of several non-steroidal anti-inflammatory drugs (NSAIDs). This analysis suggests a considerably higher BCF in fish exposed to treated sewage effluents (containing NSAIDs and other chemicals) than in fish exposed in the laboratory to a single NSAID in pure water. Basing environmental risk assessments of NSAIDs on traditional exposure studies in the laboratory may therefore underestimate their bioconcentration potential and consequently their toxicity. The aim of this study is to disentangle if the observed differences in bioconcentration are due to differences in uptake, tissue distribution, metabolism and/or excretion. We have therefore performed pharmacokinetics studies with single-dose *i.v.* administered ketoprofen (1mg/kg) in rainbow trout either kept in clean water or exposed to treated sewage effluent. The dorsal aorta was cannulated to allow repeated sampling of blood and the *i.v.* route of exposure eliminates potential differences between the groups due to uptake. The results show that the plasma elimination half life ( $t_{1/2}$ ) of ketoprofen in a fish kept in clean water is more similar to the  $t_{1/2}$  of mammalian and bird species than to the much longer half life observed in another poikilothermic vertebrate (*Iguana iguana*). The pharmacokinetic data from fish exposed to treated sewage effluent will be analyzed shortly. Liver homogenate from trout kept in either clean water or exposed to sewage effluent will be analyzed for its potential to metabolize ketoprofen. An increased understanding of how complex mixtures can affect pharmacokinetics and consequently the toxicity of pharmaceuticals has the potential to direct improvements of environmental risk assessment procedures of pharmaceuticals.

#### **61 The Effects of Anti-Androgenic Pharmaceuticals on Oestrogen Induced Feminisation in Model Fish Species**

C. Green, J. Brian, Brunel University / Institute for the Environment; R. Williams, Centre for Ecology and Hydrology; M. Scholze, S. Jobling, Brunel University / Institute for the Environment. The widespread feminisation of wild fish inhabiting UK rivers, characterised by ova-testes intersex and abnormally high concentrations of vitellogenin in males, has long been linked to the steroid oestrogens. However, in recent years concern has also grown over the presence of widespread anti-androgenic activity in sewage effluents and it has been hypothesised that anti-androgens may significantly impact feminisation in both singular and co-exposure to steroid oestrogens. This study aimed to test this hypothesis through exposure of model fish species to environmentally relevant concentrations of the anti-androgenic pharmaceuticals bicalutamide and cyproterone acetate to assess feminisation endpoints including vitellogenin and intersex induction. Predictive hydrological modelling was used to assess their concentrations in UK river catchments

following outflow from sewage treatment works using LowFlows 2000 WQX, which ranged from 0-149ng/L and 0-62ng/L of bicalutamide and cyproterone acetate respectively. Based on modelled data, fathead minnow (*Pimephales promelas*) were exposed to a mixture of bicalutamide (100ng/L) and cyproterone acetate (70ng/L) as a single treatment and in combination with environmentally relevant levels of steroid oestrogens: 17 $\beta$ -oestradiol (2.1ng/L), oestrone (9.6ng/L) and 17 $\beta$ -ethinylestradiol (0.3ng/L) for 14 days to assess vitellogenin induction. In a second experiment, Japanese medaka (*Oryzias latipes*) were exposed from embryos for 98 days to mixtures of anti-androgens and oestrogens, both alone and in combination. Two sets of concentrations were investigated, representative of sewage effluent (bicalutamide 100ng/L; cyproterone acetate 70ng/L; steroid oestrogen mixture 18ng/L combined oestradiol equivalent EEQ) and river concentrations (bicalutamide 47ng/L; cyproterone acetate 30ng/L; steroid oestrogen mixture at 8ng/L EEQ), to assess the impact on intersex. Preliminary data has found no evidence of a significant effect of the anti-androgenic pharmaceuticals bicalutamide and cyproterone acetate on either vitellogenin or intersex induction in the two model fish species at environmentally relevant concentrations alone or in combination with steroid oestrogens. Further analysis of ovarian cavities and fibrotic tissue induced in male gonads as well as the intersex severity under each treatment is required.

#### **62 Predicting the impact of endocrine disrupters on the environmental health: an ecosystem model.**

L. Clouzot, Université Laval / Département de génie civil et de génie des eaux; M. Paterson, A. Dupuis, P. Blanchfield, M. Rennie, Fisheries and Oceans Canada; K. Kidd, Canadian River Institute; P.A. Vanrolleghem, modelEAU Université Laval / Département de génie civil et de génie des eaux. Literature indicates an increasing concern about the release of pharmaceuticals, especially endocrine disrupters, through the effluent of wastewater treatment plants. Endocrine disruption has been measured in aquatic environments across the world. However, experimental approaches to characterize the ecological impact are costly and time-consuming and thus, the consequences of endocrine disruption on whole ecosystems remain unclear. Mechanistic models can help understand the impact of such contaminants on aquatic environments and assess their ecological risk. This study takes up the challenge to develop an ecosystem model that considers endocrine disruption in fish and the consequences on the whole ecosystem through ecological interactions, i.e feeding and competition. The experimental data used to develop the model come from a multi-year whole-ecosystem study performed at the Experimental Lake Area (ON, Canada): (i) two years of reference data (ii) three years of exposure to environmentally-relevant concentrations of the synthetic hormone 17 $\beta$ -ethinylestradiol (EE2) and (iii) five years of recovery. EE2 is widely used in human contraceptives and it was chosen for this study because it is one of the most widespread and potent endocrine disrupters. During the experimental study, endocrine disruption was observed in the fish species with a collapse of fathead minnow after the second year of EE2 addition. The ecosystem model that is being developed is an object-oriented model based on simplified AQUATOX equations for the species naturally present in the experimental lake (benthic invertebrates, phyto- and zooplankton, fish). The novelty of the study is to add appropriate equations for endocrine disruption. Two fish classes are used in the model: juveniles and adults. The developed model can simulate endocrine disruption based on (i) an increase of gamete mortality, (ii) a decrease of gamete production or (iii) an increase of fish mortality. For example, if the sex ratio is different from 50:50 (female:male) gamete production will decrease, less juveniles will be recruited and thus, the fish population will decrease. In addition to modeling the direct effects of EE2 on fish, the ecosystem model also considers the lake dynamics. For example, two stratified layers are modeled (epilimnion and hypolimnion) with different biological and physico-chemical compositions.

#### **63 Bioaccumulation potential of pharmaceuticals in the aquatic food chain - pseudo-persistence versus lipophilicity**

A. Zenker, University of Appl Sc Northwestern Switzerland / Institute of Ecopreneurship; S. Joerg, University of Applied Sciences and Arts

Northwestern Switzerland / School of Life Sciences. Even with the best technology currently available, waste water treatment plants (WWTPs) cannot completely remove micropollutants, in particular drug residues. Pharmaceuticals have therefore been continuously discharged to water bodies worldwide, leading to high concentrations of active ingredients and their transformation products in surface waters downstream of WWTPs. On the other hand to deduce mechanisms of toxic action or to evaluate the toxicity of mixtures, and to interpret field data on bioaccumulated toxicants the tissue residue approach has been taken into account most recently. Therefore, we have focused in our study on a multi-residue analysis of ionic and lipophilic pharmaceuticals from different trophic levels of the aquatic food chain (fish prey, fish, and fish eating birds). Determined pharmaceuticals were the frequently detected ibuprofen (an analgetic), diclofenac (an analgetic), mefenamic acid (an anti-inflammatory drug), sulfamethoxazole (a bacteriostatic antibiotic), atenolol (a beta blocker), diphenhydramine (an antihistamine), diltiazem (a calcium channel blocker), carbamazepine (an anticonvulsant), fluoxetine (an antidepressant) and its main metabolite norfluoxetine. Since our preliminary results have revealed exclusively residues of norfluoxetine, fluoxetine, diphenhydramine and carbamazepine in fish and fish prey the list of analytes were extended to other antidepressants (sertraline and citalopram) but also pharmaceuticals like gemfibrozil, fenofibrate and naproxen and metabolites of diclofenac (2-[2-(chlorophenyl)amino]benzaldehyde) and ibuprofen (2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid), which have been in parts detected in surface water. Trace analysis of compounds was performed in a multi-residue method based on HPLC coupled to mass spectrometry (LC-MS) whereby identification and quantification of pharmaceuticals in biota were arranged in MS/MS mode. MRM transitions were classified in a different elution time window to increase measurement sensitivity. Eleven out of 22 pharmaceuticals measured, as well as their transformation products, could be detected in biota samples. The antidepressant fluoxetine, its main metabolite norfluoxetine and diphenhydramine were predominant.

**64 Evaluating effects of a multi-generation pollution on *Caenorhabditis elegans*' population** B. Goussen, INERIS / Unit METO; R. Beaudouin, INERIS; F. Parisot, M. Dutilleul, A. Buisset-Goussen, J. Bonzom, IRSN / PRP-ENV/SERIS/LECO; A.R. Pery, INERIS / Unit METO. The assessment of toxic effects at biologically and ecologically relevant scales is an important challenge in ecosystem protection. Indeed, in most time, stressors impact populations over long-term. The selection pressure exerted by a pollutant is known to amplify the phenomenon of natural selection and could lead to evolutionary changes across generations. It is therefore important to study the evolutionary response of a population submitted to a long term stress. Regarding this background, we assessed the evolution of two populations (control and exposed to 1.1 mM of the heavy radiotoxic metal, uranium) of the ubiquitous nematode *Caenorhabditis elegans* submitted to a long-term exposure to uranium. The experimentation was conducted over 16 generations and life history traits (growth, reproduction and survival) as well as dose-response evolution were assessed. These parameters were followed daily on individuals extracted from the populations and exposed to a range of concentration (from 0 to 1.2 mM U). Our experiment showed an increase of adverse effects as a function of uranium concentration. Indeed the NOEC for reproduction and growth traits were respectively of 0.5 mM U and 0.9 mM U. Moreover, reproduction and growth were respectively reduced by over 60% and 20% for individual exposed at 1.1 mM U. This reduction remained constant throughout the generations. We also pointed out the appearance of genetics differentiations on reproduction traits throughout the generations. This differentiation, observed from generation 3, showed us that the total egg-laying of the uranium population was significantly decreased compared with the control population. In contrast, no differentiations were highlighted on growth traits. Our results confirm the importance of studying environmental risks related to pollutant through multi-generational studies in order to capture effects that may appear after several generation of exposition.

**65 Multi-generational exposure of *Folsomia candida* to Cd – organisms size distribution, reproduction and gene expression** C.M. Pereira, S.I. Gomes, A.M. Soares, M.J. Amorim, University of Aveiro / Departement of Biology. Because contamination can be historical or due to long term scenarios organisms may be exposed throughout many generations. Due to the required effort, few studies are available on multi-generational effects. The aim of this study was to assess the effects of multigenerational exposure of *Folsomia candida* to Cadmium [reproduction EC10 and EC50]. Results are presented until F35, to our knowledge no study has been performed along so many generations in soil invertebrates. Measured endpoints were survival, reproduction, size and the expression level of the metallothionein coding gene. Further, toxicity was monitored at generations 6, 10, 26 and 34. Continuous exposure to Cd (EC10 and EC50) caused an improvement in reproduction (number of juveniles) performance until F6, after which it started to decline and for EC10 exposure failed at the 13th generation, whereas for the EC50 exposure it continued at lower performance. Changes in reproduction occur simultaneously with changes in the size of produced juveniles. The organisms seem to be responding via Mt gene expression, which is concentration and generation dependent. Organisms are acclimated to the stress but do not seem to become resistant, given that once in clean soil the Mt gene is less expressed. Curiously, the higher tolerance observed in the population exposed to the EC50 compared to the EC10 could be related to the fact that higher Mt levels conferred larger detoxification and tolerance/longevity to Cd exposure. Effects also indicate that it is not possible to extrapolate effects from generation to generation which could be important to consider in a risk assessment framework. Keywords: adaptation dynamic, Population effects; Collembola.

**66 Linking DNA damage and effects on life history in a multigenerational exposure of *Daphnia magna* to uranium** Plaire, IRSN; J. bourdineaud, Université Bordeaux 1; L. Garcia-Sanchez, IRSN; J. Poggiale, Université de la Méditerranée; F. Alonzo, . Chronic effects of waterborne depleted uranium (U) were previously studied under laboratory conditions on successive generations of *Daphnia magna*. Results showed that U effects on physiology and life history increase across generations. Observed reduction in somatic growth and reproduction was analysed using the DEBtox approach suggesting that U primarily affects assimilation. This mode of action was confirmed by measurements of assimilation reduction and observations of histological alteration of the digestive epithelium. However the mechanisms involved in the transgenerational increase in sensitivity remained unknown, results pointing the egg as a potentially sensitive life stage. The present study aimed to evaluate how U alters DNA and affects physiology and life history of *Daphnia magna*. We investigated chronic effects of U on two successive generations, with different objectives: 1) to obtain a better estimation of DEB parameters at concentrations lower than 10 µg L<sup>-1</sup> 2) to evaluate how exposure to U during the egg stage may explain the increase in severity observed between a F0 generation (exposed from the neonate stage) and a F1 generation (exposed from the egg stage). 3) to determine concentrations at which DNA damage become significant with a special emphasis on linking perturbations between the molecular and the individual levels. 4) to elucidate the mechanism causing the increase in effect severity over successive generations. Daphnids were exposed to waterborne uranium at concentrations ranging from 2 to 50 µg L

**67 Long term effects of an early exposure to PAHs on zebrafish behavioural responses** C. Vignet, IFREMER; K. LE MENACH, LPTC; S. PEAN, IFREMER; L. LYPHOUT, D. LEGUAY, M. BEGOUT, IFREMER; X. COUSIN, INRA. Polycyclic aromatic hydrocarbons (PAH) emission in the environment is constantly increasing with human activity. In this study, zebrafish embryos were exposed to a mixture of three PAH molecules dosed at environmental concentrations based on values measured in the Seine Estuary (France) a highly polluted site. Eggs were collected from AB zebrafish strain and were placed at 4 hours post fertilization (hpf) until 96 hpf in a 3 cm diameter Petri dish containing 3 g reference sediment either plain

(control) or spiked with Benzo[a]pyrene, Pyrene and Phenanthrene at concentration 600 ng/g, 1600 ng/g and 2100 ng/g respectively. Thereafter, larvae were transferred in clean water and raised until adulthood. Behavioural tests like locomotion during 24-hrs, dark sudden change challenge and T-maze were performed at adults' stage (F0) and sudden dark change on larvae from F0 adults. In adults, during night period, contaminated fish were significantly less active than control fish. Contaminated F1 larvae were more active when light was on than control and less active when the light was off. Contamination using environmentally relevant concentration during the very first stage of development with a PAH mixture of 3 molecules on zebrafish induced behavioural effects measured at the adult stage. Further, effect was transmitted to the next generation and behavioural responses of F1 larvae were different to that of F1 control larvae. Keyword: fish, locomotion activity, oil

**68 Co-tolerance of oxidative stress in *Orchesella cincta* (Collembola) populations genetically adapted to heavy metal stress** D. Roelofs, Vrije Universiteit / Inst. of Ecological Science; T. Brand, VU University / Inst. of Ecological Science; N.M. van Straalen, Vrije Universiteit Amsterdam / Inst. of Ecological Science. Field-selected tolerance to heavy metals has been reported for *Orchesella cincta* populations occurring at metal-contaminated mining sites. This tolerance is correlated with heritable increase of metal excretion efficiency and over-expression of the metallothionein gene. A transcriptomic study showed that population-specific responses were identified for genes involved in carbohydrate metabolic processes,  $Ca^{2+}$  dependent stress signaling and cuticle synthesis. It is well known that heavy metals such as Cadmium exert oxidative stress. Here we asked the question whether metal-tolerant *O. cincta* populations exert co-tolerance to oxidative stress. To that end, we exposed metal-tolerant and reference *O. cincta* populations to paraquat, which induces oxidative stress. Survival during paraquat exposure confirmed that the resistance of the tolerant populations to stress caused by cadmium exposure, contributed to increased tolerance against oxidative stress induced by paraquat. A number of relevant genes were selected to study differential expression due to paraquat at the genetic level. Metallothionein and CYP450s showed highly similar transcriptional responses when paraquat exposed animals were compared to Cd exposed animals. However, genes involved in stress-activated protein kinase signalling differently regulated between paraquat exposed animals and Cd-exposed animals. We conclude that tolerance to oxidative stress and heavy metals is very similar at the phenotypic level, but deviates significantly at the transcriptional level. Furthermore, the data suggest that tolerant phenotypes exert a broader range tolerance to withstand additional stressors.

**69 Phenotypic evolution in response to chemical dose** S. Gerber, Eawag; F. Pomati, Aquatic Ecology. The resilience of biological systems to stress depends on the capacity of constituting organisms to adapt and evolve. Aquatic organisms are increasingly exposed to mixture of emerging contaminants. Natural populations may acclimate physiologically or evolve resistance in response to this novel pollution. Which levels of exposure result in evolution of resistance traits and whether physiological acclimation plays a leading role is not well understood. While it appears that resistance to anthropogenic pollutants in microalgae and cyanobacteria arises at lethal exposure levels as a result of selection on standing genetic diversity, phytoplankton are able to survive in adverse habitats as a result of physiological acclimation, which is supported by generally plastic phenotypes. Here we investigated the dose-dependency of changes in population abundance and phenotypic traits in toxic and non-toxic *Microcystis aeruginosa* (cyanobacteria) strains as a response to a biocide of emerging concern, triclosan. We explored if induced phenotypic change corresponds to an evolutionary adaptation and therefore was maintained across generations, and whether trait changes could be reverted to the original phenotype after a period of detoxification and recovery. The non-toxic mutant appeared to be more resistant to the chemical and showed an overall different phenotype, with larger cell size. Significant observable changes in cellular phenotype occurred for the wild type (WT) at IC50

levels and above. Stressed WT cells were phenotypically more similar to unstressed mutant cultures than to control cultures of their own strain. The non-toxic mutant showed less phenotypic plasticity and a phenotype resembling the stressed one in the WT. Changes in the WT phenotype, acquired after exposure to triclosan, were maintained after cultures were released from chemical stress, suggesting a form of phenotypic inheritance after few generations such as epigenetic effects. We provide original findings that advocate for a more careful consideration of sub-lethal doses and environmentally relevant exposure scenarios in the risk assessment of chemicals, since physiological and evolutionary processes, e.g. phenotypic transitions, can interact and occur at low levels of exposure to anthropogenic chemicals.

## **70 Simulating fate of pesticides and potential mortalities of *Asellus aquaticus* in the water network of an agricultural landscape**

Ter Horst, Alterra; A. Focks, Wageningen UR / Mathematics/Computer Science; E. van den Berg, Alterra. For aquatic ecological risk assessment possible ecotoxicological effects on aquatic organisms are considered on an edge of field scale in the lower tiers of the European pesticide regulation framework. Upscaling exposure simulations to the landscape scale improves the realism of the risk assessments. The CASCADE model has been developed to assess the fate of pesticides in the water network at the landscape scale. The pesticide fate module of CASCADE is based on the TOXSWA model, that calculates the behaviour of a pesticide in a single watercourse and includes the processes of transport, transformation, volatilisation and sorption. The model couples single watercourses into a system of communicating watercourses. At junctions all incoming pesticide mass fluxes are distributed proportionally between the outgoing water fluxes. The present research version model simulates fate processes in the water layer only; accordingly there is no interaction with sediment. CASCADE was parameterised for a 10 km<sup>2</sup> Dutch polder area, the Drentse Veenkoloniën. Exposure calculations were done for 15 applications of 0.005 kg/ha lambda-cyhalothrin in seed potatoes, starting from May 1<sup>st</sup> with intervals of 7 days. Spray drift was the only entry route of pesticide in the water courses and was fixed to 5%. Only part of the water courses in the catchment received spray drift. The half-life in water was set to 1 d. Daily maximum concentrations per numerical compartment were coupled to a dose response relation to calculate the potential for chemical induced mortality of *Asellus aquaticus*. The dose response relation used an LC50 value of 48 ng/L. It was found that decrease and increase of mortality corresponded to respectively decrease and increase in concentration. The effect of either polluted or non polluted tributaries on pesticide concentrations in water and the corresponding mortalities were clearly visible. Despite the fast dissipation in the water layer, pesticide applications caused mortality at the outlet in two km distance from the application area. On a landscape scale the connections of the water courses within the catchment and the pesticide application pattern on a spatial scale are important additional drivers next to those already known for edge of field scales (i.e. toxicity, water volume, hydraulic residence time, dissipation). The spatio-temporal explicit exposure patterns could directly be used as input for population-level effect modelling.

## **71 Evaluation of a spatially explicit model for predicting exposure of bats to soil-associated metals** B.V. Hernout, University of York / Environment; A. Boxall, University of York / Environment Department.

Bats may be exposed to metals by consuming insects that have accumulated soil-associated metals. Food chain models can be used to estimate potential risks of metals to bat health. A recent modelling framework has been developed to predict metal risks to bats in England. However, due to a lack of data, this model has not been evaluated against experimental measurements. This study was therefore performed to develop data on concentrations of metals in soils, soil invertebrate and bat samples in order to test different steps in the modelling framework. While correlations between model outputs and measurements were obtained for some components of the model and some metals, for the majority of metals and model steps, no correlations were obtained. These results point to the fact that we need a much better understand of

spatial metal concentrations, metals uptake into insect orders and bioaccessibility and regulation in the bat in order to adequately predict risks.

**72 Validation of mechanistic effect models for ecological risk assessments: A practical approach** J. Augusiak, Wageningen UR / Environmental Sciences - AEW; V. Grimm, Helmholtz Centre for Environmental Research UFZ / Department of Ecological Modelling; P.J. van den Brink, Alterra and Wageningen University. Common European environmental risk assessments for pesticides focus on standardized procedures that inherently induce limitations to a full understanding of adverse effects on non-target species populations and ecosystems. Ecological effect models have long been identified as useful tools to extrapolate limited experimental findings to more realistic conditions, such as larger temporal or spatial scales, or higher levels of biological organization, which can make a risk assessment ecologically more relevant. However, ecological effect models are not often used or accepted in regulatory risk assessments due to doubts about whether a given model represents the real world sufficiently well. Overall model credibility and a lack of validation have been raised as causes of doubt from regulators. Additionally, usually different educational backgrounds and levels of experience among involved stakeholder groups often induce time-demanding discussions concerning functional terminology and methodology. In order to support a better understanding of applied modelling and model evaluation techniques, we reviewed a number of publications that addressed model quality assessment and validation efforts across various scientific fields with a close connection to policy and decision making. We found that some terms were used with rather strong consistency whilst others caused conflict and were used interchangeably with varyingly broad background considerations. Aside from identifying a suitable system of terminology, we classified several methods that can be employed for a majority of existing modelling approaches. The classification was based on the degree to which a given method addresses underlying uncertainties but also how generally applicable the method is to different modelling approaches, e.g. stochastic vs deterministic modelling. We found, that in order to introduce ecological effect models as valuable decision support tools, particularly more complex models such as spatially explicit ones, more comprehensive quality assessment tools and protocols need to be developed and established. Such protocols should contain a glossary to enable an easier communication between involved stakeholder groups. Additionally, following a concise documentation scheme that includes explicit descriptions of model uncertainties and applied evaluation methods would facilitate a ready illustration of how a given model's output information relate to the question addressed by the model.

**73 An individual-based vertical distribution model of *Folsomia candida* (Collembola) in an agricultural soil column** Y. Roeben, RWTH Aachen University Institute for Environmental Research / Environmental Safety; F. Scherr, Bayer CropScience AG; G. Goerlitz, Bayer CropScience AG / Environmental Safety. *Folsomia candida* is an ubiquitous soil inhabitant often referred to as the "Standard Soil Arthropod" and is part of the regulatory framework of pesticide risk assessment. With respect to risk assessment it is crucial to know where and when to protect an organism but still little is known on the vertical dispersal and seasonal fluctuations of collembolan communities in agricultural landscapes. To overcome this lack of knowledge, ecological modelling offers a powerful tool. This work presents a vertical distribution model of the soil-dwelling collembolan *Folsomia candida* in an agricultural soil column to demonstrate the effect of variations in environmental parameters on the population. In addition, the model features the option to evaluate the effect of a pesticide application. The model is implemented in the modelling environment of NetLogo and is constructed based on literature data on the biology and life-history trait of *F. candida*. It represents a vertical soil column with heterogeneous layers of 1 cm. The environmental parameters and concentrations of pesticide in the soil column are supplied through outputs of PEARL calculations. The movement patterns of *F. candida* observed in laboratory studies are reproduced by the model. Temperature and

organic matter content proved to have a major influence on the movement and therefore on the vertical distribution of the population. In the model the population shows response to a pesticide application considering the population density and the reproductive success. On the basis of current literature, the model is able to simulate the possible spatial and temporal distribution of a *Folsomia candida* population in an agricultural field and emphasizes the effect of fluctuating environmental parameters, such as temperature on the population. In the next step the model will be validated and verified by field studies and/or terrestrial ecosystem models (TMEs).

**74 Consequences of choice of landscape scale for a spatial modelling ERA** C.J. Topping, bioscience; W. Schmitt, Bayer CropScience AG / Environmental Modelling; M. Ebeling, Environmental Safety; T. Schad, Bayer CropScience AG; G. Goerlitz, Bayer CropScience AG / Environmental Safety. The use of modelling for environmental risk assessment is becoming more commonplace, but this raises questions of exactly how to best use these models. There is also a greater awareness that spatial and temporal scale are important in determining the impacts of toxicants in the environment. This awareness comes with an increasing cost of complexity in the risk assessment. Here we address the question of the consequence of landscape scale and structure on a spatial modelling ERA for a small mammal non-target species. The analysis was carried out using the field vole (*Microtus agrestis*) model of the ALMaSS, a simulation system designed to evaluate the impact of management on wildlife populations. Danish 10 x 10 km landscapes were simulated with orchards covering 2.5% of the total landscape area. These orchards were subject to a single yearly pesticide application. To ensure a clear effect, the fictitious pesticide was assumed to have either a direct lethal effect above a NOEC for all voles exposed, or a severe endocrine disruption effect. A number of scenarios were evaluated assuming different landscape configurations with both pesticide types. A spatially structured ERA was carried out by considering the impact of the pesticide against a baseline where no pesticide was applied, and making this evaluation on the whole landscape, then subdividing the landscape into 4, 16, and 64 equal sized grid squares and repeating the analysis per grid square. Different topographies predicted different impacts and recovery with an interaction between pesticide type and landscape structure. When carrying out the spatially structured ERA, the size of the landscape grid evaluated had a clear impact on the variability of the result. Smaller scale landscapes increased the variability around the predicted impact. This variation showed that the conclusion reached by an ERA could be very misleading if only a single small area was assessed. There was no one simple landscape metric that could explain this variation, and general rules may be difficult to find due to differential species ecology and behaviour. In conclusion, the larger the landscape scale the lower the variability of the ERA result. If simple landscapes are used then it is important to consider a wide range of spatial scenarios to avoid bias. If large scale landscapes are used then this requires a re-focussing of the ERA to the landscape level population and greater ecological realism.

**75 Effects of a non-mitigated exposure to a mixture of pesticides used in a fruit and tuber crop on the recovery of aquatic invertebrates.** P.J. van den Brink, Alterra and Wageningen University; M. Zorn, Board for the Authorisation of Plant Protection Products and Biocides (Ctgb); T. Brock, Alterra; E. Roex, DELTARES; T. Van der Linden, RIVM, National Institute for Public Health and the Environment; R. Luttik; A. Focks, Wageningen UR / Mathematics/Computer Science. The risk assessment of pesticides in the legislative process is based on active substance by active substance or preparation by preparation. In addition the risk analysis may be based not only on the expected ecological effect but also on the potential for ecological recovery. However, implementing the recovery principle in the aquatic risk assessment of individual compounds could be unrealistic if in most crops pesticides are repeatedly applied during the growing season or multiple pesticides are applied at the same time. The aim of the conducted work was to assess whether recovery times increase when a population of a vulnerable aquatic invertebrate is exposed to the

exposure profile of one pesticide or to exposure profiles of multiple pesticides. The evaluated two sets of mixture of pesticides is representative for pesticides packages that are used on a fruit and tuber crop in The Netherlands. Exposure concentrations were predicted using the FOCUS step three modelling framework and the Dutch drainage ditch scenario. Recovery times were assessed using the MASTEP individual based population model. MASTEP is an individual-based model where Asellids are individually simulated, and some of the most basic model parameters such as time of proliferation and natural mortality are varied stochastically between the individuals. We simulated the population dynamics and pesticide effects in a Monte Carlo style, by varying the sensitivities of the simulated population by generating random LC50 values from the arthropod species sensitivity distribution of the respective pesticide. For the tuber scenario lambda-cyhalothrin resulted in long-term effects, while fluzinam hardly resulted in effects. This absence of interaction of effects of the mixture of chemicals used in the tuber scenario on effect size and recovery is also shown by the distribution of the recovery times. In the fruit scenario the applications of all three chemicals alone already resulted in large effect sizes just after exposure. The recovery times, however, are not higher for the mixture compared to those associated with exposure to the individual compounds. The conclusion is that exposure to the evaluated mixtures of chemicals that are representative for the tuber and fruit scenarios does not lead to longer recovery times than when exposed to the individual compounds alone.

**76 Dynamic modeling of toxicokinetics of short- and long-term pulsed copper in tilapia** W. Chen, National Taiwan University / Dept Bioenviron Sys Eng; J. Tsai, China Medical University / Graduate Institute of Ecology and Evolutionary Biology; C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering. The variations in toxicant concentration can generate either pulsed or fluctuating exposures in aquatic ecosystems. Aquatic organisms living in aquatic systems would positively experience pulsed/fluctuating contaminant exposures. Moreover, the organ-specific copper (Cu) accumulations in fish are critical indicators for Cu exposure. The purpose of this study was to combine mechanistic and statistical models-based data from short- and long-term pulsed Cu accumulation of tilapia (*Oreochromis mossambicus*) to examine the organ specific toxicokinetics. The accumulation, distribution dynamics, and correlation between organs Cu burden and that of whole body were also investigated. Results found that the majority of organs in response to short-term pulsed exposure had higher bioconcentration factor than those of long-term pulsed exposure, except blood, muscle, and carcass. The pulsed Cu burdens estimated by the physiologically-based pharmacokinetic (PBPK) models were consistent with the experimental data. The experimental results indicated that muscle plays the main distributive dynamics of Cu burden (34.63–50.38%) in the initial exposure time, whereas liver is the most contribution tissue (18.77–64.83%) during the exposure periods. Based on the multivariate analyses, this study found the similarity of organ accumulation property for pulsed or non-pulsed periods in tilapia occurred in acute pulsed Cu exposure. The positive correlations were found between Cu burdens of whole body and liver in acute ( $r=0.748$ ) and chronic exposures ( $r=0.607$ ). This study provided a first stage that the integrated models contribute to better realizing the fundamental toxicokinetic processes. It may also provide the opportunity to construct a preferable understanding of dynamics relationship between organ Cu concentration and adverse effect for biomonitoring and optimizing ecotoxicological risk assessment in the realistic situations.

**77 The role of feeding habits in controlling Hg bioaccumulation and biomagnification in freshwater tilapia** R. WANG, College of Environmental Science and Technology; W. Wang, Hong Kong University Sci Technol. The bioaccumulation and biomagnification of mercury (Hg), especially methylmercury (MeHg), is commonly found in aquatic organisms. The mercury level in fish is not only related to its surrounding water conditions but also the feeding habit as well, and predatory fish always has high Hg level because MeHg could be

biomagnified through trophic transfer. Whether the biomagnification potential (in terms of trophic transfer factor, TTF) could be influenced by feeding habit is an intriguing but unknown question. To explore this issue, we conducted a 40-days Hg (both inorganic and organic form) accumulation experiment, by feeding freshwater tilapia with three types of Hg labeled food (aquatic grass, freshwater shrimps, commercial pellets) at a certain ingestion rate. The results showed great differences between inorganic Hg and MeHg accumulation patterns. Interestingly, the determined TTF varied significantly with food type for Hg(II), but not for MeHg, which could be well explained by variation of assimilation efficiency (one important parameter in the energetic-based biokinetic model, could be determined by using radioisotope technique). Moreover, a three-month field study was designed to investigate the influences of feeding condition to Hg accumulation process in tilapia under local conditions, by comparing the growth and Hg accumulation process of fish feeding on additional commercial food and natural food. These results again showed the important role of feeding habit in controlling the Hg bioaccumulation and biomagnification process in fish.

**78 A benchmarking method to measure dietary absorption efficiency of chemicals by fish** R. Xiao, Stockholm University / Applied Environmental Science; M. Adolfsson-Erici; M.S. McLachlan, Stockholm University; M. MacLeod, ITM Stockholm University / Department of applied environmental science. Understanding dietary absorption efficiency ( $E_d$ ) for different organic chemicals in fish is of critical importance from both a scientific and a regulatory point of view. However, reported fish  $E_d$  for well-studied chemicals are highly variable. We applied an internal chemical benchmarking method to measure  $E_d$  of 15 chemicals. 2,2',5,6'-tetrachlorobiphenyl (PCB53) and decabromodiphenyl ethane (DBDPE) were selected as an absorbable and a non-absorbable benchmark chemical to reduce the uncertainty and variability in each individual fish response and to correct for bias that results from incomplete collection of feces from different tests. Due to the lack of standard  $E_d$  measurement, the performance of the benchmarking method was evaluated in recovery statistics of the test chemicals. Our results showed that, after benchmarking, the recovery statistics of the test chemicals was significantly increased and variability of recovery of the test chemical was reduced. The benchmarking method has considerable promise to reduce variability in measurements of fish  $E_d$ .

**79 Matching Metal Pollution with Bioavailability, Bioaccumulation and Biomarkers Response in Fish Resident in Neotropical Estuaries** L. Souza; I. Duarte, Universidade Federal do Espírito Santo / Departamento de Ciências Biológicas; M.N. Fernandes, Universidade Federal de São Carlos / Ciências Fisiológicas; J.B. Fernandes, Universidade Federal de São Carlos / Química; M. Bonomo, Universidade Federal do Espírito Santo / Departamento de Ciências Biológicas; V.C. Cavicchioli Azevedo, Instituto de Ciências Biológicas; N. Pimentel, Universidade Federal de São Carlos / Departamento de Ciências Fisiológicas; C. Pereira, Universidade de Santa Cecília; M. Monferran, Universidad Nacional de Córdoba; S. Matsumoto, Universidade Federal do Espírito Santo; D.A. Wunderlin, Universidad Nacional de Córdoba; L. Rocha, Universidade Federal do Espírito Santo / Departamento de Ciências Biológicas; M. Morozesk, Universidade Federal do Espírito Santo. Two neotropical estuaries, affected by different anthropogenic factors, were studied. We report levels of metals in water and sediment, spatial and temporal changes as well as their influence on genetic, biochemical and morphological biomarkers in the native fish *Centropomus parallelus*. Multivariate data treatment (discriminant and procrustes analyses) showed both spatial and temporal differences between studied areas throughout studied matrixes (water, sediment and fish). High concentrations of metals in sediments did not match metal bioaccumulation in fish. Conversely, we observed better correlation between interstitial water and levels of metals in fish. Morphological and enzymatic biomarkers reflected the condition of fish but genetic biomarkers did not. So far, current results highlight the need of an integrated approach to evaluate the influence of estuary pollution on native fish, considering swimming and feeding habits. Multivariate

statistics present a quite novel approach to integrate data from different origin allocated in the same estuary. Financial support: Prefeitura de Vitória/ES, FAPESP, CNPq

#### **80 Temporal changes in Stable Isotope Analysis in Baltic herring (*Clupea harengus*): an explanation for observed PCDD/F concentrations?**

**A. Miller**, Naturhistoriska Riksmuseet / Contaminant Research Department; J. Hedman, Policy Instruments for Natural Resources, Swedish Environmental Protection Agency; A. Bignert, Swedish Museum of Natural History; M. Kiljunen, University of Jyväskylä; J. Duberg, E. Gorokhova, Stockholm University. Extensive measures have been taken to reduce dibenzo-*p*-dioxin (PCDD) and dibenzofuran (PCDF) emissions within the EU. However, despite continual decreases in dioxin air emissions and the Baltic Sea abiotic environment (water and sediments), temporal trends in PCDD/Fs (l.w. and w.w.) in herring from various sites within the Baltic Sea region have shown inconsistencies in decreases or no significant decreases. This suggests that other factor(s) may be preventing herring PCDD/F concentrations from responding more quickly to decreased emissions. Diet is an important determinant of PCDD/F body burden, due to bioaccumulation and biomagnification of these chemicals in the food chain. Here, we use long-term trends of stable isotope signal to examine whether changes in trophic level may explain the observed PCDD/F concentrations in Baltic herring. Changes in herring nitrogen isotope signature ( $\delta^{15}\text{N}$ ) using muscle tissue of autumn-caught fish were evaluated in relation to the baseline represented by zooplankton (adult copepods *Acartia* sp. and *Eurytemora affinis*).  $\delta^{15}\text{N}$  in herring varied from 8.4 to 11.0 (mean  $9.6 \pm 0.6$ ), and increased by  $\sim 3\text{‰}$ , whereas zooplankton  $\delta^{15}\text{N}$  showed a simultaneous decrease of the same magnitude. This implies a change of more than one step in herring trophic level over the last 30 years, assuming a fractionation factor of 3.2‰. Due to the bioaccumulation and biomagnification properties of PCDD/Fs, the increase in trophic level observed in Baltic herring from the southern Bothnian Sea over time is one plausible reason for the observed inconsistent or absent decreases in these contaminants at various sites throughout the Baltic Sea region. However, to be certain this is not just a local phenomenon, SIA should be conducted using herring from different areas together with dietary studies.

**KEYWORDS:** Baltic herring, stable isotope analysis, dioxins, PCDD/Fs

#### **81 Effect of food provisioning on persistent organic pollutant bioamplification in Chinook salmon larvae**

**J.M. Daley**, Great Lakes Institute of Environmental Research; T.A. Leadley, University of Windsor / Great Lakes Institute for Environmental Research; T.E. Pitcher, University of Windsor / Department of Biological Sciences; **K.G. Drouillard**, University of Windsor / Great Lakes Institute for Environmental Research. Fall spawning pacific salmon provision large amounts of yolk to their eggs to allow survival of larvae during under the ice winter conditions. This yolk provisioning leads to maternal offloading of persistent organic pollutants (POPs) to eggs and larvae. Previous research has shown that Chinook salmon larvae exhibit limited capacity to eliminate POPs during the cold water period resulting in bioamplification of POP residues. This study compared POPs bioamplification in Chinook salmon larvae under a high food provisioning treatment and a non-fed treatment to test whether or not food availability attenuates POPs bioamplification via growth dilution. Results demonstrate that larvae in the food provisioning treatment did not gain weight until after day 129. Between hatching and day 129, fed and non-fed treatments exhibited similar decreases in whole body lipid content, negligible POPs elimination and POPs bioamplification factors approaching 1.5. By day 184 of the study, POPs bioamplification factors in the non-fed treatment were as high as 5.3 across chemicals but ranged from non-detectable to approaching 1 in the fed group. This study demonstrates that POPs bioamplification occurs in Chinook salmon larvae even under ideal rearing conditions but peaks after day 129, following which growth dilution can attenuate bioamplification relative to starved individuals. While bioamplification in a real population would likely be captured between the two extremes of maximum food provisioning and the starved group, other stressors could interact with

the toxicokinetics of POPs. Based on the observations from the current study, it is predicted that multiple stressors such as resource limitation, habitat quality and climate change could potentially push bioamplification factors closer to the non-fed state observed in the present research.

#### **82 Screening organic contaminants in tree cores using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry**

**C. Gauchotte-Lindsay**, University of Glasgow / Infrastructure and Environment; A. Cooper, University of Strathclyde / Department of Civil and Environmental Engineering; H. Roussel, ADEME / Urban Brownfield and Polluted Sites; M. Chalot, Université de Franche-Comté / UMR 6249 Laboratoire Chrono-environnement; L.A. McGregor, R. Kalin, University of Strathclyde / Department of Civil and Environmental Engineering; J. Balouet, Environment International. Pollution investigation by trees (PIT) is an international research programme funded by the French agency for environment and energy management (ADEME) to develop the use of phytoscreening and dendrochemical applications at polluted sites. The term phytoscreening was coined to describe the use of plants to characterise polluted sites. Outermost rings of tree cores are microsampled and tested for sap-transported contaminants such as volatiles, polycyclic aromatic hydrocarbons (PAHs), heavy metals, polychlorinated biphenyls (PCBs) and dioxins. Phytoscreening allows semi-quantitative delineation and mapping of plumes in a non-invasive, low cost and rapid manner. Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOFMS) is a chromatographic technique with a resolution power an order of magnitude greater than that of classic gas chromatographic techniques. Two columns with different selectivity are placed in series and permit planar resolution of the separation. The use of a time of flight mass spectrometer as a detector allows molecular identification of the eluted compounds. Because of its high resolution power, GCxGC-TOFMS has been employed for the characterisation of complex environmental samples in soils, sediments and waters. As part of the PIT research programme, we are developing a one-step screening method for organic contaminants in tree core samples using GCxGC-TOFMS. Organic compounds are extracted from the tree core using pressurised liquid extraction, a faster, greener option to common Soxhlet methods and extracts are analysed by GCxGC-TOFMS without further treatment thereby minimising losses. We present here preliminary results and method development stages establishing the potential of the technique for phytoscreening of PAHs and PCBs. Additionally, through the analysis of a series of Arcolors by GCxGC-TOFMS, we show that multivariate data analysis methods such as principal components analysis further improve the technique by potentially making source identification possible.

#### **83 Phytoforensics Analytics: Delineating Solvents and Explosives through Novel Plant Sampling and Analysis**

**J.G. Burken**, Missouri University of Science and Technology / Civil Architectural and Environmental; M.A. Limmer, Missouri University of Science and Technology / Civil, Architectural, Environmental Engineering; Y. Yuan, Missouri University of Science and Technology / Environmental Engineering; H. Shi, Missouri University of Science & Technology / Chemistry; Y. Ma, R. Mu, Missouri University of Science and Technology / Chemistry; C. Balouet, Environment International. Harnessing tremendous energy of evapotranspiration, plants extract nutrients and water from their surrounding environment and concurrently accumulate contaminants. Novel and innovative analytic approaches were developed to access this data and provide information on toxic compounds distribution and exposure easily, rapidly, inexpensively and with little impact to ecology or private property. Fugitive contaminants are difficult to detect in groundwater and the potential risk assessment and remediation efficacy relies on accurate delineation of contaminated areas. Methods: Novel centrifugation, solvent-free extraction techniques were developed to collect tissue fluids, i.e. sap, mechanically. Collected fluid was filtered and injected directly. LC-MS-MS and IC analytic methods were developed to



analyze sap for PETN, HMX, RDX, TNT, DNAN and Perchlorate. Method detection limits (MDLs) are the lowest reported for many compounds, down to 20 ng/l. Volatile organic compounds (VOCs) analysis was done by collecting tree cores, followed by headspace solid phase microextraction (SPME) sampling and GC-<sup>2</sup>ECD/FID or GC-MS analysis. This method requires no processing and can reach ng/l levels for sap concentrations. *In-planta* SPME and direct GC-MS analysis methods were developed to analyze contaminants in trees. Solvent-free sampling methods were applied for a variety of monocot and dicot species. Results compared favourably to traditional solvent extractions, which are expensive, time consuming and use hazardous solvents. Linear relationships for *in-planta* sap and pore water concentrations for HMX, RDX, and Perchlorate confirmed the successful phytoforensic methods development for explosives and energetics. The methods were applied at a perchlorate field site to delineate the groundwater plume, also confirmed with groundwater sampling, revealing potential for using native plant species in phytoforensics. *In-planta* SPME and real time/*in-field* GC-MS analysis of VOCs was developed, and using specially designed ports, repetitious sampling of the same tree over years offers a novel long term monitoring approach. Detection limits as low as 0.5 ng/l (parts per trillion) in the xylem tissue were reached. Plumes have been delineated in many settings including urbanized areas, where sampling landscaping trees on personal properties indicated presence of indoor contaminants (vapor intrusion potential), thereby directly assessing exposure pathways to protect human health.

**84 Leaching of additives by artificial rain fall under laboratory conditions** J. Eltfeld, Instrumental Analytical Chemistry; T. Schmidt, University of Duisburg-Essen. New thermal regulations in Germany have resulted that the outer shells of buildings have to be more equipped with polymer materials as in the past. This has the consequence that insulation layers are increasingly equipped with additives: biocides, fungicides, flame retardants, and other organic substances. Forecasts predict that, due to climate change, heavy rainfall coupled with prolonged dry periods will increase. Particularly heavy rainfall events lead to increased leaching of these substances from building facades and thus make their entry more likely to surface water, as there is currently no treatment of this materials provided in the treatment plants. In this project, the mass flows of selected markers (diuron, octylisothiazolone, terbutryn, TCPP, ...) was estimated in order to make predictions about future developments. In addition, it was also assessed whether currently or in the future these substances are expected to exceed the thresholds in waters. Aim of the laboratory experiment was to determine which kind of insulation material is leading to the release of organic trace elements and in which extent they were exposed into the environment. The experiment was performed under realistic rain conditions, wherefore four differently equipped facades (1 x 1 m) were irrigated. The runoff water was collected in 1 Litre bottles each and was enriched by solid phase extraction for further GC-MS analysis. Based on the obtained data, water pollution will be estimated for urban areas. This will also be verified by spot sampling from rivers of these particular areas. Additionally it will quantitatively be examined whether "first flush events" were more relevant as the following rain. These results will be compared with the investigation of a large-scale basin. From this work, existing toxicological data and still to be collected dose-response relationships will be estimated for the ecological risk of individual substances and mixtures, which are valued according to the Water Framework Directive.

**85 Uranium distribution among cytosolic proteins of *Danio rerio* gills after different exposure conditions** G. Bucher, IRSN / L2BT; S. Mounicou, LCABIE; O. Simon, IRSN; L. Fevrier, IRSN / LRE; R. Lobinski, LCABIE; S. Frelon, IRSN. Uranium (U), found in a wide range of concentrations in aquatic ecosystems, was shown to potentially exhibit toxicity against aquatic organisms, but all mechanisms have not been elucidated yet. To better understand intake, incorporation, storage and elimination mechanisms and then potentially elucidating its associated toxicity, the study of U compartmentalisation and more specifically the identification of U target biomolecules is a key step.

Indeed U, mainly uranyl cation in aerobic biological matrices, is known to be likely to bind to proteins, then inducing a potential competition with endogenous essential metals. Thus, U speciation in cytosol of cells is of great importance to identify metal toxic fraction as this compartment, containing hydrosoluble proteins, is known to have an important role in the toxicokinetics and toxicodynamics of metals. Therefore, this study focused (i) on the development of non-denaturing and highly sensitive hyphenated analytical techniques (SEC-ICP SFMS presented here) and (ii) on their application to better describe U speciation in cytosols. The ecotoxicological objective was to investigate the influence of different waterborne exposure conditions to U (environmental 20 µg L<sup>-1</sup> (c<sub>0</sub>), incidental-like 250 µg L<sup>-1</sup> (c<sub>250</sub>)) on its distribution among cytosolic proteins in *Danio rerio* gills<sup>250</sup>, the latter being one of the first barriers against dissolved pollutants. Consequences on endogenous essential metals distribution in the cytosol have also been studied. Results show that U accumulation in exposed zebrafish gills was significantly different from control (c<sub>0</sub>) and rather depended on contamination level than on duration. At the subcellular level, a larger extent of U was accessible to cytosolic proteins in c<sub>250</sub> compared to c<sub>0</sub> exposure (*i.e.* 32 vs. 24%) and cytosolic Zn burden increased noticeably in the case of c<sub>250</sub> exposure while Fe and Cu burden remained unchanged. SEC-ICP SFMS chromatographic patterns of gill cytosols were similar for all conditions but U distribution within the pool of proteins varied from c<sub>0</sub> and c<sub>250</sub> exposure with a larger extent of U bound to high molecular weight<sup>250</sup> proteins. Co-elution of U with some Fe, Cu and Zn peaks let suppose a possible competition for endogenous metal binding proteins. Results confirmed U accumulation on proteins of gill cytosols and provided new information (i) to identify the U toxic fraction and (ii) on potential interaction and competition with endogenous essential metals.

**86 Determination of selenium species and some other elements in wild and fish farm trout (*Oncorhynchus mykiss*) and seabass (*Dicentrarchus labrax*)** U. Kristan, Jozef Stefan Institute / Environmental Sciences; V. Stibilj, Jozef Stefan Institute. Fish are an important source of protein, micro- and macro elements, fatty acids and fat-soluble vitamins and therefore beneficial to human health, because of their high nutritional value. Selenium (Se) as an essential trace element plays a fundamental role in human health, as a component of several major metabolic pathways including thyroid hormone metabolism, antioxidant defence systems and immune function [1]. Its determination and speciation is of strong importance, since in humans, selenium has one of the narrowest ranges between dietary deficiency and toxic levels. The recommended adequate intakes of Se published by DACH are 30 - 70 µg/day for adults [2]. Nevertheless, some fish species may contain significant levels of mono methylmercury (MeHg<sup>+</sup>), polychlorinated biphenyls (PCBs), dioxins and other contaminants. These substances bioconcentrate in the aquatic food chain in such way, that levels are generally highest in older, larger, predatory fish and marine mammals [3]. In our study we included two main fish species that represent the most commonly consumed fresh fishes in Slovenia; trout (*Oncorhynchus mykiss*) and seabass (*Dicentrarchus labrax*) both from different locations (Slovenia, Italy, Greece) and environments (fish grown up in fish farms and wild fishes). Total Se concentrations were measured by HG-AFS [4], speciation in soluble fraction after enzymatic extraction was performed by HPLC-ICP-MS, while other elements were determined after microwave digestion by ICP-MS. Content of toxic elements, such as cadmium (Cd) and lead (Pb) were under the detection limit (3 and 23 ng/g respectively) regardless fish species. Se concentrations were more variable; in trout concentrations ranged from 120 to 211 ng/g on wet mass basis while in seabass Se concentrations varied regarding fish origin and breeding method. In seabass, which were grown up in fish farms Se concentrations were in the same range as in trout, whereas seabass caught from natural environment have significantly higher concentrations around 400 ng/g. Keywords: fish, ICP-MS, selenium, speciation

**87 Rapid measurement of petroleum hydrocarbons in field soils using mid-infrared diffuse reflectance spectroscopy** L. Janik, S.



Forrester, J. SorianoDisla, CSIRO Land and Water; M. McLaughlin, CSIROUniversity of Adelaide; G. Webster, R. Stewart, Ziltek Pty Ltd. Contamination of soil by crude oil and refined oil products (total petroleum hydrocarbons – TPH) is a worldwide problem, and the quick and inexpensive analysis of soils and sediments contaminated with these materials would significantly aid remediation and risk assessment activities. Diffuse reflectance infrared Fourier transform spectroscopy, with partial least-squares (PLS) regression, was used to develop calibration models for the non-destructive and economical prediction of TPH concentrations in soils. Standard soils were spiked with known concentrations of TPH and spectral response of the spiked sampled examined. Soils were also collected from different contaminated sites in south eastern Australia known to be contaminated with TPH. A number of selected near-infrared (NIR) and mid-infrared (MIR) frequency ranges were tested for prediction of TPH concentrations. The aliphatic alkyl stretching vibration region was found to be the most sensitive to TPH concentration; NIR frequencies in the 4500-4100  $\text{cm}^{-1}$  region and MIR frequencies at 3000-4600  $\text{cm}^{-1}$ . In particular, the MIR range included two specific alkyl peaks, one at 2950  $\text{cm}^{-1}$  and the other near 2730  $\text{cm}^{-1}$ , both shown to have a strong correlation with TPH at low and high TPH concentrations respectively. These peaks were considered to be either weak or absent in natural soil organic matter relative to the usual  $-\text{CH}_2$  region at 2930-2850  $\text{cm}^{-1}$ . Using these frequencies, the technique is therefore has the potential to be a rapid and accurate non-destructive method to determine concentrations of TPH in contaminated soils and can be adapted for in-field applications.

**88 Practical Quantification of Ecosystem Services for Environmental Decision-Making in Mine Development** J. Nicolette, ENVIRON International Corporation; S. Deacon, ENVIRON UK Ltd; F. Colombo; N. Eury, ENVIRON UK Ltd; R. Wenning, M. Rockel, ENVIRON International Corporation. The incorporation of ecosystem services into international guidance has been increasing since the Millennium Ecosystem Assessment. This includes the incorporation of ecosystem services into the development of environmental and social impact assessments (ESIAs) for large scale extractive industries. In particular, the mining sector is influenced by the multilateral banks and private lenders who have adopted the Equator principles. As an example, the International Finance Corporation (IFC) has incorporated ecosystem services into large-scale impact assessment considerations with Performance Standard 6 on Biodiversity Conservation and Sustainable Management of Living Natural Resources. Although ecosystem services have been incorporated into developing guidance, there is considerable effort being conducted to develop methodologies and approaches to quantify ecosystem services to demonstrate environmental sustainability, stewardship and biodiversity. Many of these methods attempt to place a monetary value on ecosystem services when in fact, those estimates contain significant uncertainty. The aim of this paper is to demonstrate an approach that does not rely on the valuation of all the ecosystem services provided by a habitat, but a subset of representative measures in order to provide a cost-effective approach to develop information sufficient for environmental-decision-making. In addition, the approach does not necessarily require monetary valuation of services. The proposed approach will support developing guidance under the IFC and a multitude of other frameworks. In understanding the need to quantify ecosystem services, it is important to understand how that information will be used and what components of valuation are necessary. Ecosystem service quantification for impact assessment originally arose out of the United States natural resource damage assessment (NRDA) process. These tools and approaches have been used on small scale environmental issues but also have merit in evaluating ecosystem services associated with large scale ecosystems. These tools and approaches are adapted within a framework known as net ecosystem service analysis (NESA). We will demonstrate, via a large scale case study, how environmental economics models using monetary and non-monetary metrics, have been used to quantify ecosystem service losses and gains from a practical standpoint facilitating decisions by various stakeholders directly applicable to the mining industry.

## **89 Deriving a Water Quality Standard for Iron from Field Evidence**

A. Peters, WCA Environment Ltd; B.J. Adams, Rio Tinto / Product Stewardship; P. Simpson, WCA Environment Ltd; P. Whitehouse, Environment Agency. Recent studies on the ecotoxicity of iron to fish, invertebrates and algae have shown a clear effect of water chemistry on iron toxicity. As such, water chemistry should ideally be taken into account when establishing quality standards for iron. Field data can be an important line of evidence in setting quality standards alongside conventional laboratory ecotoxicity data. This study aims to take account of water chemistry conditions on the ecotoxicity of iron in assessing whether or not impacts are observed on ecological communities in real field conditions. Previous analyses of the effects of iron on aquatic communities have not taken account of the effect of water chemistry on the ecotoxicity of iron. A limiting function model was fitted describing the ecological quality, expressed relative to a reference condition, as a function of each of the three measures of iron exposure. A statistically significant decline in ecological quality was observed in each of the different measures of iron exposure, although different thresholds were derived for each exposure metric. In each of these analyses the sites with the highest iron exposures have reduced ecological quality, irrespective of whether the exposure is expressed as total iron or as one of the forms of “effective iron”. This supports the assertion that the models developed by Iron Platform for predicting the effects of iron on three different trophic levels are taking account of the principal factors which influence iron ecotoxicity.

## **90 The use of macroinvertebrates to characterise neutral mine drainage and river flow variability**

P. Byrne, Liverpool John Moores University; P. Wood, Loughborough University; J. Gunn, University of Birmingham. Contamination of aquatic environments by acid mine drainage has potentially far reaching implications for the quality of aquatic ecosystems. However, little attention has been given to sites where the mine drainage is typically circum-neutral ( $6 < \text{pH} < 8$ ). Previous research on a river receiving neutral mine drainage indicated that aquatic macroinvertebrate assemblages in metal-mine impacted regions are frequently deemed to be in relatively good condition when using standard bio-monitoring indices, despite severe metal contamination of bed sediments and river water. However, the application of multivariate analysis techniques has facilitated the identification of differences in macroinvertebrate community structure in mining impacted and un-impacted reaches of the river associated with metal mine contaminants. It is hypothesised that macroinvertebrate bio-monitoring indices fail to identify impacts of metal contamination at the community level because they either seek to identify impacts of a specific contaminant or are dependent on a model community response to a given stress, and neither of these responses are typical of neutral mine drainage. In this paper we examine how the macroinvertebrate community and biotic indices respond to changes in dissolved zinc concentrations and river discharge in the River Lathkill, Derbyshire, associated with the partial blockage by collapse of an underground drainage sough. Analysis of macroinvertebrate biomonitoring indices of water quality indicated moderate improvements in both the Biological Monitoring Working Party Score and Average Score Per-Taxon. In contrast, the Lotic Invertebrate index for Flow Evaluation score (LIFE), which provides a measure of the aquatic macroinvertebrate community response to changes in the river flow regime displayed no significant change. The use of multivariate analysis techniques examining the macroinvertebrate community (Detrended Correspondence Analysis) and both flow and water quality parameters (Canonical Correspondence Analysis) allowed the identification of the biotic and abiotic drivers of community structure. The results indicate that caution is required when interpreting the results of macroinvertebrate biomonitoring indices in regions affected by historic metaliferous mining activity, particularly when the drainage is circum-neutral. Where biotic indices are unresponsive, multivariate analysis may provide an alternative method to examine changes in community structure.

## **91 Using tissue residues to predict ecological effects of metal mining**

**on macroinvertebrate communities** M. De Jonge, University of Antwerp / Biology; E. Tipping, Centre for Ecology and Hydrology / Lancaster Environment Centre; S. Lofts, Centre for Ecology Hydrology / Shore Section; L. Bervoets, University of Antwerp / Dept. of Biology, Systemic Physiological and Ecotoxicological Research group; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology. The present study investigated whether tissue concentrations can be used to predict metal-induced effects on aquatic invertebrate communities. Total dissolved metal levels and four invertebrate taxa (*Leuctra* sp., Simuliidae, *Rhithrogena* sp. and Perlodidae) were sampled in 36 headwater streams located in the north-west part of England. Using the River Invertebrate Prediction and Classification System (RIVPACS) taxonomic completeness of invertebrate communities was assessed. Quantile regression was used to relate tissue concentrations to a maximum (90<sup>th</sup> quantile) ecological response. The current abstract presents the results for Zn, which was one of the metals showing significant impact. A significant negative relation was observed between RIVPACS-calculated taxonomic completeness and Zn accumulation in Simuliidae. Using the quantile regression model, accumulated Zn concentrations in Simuliidae corresponding to taxonomic completeness associated with 0%, 20%, 50% and 100% decrease could be calculated (4.4, 14.8, 30.3 and 56  $\mu\text{mol g}^{-1}$  Zn respectively). Based on invertebrate tissue concentrations dissolved metal concentrations representing safe levels for the protection of invertebrate communities could be derived, resulting in 0.26, 9.2, 76 and 463  $\mu\text{M}$  dissolved Zn, which are generally in good agreement with current international environmental quality standards (EQS) and field-based results from literature. Overall, the present study concludes that accumulated metal concentrations in aquatic invertebrates can be used to 1) predict metal-induced ecological effects and 2) to derive safe metal levels for the protection of aquatic invertebrate communities.

**92 Toxicity and bioaccumulation assessment with the oligochaete *Tubifex tubifex* in the Nalón River catchment: impact of abandoned metal mines in Asturias** L. Mendez, P. Rodriguez, UPV/EHU / Zoology and Cell Biology; M. Martinez-Madrid, University of Pais Vasco / Genetics, Anthropologie and Animal Physiology. Abandoned mine sites are a great environmental problem. In central Asturias (North Spain), two main focuses of metal mine activities were active until the early 1970s, one in *Texeo* Mine in Riosa district (Cu mines), and a mine group in the districts of Mieres and Pola de Lena (Hg/As mines). The main objective of this work was to assess sediment toxicity and the bioaccumulation (metal tissue residues) in the aquatic oligochaete *Tubifex tubifex* exposed to field sediment chronic bioassays. Twenty-six sites were studied in the *Nalón* Catchment, including reference and non-reference sites from the water surveillance nets in Spain. The 28-day *T. tubifex* chronic bioassay included survival, reproduction and total growth rate endpoints. Sediment metal concentration and sediment related toxicity and metal bioaccumulation in worms were analyzed using multivariate techniques. Sites from Hg/As mines were severely affected by high levels of both metals, reaching to a maximum sediment concentration of 5320.9  $\mu\text{g As g}^{-1}$  dw at *Los Rueldos* site (LRu) and 312.5  $\mu\text{g Hg g}^{-1}$  dw at *La Peña-El Tarronal* (LPET). In *Texeo* Cu Mine area, LLamo (LL) reached 115.2  $\mu\text{g Cu g}^{-1}$  dw sediment concentration. Regarding toxicity 5 different groups were distinguished showing a marked toxicity gradient (ANOSIM,  $R=0.987$ ). Eleven test sites were ordered within the same groups than reference sites and thus assessed as *Non-toxic*, including *Texeo* copper mine sites. Four sites were assessed as *Potentially toxic*, and 5 sites from Hg/As Mines with a great impairment on survival (< 20%) and almost no reproduction were assessed as *Toxic*. Evaluation through Reference Condition Approach using probability ellipses, on a database of 61 reference sites in northern Spain, agrees with nMDS classification approach. Due to high worm mortality observed in 4 sites from Hg/As mining areas, worms from only 22 sites were analyzed for metal tissue residues. Two groups (ANOSIM  $R=0.987$ ) were distinguished, one group included sites with low metal tissue residues, whereas the other included 3 sites with extremely high values of As and Zn (up to 2166.7 and 2498.1  $\mu\text{g g}^{-1}$  dw, respectively). Sites from the *Texeo* Cu Mines exhibited low values of Cu

tissue residue, indicating scarce Cu bioavailability and explaining low chronic toxicological effects observed. A great environmental problem can be inferred from Hg/As mines, and an extensive Evaluation of Risk Assessment (ERA) should be conducted in the area to undertake remediation plans.

**93 Effects of metal mixtures on fish and benthic macroinvertebrate populations in a mining-impacted stream: Railroad Creek, Washington, USA** C.B. Meyer; J.S. Meyer, ARCADIS; R.A. Hummell, MWH; W. Adams, Rio Tinto. Railroad Creek is a high-gradient tributary to Lake Chelan, at 1981 to 335 m asl in north-central Washington State in the USA. The Holden Mine, which produced Ag, Au, Cu, and Zn ore from the late 1800s until 1957, is located adjacent to the creek, approximately 16 km upstream from the lake. Concentrations of Al, Cd, Cu, Fe, and Zn have historically increased as Railroad Creek flows past the mine portal and several waste rock and tailings piles, but remediation efforts are in progress to remove metal and acid rock drainage inputs to the stream. Rainbow trout (*Oncorhynchus mykiss*) and westslope cutthroat trout (*O. lewisi clarki*) and their hybrids inhabit the entire creek and are the only fish that have been observed upstream of a set of barrier falls approximately 2.5 km from the lake. We combined fisheries, benthic macroinvertebrate, and physical-habitat data with historical and current water chemistry data to evaluate the relationships of population densities to habitat characteristics and metal concentrations in the creek [after collapsing the metals data into two principal components (PCs)]. The five strongest predictors of trout density were, in order of significance (sign of correlation in parentheses): PC1 "metals from tailings piles" (-), proximity to lake (-), proportion of pools (-), PC2 "metals from mine portal" (-), and density of rootwads (+); additionally, impacts that might be attributed to physical effects of iron-oxide deposition extended downstream from the tailings piles. Historical benthic macroinvertebrate data suggest impacts to trout food sources also extended along and downstream of the mine site, but recent data suggest impacts to macroinvertebrates are now spatially limited to areas immediately adjacent and just downstream of the tailings piles. Ferricrete has cemented some of the macroinvertebrate habitat, decreasing habitat quality. These models suggest improvement of the stream substrate and cover (e.g., adding rubble/boulders [substrate >150 mm, which was also related to trout density] and rootwads, and decreasing iron oxide precipitates and ferricrete on the stream bed) might provide benefits in a restoration project to improve trout habitat in Railroad Creek.

**94 Associating risk to chemicals with both natural and anthropogenic sources** S.M. Mudge, Exponent International Limited; P.C. DeLeo, American Cleaning Institute; S.E. Belanger, The Procter Gamble Company / Environmental Stewardship and Sustainability Organization; R.A. Stackhouse, Sasol North America Inc / Global Product Safety and Sustainability. Fatty alcohols and their derivatives are used as surfactants in many detergents and personal care products. These high production volume chemicals have been assessed under REACH and similar global chemical management programs with data requirements driven to understand environmental sources, fate and effects of these compounds. European and US regulatory bodies have indicated further interest in the whole category of long chained fatty alcohols given their widespread use in consumer products. Their usage generally results in a down-the-drain disposal pathway that leads to wastewater treatment plants (WWTPs). Recent work using stable isotopes has demonstrated that the chemicals entering the WWTPs are degraded within the system and the fatty alcohols in the liquid effluents are not the same ones that entered in the influent. Likewise, analysis of fatty alcohols in the sediments of the receiving (fresh and salt) waters shows that these are dominated by terrestrial plant derived compounds, and in situ production by bacteria and algae. PNECs for the alcohols are derived for all chain lengths through empirical and toxicity modelling whereas for many alcohol derivatives (surfactants) PNECs are derived using mesocosms and Species Sensitivity Distributions. Measured environmental concentrations are available for a range of locations including the sediments from rivers, soils and coastal environments. By

using the stable isotopic signatures and chain length profiles, it is possible to determine the risk ratios for each location and for each source thereby apportioning risk to the different compartments. Since the vast majority (>99%) of the fatty alcohols measured in the sediments are from natural sources, the anthropogenic signal and associated risks are very small.

**95 Assessment of inorganic UVCBs under REACH** F. Iaccino, K. Arijis, Arche consulting; K. Lacasse, ECI; F. Verdonck, Arche consulting; D. Vetter, EBRC; H. Waeterschoot, Eurometaux; K. Delbeke, ECI; V. Verougstraete, Eurometaux; C. Braibant, EPMF. Complex inorganic substances containing varying amounts of metals, metal compounds and/or minerals, which may occur naturally (e.g. mineral ores) or be manufactured during the various refining streams of the metal and mineral industry (e.g. inorganic intermediates) represent an important fraction of the substances manufactured within the metal industry (e.g. fluxes, slags, ...) and used for a wide range of applications. Inorganic UVCBs have unique characteristics (e.g. chemical speciation and valence bioavailability) that should be considered when assessing their risks. A specific approach has been developed to identify the inorganic UVCB substances meeting the REACH requirements and appropriately run the risk assessment. Specific data as published in the ECHA website and data from consortia have been collected and used to assess the overall risk of the complex substance without having to perform unnecessary testing on the UVCBs. In view of the range of complex inorganic UVCBs, a vast amount of testing would be required and the results would not be predictive due to the temporal and spatial uniqueness of the UVCBs. Eurometaux is working to develop ad hoc guidelines and testing them on specific industry cases.

**96 A socio-economic framework for decision-making on chemicals' authorisation: The case of PBT chemicals** S. Gabbert, Wageningen University / Social Sciences; M. Scheringer, ETH Zurich / Institute for Chemical and Bioengineering; C.A. Ng, ETH Zurich / Institute for Chemical and Bioengineering. Under the European chemicals' legislation, REACH, "substances of very high concern" (SVHC) that have been included in Annex XIV can only remain on the market if the European Commission has granted an authorisation. Companies who apply for an authorisation must submit a socio-economic assessment documenting that "social benefits" from using the chemical outweigh "negative impacts". We focus on PBT chemicals, an important subgroup of chemicals for which authorisation might be requested. Assuming that the authorisation process requires performing such impact assessment raises the problem of how coherent decisions on the authorisation of PBT chemicals can be adopted. This, clearly, depends on how benefits from the use of chemicals are weighed against impacts, in particular environmental impacts. We develop a socio-economic framework that provides conceptual guidance for regulatory decision-making on chemicals' authorisation. The framework includes cost-benefit analysis and cost-effectiveness analysis as key socio-economic tools that have become widely applied in many different domains. For both tools we develop a methodological setup for the authorisation of PBT chemicals by assuming that environmental impacts can be modelled as a stock pollution problem. Furthermore, we survey information and data requirements for their application, and we discuss decision rules for PBT chemicals' authorisation. Taking hexabromocyclododecane (HBCDD) as an illustrative example, we examine the implications from CBA and CEA for authorisation of PBTs and discuss implications for short- and long-term policy-making. The CBA model illustrates that the decision on granting or refusing an authorisation, and the optimal timing for removing a chemical from the market, depends on the emission path and the shape of the pollution cost function. An empirical application of a CBA approach seems, however, intractable as long as the relationship between risks and environmental impacts cannot be predicted in a sufficiently reliable way for all relevant ecosystems. Applying CEA requires to define policy targets for PBT use, for example in terms of maximum tolerable PBT scores. Our results, therefore, emphasise that coherent and sound decision-making requires further in depth research on assessing the risks of the use of PBT chemicals. In addition, for

regulators there is no way around defining precautionary policy targets for the use of PBTs.

**97 HC5s from Taxonomically Structured Hierarchical Species Sensitivity Distributions** P. Craig, Durham university / Mathematical Sciences; M. Galay Burgos, ECETOC / Environmental Sciences Manager; P. Chapman, Tecsolve; M. Hamer, Syngenta; A. Hart, The Food and Environment Research Agency; G. Hickey, Durham university / Mathematical Sciences; S. Marshall, Unilever; O. Price, Unilever / Colworth Science Park; W. Roelofs, The Food and Environment Research Agency. One approach to deriving the predicted no-effect concentration for a chemical is to use a species sensitivity distribution (SSD) model to estimate the hazardous concentration affecting p% of species (HCp), where p is usually 5. Many questions have been raised about both principles and application of SSDs but the concept has nevertheless been found to be useful. In refining the SSD approach, several statistical issues need to be addressed. These include: inter-species correlation; tendencies of particular species to one or other end of the sensitivity distribution; and inter-test variation. Attempts have been made at addressing each of these issues on its own. Addressing them collectively requires multivariate statistical modelling. We present a Bayesian hierarchical model of variability and uncertainty for sensitivities of species to a chemical undergoing assessment and for a database of relevant test results for other chemicals. The Bayesian approach has several advantages over traditional non-Bayesian statistical methodology aimed primarily at analysing experimental data. It can incorporate both data and other information such as expert judgements or results of meta-analyses. It provides a collective description of uncertainty for all components of a model, a coherent mechanism for revising uncertainty when additional data become available, and a decision-making framework which addresses both uncertainty and utility. Our model generalises the single chemical random-sampling model proposed by Aldenberg and Jaworska (2000) and addresses the issues raised above. It models inter-species correlation by building species tendencies and sensitivities hierarchically, based on the taxonomic classification of species. Taxonomically-related structure seems natural and makes the model a better description of the available data but means that it is necessary also to specify a taxonomic scenario: the taxonomic structure of the community being protected by the HC5. The HC5 is then scenario-specific, being the 5th percentile of sensitivity to the chemical for species in the scenario. We illustrate the application of the hierarchical model to an existing database of test data and present open-source software developed to implement the hierarchical SSD approach. The software allows a user to estimate the HC5 for a chemical based on test data provided by the user and on the user's choice of scenario. The user can also explore various related aspects of uncertainty.

**98 Trophic Magnification and the new provisions for bioaccumulation in Annex XIII of REACH –a regulatory point of view** W. Drost; J. Ackermann, U. Joehnecke, Federal Environment Agency. Bioaccumulative substances are of concern because substances which accumulate in biota are slowly eliminated and thus, can be retained in the organism for a longer time period, they can provoke adverse effects at low external concentrations and over a greater time scale and can be transferred to the food web. The assessment of the bioaccumulation potential is a key aspect of the European REACH regulation in terms of identifying substances which are persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB). The identification of PBT and vPvB substances is based on a set of criteria described in Annex XIII of the REACH regulation. The assessment of bioaccumulation is usually based on distinct cut-off values such as bioconcentration factors (BCF) with BCF>2000/5000, required for classification as "bioaccumulative (B)" or "very bioaccumulative (vB). The standardised bioaccumulation test with fish (OECD305) is used to achieve these BCF-values and reflects a chemical equilibrium between water and organism, i.e. fish. Additionally, since the amendment of Annex XIII of the REACH regulation, alternative test results such as bioaccumulation factors (BAF)

and biomagnifications (BMF) as well as trophic magnification (TMF) derived from field studies may be considered for the assessment using a weight-of-evidence approach. The amendment of Annex XIII and the consideration of other endpoints apart from the BCF help to classify substances as bioaccumulative where biomagnification is clearly shown in various field studies but the BCF is below the trigger value. However, apart from benefits TMFs and BMFs derived from field studies have limitations. These will be discussed from a regulatory point of view.

#### **99 Mining of the terrestrial toxicity data in the REACH database**

A. Kapanen, D. Vesentini, ECHA; M. Sobanska, European Chemical Agency; R. Cesnaitis, European Chemicals Agency; P. Karamertzanis, J.V. Tarazona, ECHA. The REACH Regulation (EC 1907/2006) foresees the collection and evaluation of information on industrial chemicals to ensure the protection of human health and the environment. The REACH registration database contains information for over 7 600 substances in 30 000 registration dossiers. For the most of these substances the information on environmental fate and ecotoxicological endpoints is available in the database. REACH information requirements in relation to the effects on terrestrial organisms encompass three trophic levels, invertebrates, plants and microorganisms, and the study of both long and short term exposure. The Predicted No Effect Concentration (PNEC) for the terrestrial compartment is calculated on the basis of the available hazard information on terrestrial toxicity. There is possibility also to calculate a surrogate PNEC based on aquatic toxicity data, using the Equilibrium Partitioning Method (EPM). Based on PNEC and the Predicted Environmental Concentration (PEC) for the substance, a risk for the terrestrial compartment can be characterised and risk management measures can be applied if needed. The mining of the REACH registration database for data relating to terrestrial toxicity has been performed to gain an understanding about the type and quality of data available and the extent of the terrestrial toxicity knowledge used by industry for ensuring safe uses. We will present an overview of the terrestrial data in the REACH database with focus on the toxicity to soil organisms and PNECs. In addition we will discuss sensitivity and representativeness of specific terrestrial end points. We will focus on species diversity needed for the evaluation of long term terrestrial plant toxicity, the importance of soil microbial toxicity assessment and selection criteria for long term tests on soil dwelling invertebrates in more details. Based on the data provided, it was possible to identify the most critical areas where further information could improve the scientifically-justified and consistent assessment of terrestrial risks. Consequently, the importance for more extensive information on terrestrial toxicity to support decision making processes will be discussed.

#### **100 A systematic comparison of IMPACT World+ with other existing life cycle impact assessment methodologies**

C. Bulle, CIRAIQ Polytechnique Montreal / Chemical Engineering; G. Bourgault, CIRAIQ; M. Margni, Ecole Polytechnique de Montreal / Department of Mathematical and Industrial Engineering; P. Lesage, CIRAIQ / CIRAIQ; O. Jolliet, University of Michigan / School of Public Health. This work aims at systematically compare the newly developed IMPACT World+ life cycle impact assessment (LCIA) methodology with ReCiPe and IMPACT 2002+ methodologies by characterizing the 4000 accumulated datasets of the ecoinvent 2.2 database. As comparing CF between methods does not give a sense of how the differences play out in actual LCA applications, this research project proposes to develop a systematic evaluation approach allowing a pairwise comparison of characterized inventories by two different LCIA methodologies. The methodology is applied to compare IMPACT World+ to established methods. Each ecoinvent v2.2 cradle-to-gate inventory (or accumulated dataset) is characterised for one production unit at mid-point and at end-point levels using the following LCIA methodologies: a) IMPACT World+, the global default version, in which CFs are weighted averages of the regionalised CFs using emissions proxy as weighting factors; b) IMPACT World+, 6 continental versions, in which characterization factors are weighted averages of the finest resolution scale regionalised CFs within each continent using emissions proxys as weighting factors;

c) ReCiPe and d) IMPACT 2002+ A contribution analysis by midpoint impact category to the respective damage categories human health and ecosystem quality has been performed in order to identify if recurring patterns are observed across the entire database and if they depend on the LCIA methodology. This “category fingerprints” were used to determine systematic differences of behaviour between methodologies. The modeling choices and the main contributing elementary flows underlying those differences were identified and analyzed to establish our level of confidence in those influent modeling choices and to determine if those differences are to be considered as the consequence of an improvement in the assessment or if they are reflecting model weaknesses which still has to be addressed or errors that have to be corrected. The present study allowed a better understanding of the behaviour of IMPACT World+ methodology, but also of the two other LCIA methodologies which have been compared, ReCiPe and IMPACT 2002+. An indepth understanding of the most influent underlying modelling assumptions was allowed by this systematic comparison, putting in light both the improvements brought by IMPACT World+ and its remaining weaknesses in order to better address them.

#### **101 Improving the spatial scale of impact assessment: analyzing and applying the regionalized LC-IMPACT methods**

C. Mutel, S. Hellweg, ETH Zurich / Institute of Environmental Engineering. Regionalized life cycle impact assessment has recently been an productive area of research, and the LC-IMPACT project will produce a number of regionalized characterization factor (CF) maps. However, serious questions about regionalized life cycle assessment (LCA) are still unanswered, such as the best way to choose the spatial scale of regionalized CF maps and the use of raster data in LCA calculations. Regionalized method developers use the most detailed input data available, but this does not mean that the published maps of CFs should be on this highly detailed scale. Rasters with high levels of detail present computational challenges (hundreds of thousands of individual cell and large file sizes); simplified CF maps are easier to use in LCA calculations, easier to interpret, and provide important information to inventory developers about the needed spatial scale of inventory data. The choice of spatial scale should be based on the maximization of some objective criteria. The minimization of spatial autocorrelation is one such approach. We examine regionalized characterization maps from the LC-IMPACT project for noise, emission of metals, forestry, freshwater eutrophication, acidification, and impact on wetlands from freshwater consumption. As a first step, we examine and discuss the similarities and differences in spatial pattern and range for the various methods. We improve the spatial autocorrelation algorithm by the inclusion of spatial relationships in the discretization process, and apply this approach to calculate a best spatial scale for each regionalized method. We discuss the different approaches to discretization, and quantify their effect on outputs from the minimization of spatial autocorrelation algorithm. The choice of spatial scale is important in understanding, interpreting, and applying regionalized impact assessment methods correctly and with minimal uncertainty. The analysis and comparison of multiple regionalized CF maps from the LC-IMPACT project shows the applicability of the spatial autocorrelation algorithm. In some cases, however, the spatial autocorrelation algorithm cannot help when the input data is already highly aggregated or pixelated. In this case, interpolation may help in building smoother CF surfaces. Analysis and comparison of multiple regionalized methods also increases our understanding of regionalized LCA.

#### **102 Towards specific archetypes for the impact assessment of chemicals**

B. Ciuffo, European Commission Joint Research Centre / Institute for Environment and Sustainability; S. Sala, Joint Research Centre European Commission / Sustainability Assessment Unit - Institute of Environment and Sustainability. Emission of chemicals is increasing over years and the related impacts are greatly influenced by spatial differentiation. Chemicals are usually emitted locally but, due to their physical-chemical properties and persistence, may exert both local and global impact. Besides, variability of environmental parameters of the emission compartment may affect the fate and the exposure up to

orders of magnitude of difference. Several spatially distributed fate and transport models of chemicals, have been therefore developed at various resolutions. These models allow assessing the distribution and fate of chemicals in the environment after their emissions, on the basis of chemical (viz. physical chemical) and landscape related properties. Unfortunately, most of the models adopted so far are mainly simplistic box models in which the concept of spatial differentiation is based on the scale/resolution. This approach, however, can reduce the uncertainty of the assessment to the extent in which the “box” is really representative of the removal/transport processes. Since this is not necessarily the case, in the present work the authors try to propose a novel approach in which emission archetypes are defined on the basis of the quantitative results achieved by means of a global sensitivity analysis of a complex spatially resolved model. In particular, global sensitivity analysis techniques have been applied to the MAPPE with two aims: to assess the variability in removal rates, focusing on the relative influence of substance properties and of environmental characteristics; and to support the development of chemical specific and compartment specific archetypes. Results strongly call for a “scenario-oriented” approach in the archetype definition. In particular given the underlying variability in the behaviour of chemicals, archetypes should be: (i) compartment specific; (ii) chemical specific; and (iii) target specific. In particular, from the results of the sensitivity analysis the authors attempted defining climate-based archetypes, testing their suitability with respect to the more classical geographical approaches (e.g. continents or countries). Results confirm their expectations, even though, the necessity for further work is also acknowledged.

### **103 Considering local variability in lignocellulosic feedstock for 2nd generation biofuel LCA thanks to agro-ecosystem modellingK.**

**Dufosse**, INRA / Environnement and Arable Crops; **B. Gabrielle**, AgroParisTech; **J. Drouet**, INRA. **Context** Agriculture represents 10 to 15% of GHG emissions in France and 65% of N O emissions (GWP = 298). Owing to these facts and new regulations (RED) it is essential to carefully develop, evaluate and utilise methods to assess the environmental balance of lignocellulosic crops used as 2<sup>nd</sup> generation (G2) biofuel feedstock. However, the literature has pointed out the lack of common methodology for G2 biofuel LCA, which could counterbalance the general results of net reduction in GHG emissions. They especially put stress on the lack of consideration for land use change and use of the generic factors used to calculate greenhouse gases (GHG) emissions, strongly bound with local pedo-climatic conditions and technological options (especially N application rates). A multi-scale approach was required to fulfill a complete LCA. This approach was used to estimate direct N O emissions, downstream indirect N O emissions and emissions<sup>2</sup> due to land use change. **Materials and methods** Firstly, the agro-ecosystem model CERES-EGC was developed and used to simulate crop growth and related C and N cycles. Simulations were carried out at plot (i.e. a few hectares) and regional scales (i.e. hundreds of hectares) for a wide range of crops including G2 lignocellulosic feedstock. In future work, it will be combined with prospective scenarios of land use change towards lignocellulosic crops at regional scale. Secondly, downstream indirect emissions will be simulated from the NitroScape model, which accounts for hydrological and atmospheric transfers of reactive nitrogen between landscape elements (e.g. plots, farm buildings). This model typically works at a scale of a few (20-30) square kilometers. **Results and discussion** First results on an example region (Picardy, France) showed ranges of yields and direct N O emissions for *Miscanthus* (14.3 to 20.5 t DM ha<sup>-1</sup> yr<sup>-1</sup> and 0.18 – 0.38 kg N O-N ha<sup>-1</sup> yr<sup>-1</sup>) depending on the selected crop management strategies and local pedoclimatic conditions. Future work will present LCA simulated at farm gate for a mixture of feedstock and integrated at regional scales. **Conclusions** This multi-scale method helps at integrating local variability in estimates of GHG emission, an important step in biofuel LCA. Moreover, it will be applied in the French project FUTUROL to assess the sustainability of a proposed bio-ethanol production and supplied by a mixture of feedstock (annual and perennial crops, especially *Miscanthus*, and crop residues).

**104 Opening Pandora's box: Uncertainty propagation in life cycle impact assessment** **P. Lesage**, CIRAIG / CIRAIG; **G. Bourgault**, R. Samson, Ecole Polytechnique de Montreal / CIRAIG; **M. Margni**, Ecole Polytechnique de Montreal / Department of Mathematical and Industrial Engineering. Uncertainty calculation is receiving more and more attention in Life Cycle Impact Assessment (LCIA). For the first time, statistical distributions are being reported for characterisation factors (CF). This uncertainty is based on statistical distribution on the input parameters, which is then propagated through the models. Due to strong non-linear equations in the impact models, unexpected phenomenon occur that have not been reported so far in the LCA field. The case of a the model used to estimate direct human health impact of water consumption in IMPACT World+ is presented. One parameter in the model,  $y$ , is expressed as a function of  $x$ , with  $f(x)$  being a sigmoidal (s-shaped) function, and the input parameter  $x$  being lognormally distributed. This type of model is common in LCIA. The quantitative example shows that the probability distribution function (PDF) of the output parameter  $y$  is a bimodal that could be approximated with a beta distribution, far from the lognormal distribution of input parameter  $x$ . More importantly, if a deterministic value for  $y$  had been calculated using the arithmetic mean of  $x$ , it would have been far from the observed mean of the function  $f(x)$ . In fact, in the case of non-monotonic functions, it is even possible that the deterministic result of the model do not fall within the 95% confidence interval calculated by the Monte Carlo. This raises many important questions for LCA as a whole. Should we avoid calculating impact scores with deterministic values? Are the current tools for uncertainty assessment in LCA appropriate for this kind of phenomenon? The purpose of this intervention is to raise awareness about the complexity of the issues at hand and to urge people working in LCA to consult with mathematicians or statisticians to gain better insights about the implication of uncertainty in input parameters of strongly non-linear systems.

**105 The first steps towards simplifying the Northern Zone groundwater requirements** **A. Gimsing**, The Danish Environmental Protection Agency / Pesticides and Gentechology; **F. Stenemo**, Geosigma AB; **J. Asp**, G. Czub, J. Ostgren, Swedish Chemicals Agency; **U. Bukss**, State Plant Protection Service; **R. Holten**, P. Mulder, The Norwegian Food Safety Authority; **D. Kavaliauskaite**, The State Plant Service under the Ministry of Agriculture; **R. Silvo**, A. Sari, Finnish Safety and Chemicals Agency; **J. Raukas**, Estonian Agricultural Board. Regulation EC 1107/2009 concerning the placing of plant protection products on the market in the EU entered into force on 14 June 2011. A central aspect in the new regulation is worksharing and harmonization within and among the three European zones. In the Northern Zone there are different national requirements for assessing the risk of leaching of active substances and metabolites to groundwater. No harmonisation on this point has yet been achieved and the Member States are reluctant to accept assessments on the basis of the other Member States requirements. For the core assessment simulations with the following FOCUS models and scenarios are currently required, as described in the Northern zone guidance document: - PEARL with the Jokioinen scenario (Finnish and Latvian approach) - PELMO Hamburg or MACRO Danish scenarios Karup and Langvad with Danish input parameters - MACRO with Norwegian scenarios Rustad og Heia - MACRO with Swedish scenarios Önnestad, Krusenberga and Näsbygård \nDue to strict deadlines in the regulation EC 1107/2009, it is imperative to minimize the work load for both companies and regulators. A first step towards harmonisation has been to compare the different models and approaches to assess how much the model predictions differ from each other and how they are influenced by external preconditions. A project has been carried out with the purpose of running the different models with hypothetical substances (different combinations of half-lives and sorption properties) and comparing the model predictions with the aim of finding a worst-case ranking order of the models to allow for a stepwise approach to groundwater modelling. The results of this project will be presented and the conclusions and perspectives for the Northern Zone guidance will be discussed. Session: Modelling of chemical fate and exposure in the context of pesticide and biocide

regulations Keywords: Groundwater modelling, pesticides, Northern zone, authorisation Presentation preference: Platform and poster

**106 Modelling in Support of an Extended Groundwater Monitoring Study in the EU** P. Sweeney, Syngenta; G. Hoogeweg, S. Zelonis, Waterborne Environmental; P. Hendley, Syngenta Crop Protection Inc; S. Hayes, Syngenta. The groundwater assessment for agrochemicals in the EU has become increasingly hard to pass due to changing parameterisation of models. Registrants have increasingly resorted to monitoring studies to provide higher-tier support for groundwater assessments in addition to modelling. The data available to identify candidate sites for monitoring in the EU are of variable quality and this limits the ability of registrants to identify credible areas to monitor in locations where there is no existing network of monitoring wells. We show how modeling can be used with the data available at a consistent resolution for the EU 27 with reference to an example of a weakly sorbed metabolite of a maize herbicide. Modelling is used from the initial stages of defining a conceptual model of leaching - identifying likely travel times to groundwater and identifying environmental parameters controlling movement of the substance to groundwater - to providing estimates of leaching using a spatial model. Mass flux is the most relevant quantity to estimate and compare likely leaching of particular soil/weather combinations and we propose that a 10km x 10km grid cell is a relevant spatial unit to consider leaching, given the spatial uncertainty inherent in pan-European datasets such as CAPRI. Calculated median mass flux at a European level is combined with estimates of shallow groundwater (defined as < 10m) and cropping density to provide candidate regions that can be sampled at random as part of a statistically robust monitoring program relevant to leaching across the EU. Comparison with field data show the effectiveness of this approach in identifying high density maize-growing regions overlying shallow groundwater. Finally we show how the sites selected for monitoring can be placed in context of vulnerability for the EU.

**107 Characterizing pesticide dissipation in food crops** P. Fantke, Technical University of Denmark; R. Juraske, ETH Zurich; O. Jolliet, University of Michigan / School of Public Health. Ingestion of residues via consumption of food crops is the predominant exposure route of the general population toward pesticides. However, pesticide dissipation in crops constitutes a main source of uncertainty in estimating residues in harvested crop parts and subsequent human exposure. Nevertheless, dissipation is a key mechanism in models assessing pesticide distribution in the crop-environment and the magnitude of residues in harvest. We provide a consistent framework for characterizing pesticide dissipation in food crops for use in modeling approaches applied in health risk and impact assessment. We collected 4,482 unique dissipation half-lives for 341 substances applied to 182 different crop species and fully characterize these data by describing their variance, distribution and uncertainty as well as by identifying the influence of substance, crop and environmental characteristics. We obtain an overall geo-mean half-life over all data points of 3.9 days with 95% of all half-lives falling within the range between 0.6 and 29 days. Uncertainty in predicting a substance-specific geo-mean half-life varies with varying numbers of available data points with the highest uncertainty associated to pesticides with less than seven reported half-lives. Temperature in air was identified to have a significant influence on dissipation kinetics. We, hence, provide estimated half-lives for a default temperature of 20°C, while introducing a correction term for deviating temperature conditions. Diffusive exchange processes also have a significant influence on pesticide dissipation, wherever these processes dominate dissipation rates compared to degradation. In these cases, we recommend not to use measured dissipation half-lives as basis for estimating degradation, which is recommended in cases, where degradation is dominating. We are currently testing the regression to predict degradation half-lives in crops. By providing mean degradation half-lives at 20°C for more than 300 pesticides, we reduce uncertainty and improve assumptions in current practice of health risk and impact assessments.

**108 Characterizing exposure of bystanders and residents to pesticides applied in agricultural fields** M. Ryberg, P. Fantke, Technical University of Denmark; R.K. Rosenbaum, Technical University of Denmark / Management Engineering. Humans are exposed to agricultural pesticides via different pathways. Bystanders and residents living near agricultural fields, in particular, are potentially exposed to pesticides primarily via inhalation. However, bystander/resident exposure has not yet been considered in life cycle impact assessment (LCIA), even though bystander/resident exposure is expected to contribute significantly to overall human exposure to pesticides. Therefore, we aim at quantifying human exposure of bystanders/residents to agricultural pesticides applied under realistic field conditions. We start from a pulse application, of which a certain fraction is subsequently lost to air. We thereby build upon an existing model for quantifying pesticide emissions from field applications. The model will calculate the fraction from wind drift and volatilization leaving the field, based on the quantity of pesticide applied to the field. From the emission, the concentration near the receptor – either bystanders or residents living near the field – will be modelled as a function of the distance to the field. Human exposure will furthermore be depending on the duration of the exposure and the inhalation rate. Hence, the exposure differs between bystanders and residents due to different activity patterns. Based on this, intake fractions and – after combination with respective effect information – characterization factors will be derived. Because the impact only affects a fraction of the total population, the results will be normalized, for the characterization to be used together with other exposure pathways where the total population is included. Bystander and resident exposure is expected to be in the same range as exposure via food consumption and is furthermore expected to be higher than exposure of the general public via exposure to environmental emissions drifting far beyond the treated field. Hence, it is necessary to include this exposure into current LCIA methodologies for pesticides to provide a holistic view of the impacts related to pesticides use.

**109 Comparison between a priori regulatory predicted concentrations and measured ones from monitoring: the French case** A. Dubois, A. Boivin, A. Conrad, V. Poulsen, ANSES. In France, risk assessment of surface water contamination required for pesticide authorization is conducted with European FOCUS (Forum for the Co-ordination of pesticide fate models and their Use) exposure models. Recent publications have raised potential concern about the reliability of the FOCUS predictions compared to the real concentrations from monitoring. In the present communication, based on a dataset of 28 active substances recently assessed (national re-registration process), a comparison of FOCUS step 1 - 4 PEC<sub>sw</sub> to measured concentrations from SOEs (Observation and Statistics Office of the French Ministry for Ecology) monitoring network was performed. It does not show any overall underestimation of the FOCUS *sw* concentrations compared to the measured concentrations. This comparison offers the opportunity to remind that the FOCUS modelling approach is not aimed at describing pesticide transport at specific field or location (i.e. scenarios) after which they are named. For French risk assessment, all the FOCUS *sw* scenarios used together are considered, as covering the national variety of agro-pedo-climatic conditions, in order to identify acceptable and/or unacceptable risks for aquatic organisms, including mitigation measures when appropriate. However, the actual reliability and sensitivity of the FOCUS tools used for regulatory purposes is not straightforward. They have indeed to reflect complex and transient processes (e.g. runoff due to heavy rainfalls). The high temporal variability of the processes involved remains a key issue to be faced for regulatory modelling (e.g. the choice of application date or the time period and resolution to consider for climatic data). At the light of results and Anses experience of regulatory modelling, suggestions for improvement are made. Updating FOCUS surface water in order to produce more accurate PEC<sub>sw</sub> by taking into account several years of repeated applications would be welcome. The new PEC<sub>sw</sub> to be account for risk assessment would have to be defined according to protection goals.

**110 Using modelling and GIS analyses to identify appropriate**

**measures for product stewardship of bentazone** B. Jene, T. Haering, BASF SE. The registration process for plant protection products in the EU according to Regulation 1107/2009 is very stringent and protective. This assures a high level of safety for groundwater regarding the entry of pesticides via leaching after application according to good agricultural practice. Nevertheless, extreme or unusual hydrogeological situations can be relevant at local scale, leading in exceptional cases to exceedances of the drinking water threshold 0.1 µg/L for mobile pesticides such as bentazone. In order to further increase the margin of safety and reduce the number of situations where leaching of bentazone can occur, product stewardship measures have been implemented on the basis of analyses using efate models. The modelling analysis includes local as well as geographical aspects. The sensitivity of the leaching of bentazone to factors such as application time and rate in combination with the type of crop and the respective growth stage was tested under relevant pedo-climatic boundary conditions. The sensitivity of bentazone leaching as a function of site specific parameters such as soil organic carbon content, which determines the actual sorption, and the soil hydrology was also tested. Furthermore, the soil profile depth, the travel distance through the unsaturated zone, was analysed with respect to its effect on the leaching of bentazone. The most important influencing factors on the leaching of bentazone were the application timing, organic carbon content and the depth of the unsaturated soil profile. It was found that the total application rate has an overproportional effect on the predicted leaching of bentazone. The sensitivity analysis therefore resulted in a maximum application rate of 1.0 kg active substance per ha according to product stewardship. As a further important product stewardship measure no autumn or winter applications of bentazone containing products are supported. Furthermore bentazone should not be applied in soils with an organic carbon content below 1.0%. Finally it was specified that bentazone containing products should not be applied to areas with shallow groundwater at less than 1 m depth or shallow soils above karst groundwater aquifers, where water can easily flow through cracks fissures or caves and rapidly reach the groundwater. Spatial analysis using geographical data was carried out to identify areas with shallow soils and karst geology or areas where shallow groundwater can be expected.

**111 Can Life Cycle Assessment contribute to strengthen Responsible Research and Innovation?** P. Masoni, ENEA / Protezione e Sviluppo dell'Ambiente e del Territorio. Responsible research and innovation (RRI) is a novel approach to develop a legitimate, inclusive, and transparent decision-making process, taking into account wider social, ethical and environmental issues and assessing potential impact and potential for unintended consequences of innovative products and technologies. Life Cycle Assessment can provide relevant contributions to RRI. Life cycle approach is generally considered the best way to assess potential impacts avoiding possible problem shifting. Research in LCA is addressing the question of how better to assess potential impacts in a perspective way with: dynamic LCA, scenario analysis, spatially differentiated LCA, risk based LCA, environmental input-output based LCA (EIO-LCA) and hybrid LCA. Moreover, LCA is broadening the scope of the analysis to include not only environmental but also economic and social aspects (life cycle costing and social life cycle assessment), and broadening the object of the analysis from product systems to technologies, industrial sectors up to the whole economy. Recently, a novel framework for the life cycle sustainability analysis (LCSA) has been proposed, linking life cycle sustainability questions to knowledge needed for addressing them, identifying available knowledge and related models, knowledge gaps and defining research programmes to fill these gaps. In addition, LCSA deepens current LCA to also include other than just technological relations, e.g. physical relations (including limitations in available resources and land), economic and behavioural relations, etc. Moreover, as part of deepening, normative aspects can be explicitly incorporated. Concisely, LCSA is a trans-disciplinary integration framework of models: it integrates empirical facts, scientific knowledge and normative positions, values and rules, in a coherent way. LCSA could therefore

contribute to strengthen RRI.

**112 Good Science, Bad Researchers and Ugly Politics: Reflecting on Ethics in an Ecotoxicology Laboratory** F. Wickson, . In aiming to develop “responsible research and innovation”, the Research Council of Norway is currently pursuing a model in which scholars from the social sciences and/or humanities are “integrated” into scientific or technological research projects. This presentation will reflect on my own experience working as an integrated scholar embedded in ecotoxicology laboratories. Ecotoxicology plays a significant role in shaping our understandings of environmental harm and influences how new and emerging technologies are understood, managed, and governed within the industries that are developing them, the policy circles that are regulating them, and the societies that are using them. Ecotoxicology research is therefore a highly relevant site for an integration that seeks to further responsible innovation. The ecotoxicology laboratories that I have been involved with are studying the controversial fields of biotechnology and nanotechnology and in this presentation I will discuss some of the ethical issues that have emerged during our collaboration. These include: 1) *Good Science*: the role of international standards in defining quality in science for policy; 2) *Bad Researchers*: factors that currently inhibit honest practice; and 3) *Ugly Politics*: how the interface between science and politics is generating ethical dilemmas. In concluding the presentation I will open for a discussion about the extent to which this type of socio-technical integration can in fact facilitate the development of responsible innovation.

**113** J. van den Hoven, TU Delft - Centre for Ethics and Technology.

**114 Plenary panel discussion** R. Owen, .

**115 Questions** R. Owen, .

**116 Development of a plant toxicity test using species native to the Canadian boreal wetlands** M.J. Moody, Saskatchewan Research Council / Environment Division; R.P. Scroggins, Environment Canada / Biological Methods. Development of a plant toxicity test specific to wetland habitats of the boreal eco-zone is a priority recognized by governments and industry in Canada. Hundreds of accidental spills of crude and produced water from oil and gas production pipelines into boreal wetlands across Western Canada happen each year. Relevant testing tools are needed to quantify biological impacts from pipeline spills or poor waste treatment practices which lead to releases of high levels of salts and hydrocarbons into wetland environments. Candidate test species native to three wetland habitat types (bogs, fens and shallow water marshes) were chosen to offer an ecologically relevant test battery. Achieving consistent germination and growth in preliminary tests led to adoption of suitable test durations and measurements of growth in substrates and waters collected from clean reference sites. As a first method validation step, the sensitivity of the candidate plant species exposed to produced water brine was measured. The next step was to test samples from wetland locations impacted by brine salts and/or petroleum hydrocarbons from pipeline spills or former land treatment sites. The goal of the research and validation efforts is development of a plant testing method using ecologically relevant species that are capable of quantifying the impacts on vulnerable wetland habitats.

**117 Effects of salinity and freeze temperatures in *Enchytraeus albidus* – increased freeze tolerance and changing impact on toxicity** A.P. Silva, University / Biology; M. Holmstrup, Aarhus University / Department of Bioscience; M.J. Amorim, Universidade de Aveiro / Department of Biology and CESAM. *Enchytraeus albidus* is a freeze-tolerant enchytraeid found in diverse habitats, ranging from supralittoral to terrestrial ecosystems and spanning temperate and arctic regions. Therefore, this species is not only frequently exposed to salinity and temperature fluctuations as well as pollutants. Freeze-tolerance of *E. albidus* is known but the effect of other natural stressors and/or



chemicals in this winter-survival strategy is still poorly understood. We studied the effect of salinity and/or nonylphenol (as an example of an organic pollutant) on the freeze-tolerance of *E. albidus*, when exposed to different temperature regimes. Several endpoints were addressed, including survival, reproduction, physiological and biochemical parameters. Pre-acclimation to even modest salinities of soil water improved worms' survival to freezing at low temperature considerably, mainly due to increase in osmolality, decrease in melting point and water content, depletion of glycogen reserves and accumulation of glucose. These physiological and biochemical readjustments led to a lower internal ice fraction during freezing and membrane adjustments, that were crucial to guarantee the survival of worms in extreme temperatures. The presence of Nonylphenol affected negatively the survival of *E. albidus*, particularly under combined effect with constant frost temperatures or daily temperature fluctuations (freeze-thaw cycles), the latter being the worst. Glycogen was significantly depleted in worms exposed to higher concentrations of nonylphenol in the soil, with more prominence in worms exposed to the combined effect with constant frost temperatures. Lower glucose levels in worms exposed to the combined effect of nonylphenol and daily freeze-thaw cycles were probably due to its use as energy source during thawing processes. Higher glucose levels in worms exposed to the combined effect of nonylphenol and constant frost-temperatures may act as cryoprotectant, which can also explain their better overall survival compared with worms exposed to nonylphenol combined with freeze-thaw cycles. The present results are highly relevant, not only in terms of physiology of invertebrates but also in the ecotoxicology field, where the level of information outside temperate regions and controlled conditions is virtually absent.

**118 Sensitivity of non-temperate versus standard-test species to pesticides: a case-study with carbofuran and Collembola in Brazil** S. Chelinho, IMAR CMA / Department of Zoology; X. Domene, CREAM / Universitat Autònoma de Barcelona; T. Natal da Luz, University of Coimbra / Department of Life Sciences, University of Coimbra; P. Andres, CREAM - Centre de Recerca Ecològica i Aplicacions Forestals / Universitat Autònoma de Barcelona; I. Lopes, University of Aveiro / CESAM; E.L. Espindola, Universidade de São Paulo - USP / Escola de Engenharia de São Carlos - EESC; R. Ribeiro, Universidade de Coimbra / IMAR-CMA, Dept. of Life Sciences; P. Sousa, IMAR-CMA / Department of Life Sciences, University of Coimbra. In the present contribution, the effects of carbofuran applications on a tropical collembolan community and also on the standard collembolan *Folsomia candida* were assessed. The main goal was to compare the sensitivity of both collembolan community and *F. candida* towards this insecticide. The endpoints evaluated were changes in richness and abundance of Collembola families and mortality and reproduction of *F. candida*. After field contamination of an agricultural soil from Brazil, a gradient of carbofuran dilutions was prepared. Soil cores were taken from the respective uncontaminated surrounding areas and the Collembola community of three cores was extracted directly to the test soil. After extracting the collembolans to the test soil, these were incubated under laboratory conditions for 4 weeks, after which the mesofauna was extracted again. The organisms were assorted into 5 families and also classified in different morphotypes, according to five morphological traits related their life-form (namely: ocelli, furca, antenna, pigmentation and the presence of hairs and scales). In parallel, the treated soil samples were also used to conduct a standardized reproduction test with *F. candida*. Results showed that the community of collembolans was negatively affected by soil contamination and a dose-response pattern was observed along the contamination gradient, with a lower abundance and taxonomic diversity relatively to the control. With respect *F. candida*, very low concentrations of carbofuran (0.460 mg/kg) caused the concurrent impairment of both survival and reproduction of *F. candida*. For this particular soil community, the derived toxicity parameters suggest that the intrinsic sensitivity of local Collembola community (EC50 for decrease in abundance = 0.061 mg/kg) to carbofuran was rather similar to the one found for *Folsomia candida* in the same test-soil (LC50 for survival = 0.057 mg/kg). Furthermore,

regarding the trait-classification of collembolans, the effects of carbofuran on the abundance of local morphospecies (within the family Isotomidae) possessing the same combination of traits scores as *F. candida*, and the survival of latter species is also alike. As this was only a case-study, further studies, including other pesticide classes, groups of organisms and climate regions are urgent to confirm or not this similarity as well as to enlarge the database on pesticide toxicity under non-temperate conditions.

**119 Applying laboratory and in situ ecotoxicological and ecological tools in ERA in the tropics: the experience on a metal contaminated site in Brazil** J.C. Niemeyer, Rua Prof Sabino Silva / Department of Life Sciences; M. Moreira dos Santos, IMAR-CMA Instituto do Mar / IMAR-CMA, Dept. of Life Sciences; R. Ribeiro, Universidade de Coimbra / IMAR-CMA, Dept. of Life Sciences; E.M. da Silva, Federal University of Bahia / Institute of Biology, Department of Botany; J. Sousa, University of Coimbra / IMAR-CMA, Dept. of Life Sciences; S. Chelinho, University of Coimbra. This study was carried out in a metal contaminated area in Brazil, with the major goal to further contribute to the application of a tiered ecological risk assessment (ERA) framework to tropical environments, evaluating the feasibility and usefulness of different assessment tools to be used in different tiers within a Triad approach. This presentation is focused on the ecotoxicological and ecological tools in complementing the chemical characterization of the site. Avoidance behavior tests with *Eisenia andrei* and *Folsomia candida* were included in the Ecotoxicological LoE of tier 1, while soil basal respiration, *in situ* bait lamina test and evaluation of vegetation cover were included in the Ecological LoE of this tier. At tier 2, the Ecotoxicological LoE integrated the results on growth and biomass of plants, *Avena sativa* and *Brassica rapa*; the reproduction of the oligochaete species *Eisenia andrei* and *Enchytraeus crypticus*, and of the collembolan *Folsomia candida*. In the Ecological LoE of tier 2, plant litter decomposition (litter bag test), ecological surveys on plant community and surface dwelling invertebrates (using pitfall traps) and conventional microbial parameters were carried out. A sensitivity analysis was conducted taking into account not only the ability of each parameter to detect differences between contaminated and non-contaminated points (outside the area), but also their ability to detect a gradient of contamination, and the time necessary to obtain the parameter. The ability of the avoidance tests to detect toxicity within a short test period and at low costs makes them suitable for use in decision processes. In general, the risk values pointed by them in tier 1 were confirmed in tier 2 with the reproduction tests. Oligochaete species were the most sensitive. The high sensitivity of feeding activity of soil fauna and its practicability make the bait-lamina test a definitive parameter to be included in the ecological LoE of tier 1. Litter bags showed a high sensitivity to contamination and derived habitat disruption, but presented a low capacity to differentiate the level of contamination. Soil basal respiration and microbial biomass carbon were the most promising microbial parameters in distinguish the level of soil contamination. Soil fauna structural parameters were not able to detect contamination gradients, but they were able to differentiate the sites inside and outside the smelter area.

**120 Development and Application of Toxicity Tests for Plants and Soil Invertebrates of Canada's Subarctic Taiga Plains Eco Zone** G. Leighton-Boyce, WorleyParsons / Infrastructure and Environment; M.J. Moody, Saskatchewan Research Council / Environment Division; J. Battigelli, Stantec; C. Fraser, Environment Canada / Ecotoxicology and Wildlife Health Division; J. Princz, Environment Canada; R.P. Scroggins, Environment Canada / Biological Methods. In Canada, generic, Canada-wide soil quality guidelines protective of plants and invertebrates (the soil eco-contact pathway) are available for use in the assessment and remediation of contaminated sites. These guidelines were generally developed using toxicity testing on agronomic plant species and earthworms. Guidelines for petroleum hydrocarbons (PHCs) were typically developed using fresh crude. The eco-contact pathway is commonly included for soils from surface to 3 m depth. In sub-arctic regions with intermittent permafrost, endemic plant and invertebrate



species may have a different sensitivity to contaminants than agronomic species used in the generic guidelines. Distribution of soil invertebrates within the soil profile and the rooting depth of plants in sub-arctic regions may also differ from the distribution within temperate regions. Also, at some sites with historic impacts, or sites where soils have been bioremediated, the objective may be the management of weathered or aged as opposed to fresh PHC impacts to soils. A collaborative research project between industry and government was initiated in 2009 with the overall objective of developing site-specific remediation guidelines for a range of contaminants for the eco-contact pathway that are protective of native plants and invertebrates found at sites located in the Canadian sub-arctic. For plants, method development has progressed through initial species selection from published surveys, germination, growth and test duration trials, assessment of the need for fertilizers, and an evaluation of the applicability of using the standard Environment Canada test climate or a northern climate. For the invertebrate tests, a literature search found very few surveys of soil mesofauna in Canada's north. A field investigation of the distribution, density and diversity of soil invertebrates, and of plant rooting depths, through the soil profile from surface to either permafrost or 3 m was completed. Bulk soil samples were also collected from the study area for extraction and culturing of soil invertebrates, with a goal of establishing laboratory cultures to be used in future toxicity tests. Results of the plant method development and of the field invertebrate survey and culturing will be presented and implications for the depth to which the eco-contact pathway may be applicable in sub-arctic regions will be discussed.

**121 Assessing microbial toxicity of heavy metals using flow cytometry** S. Kim, Konkuk University; Y. An, Konkuk University / Department of Environmental Sciences. The effects of heavy metals on microbial growth were investigated on liquid media, and the colony forming unit (CFU) assay and colony area evaluation were performed on agar media. The *Escherichia coli* and *Bacillus subtilis* were chosen as a test species. In order to assess the live cell ability and size distribution, we used the Fluorescence Activated Cell sorting (FACs) and calcein acetoxymethyl ester (CAM) as a fluorescent dye of live cell. On agar media, the colony area evaluation shows higher sensitivity than CFU assay. Also, Cu and Ni induced the early developmental inhibition of *E. coli* and *B. subtilis* on liquid media. On flow cytometer analysis, live cell ability, side and forward scattering of cells were measured. The live cell abilities of exposure groups were decreased, and the cell size and granularity show lower than control group. This phenomenon can be related with small colony formation and growth inhibition. Our results suggested that effects of heavy metal may be assay-dependent, because heavy metal can change the cell morphology characterization. *This subject is supported by Korea Ministry of Environment as the GAIA project (2012000540011).*

**122 Unexpected dose response of repeated annual veterinary drug applications on microbial diversity and functions in an agricultural soil** E. Topp, Agriculture and Agri-Food Canada; A. Scott, L. Sabourin, Agriculture and Agri-Food Canada; P. Grenni, National Research Council CNR / Water Research Institute; A. Barra Caracciolo, National Research Council / Water Research Institute; R. Marti, Agriculture and Agri-Food Canada. Use of antibiotics as growth promoting agents in livestock production contributes to the increasingly worrisome development of antibiotic resistance. In order to evaluate the long term impacts of antibiotic exposure on soil microbial populations, a series of field plots were established in 1999 that have since received annual applications of a mixture of sulfamethazine, tylosin and chlortetracycline at concentrations (0, 0.1, 1.0 and 10 mg/kg soil) bracketing that which would result from an annual application of manure from medicated swine. Soils were evaluated for drug persistence, herbicide and crop residue decomposition, distribution of major bacterial groups by FISH analysis, abundance of *Sul1* by qPCR, and tolerance to the antibiotics by PICT. Sulfamethazine and tylosin were biodegraded significantly more rapidly in the soils historically exposed to 10 mg/kg drugs than in control soils. Most endpoints were significantly affected by historical drug exposure at the lower and

intermediate drug exposure concentrations, compared to untreated and highest level of exposure. We speculate that the evolution of a biodegradative population enriched at the highest exposure concentration results in reduced exposure of the overall soil population to the drugs.

**123 Fate and transport of chlortetracycline and resistance genes in the environment after land application of swine manure** R. Joy, Civil Engineering Dept / Civil Engineering Dept.; S. Bartelt-Hunt, X. Li, University of Nebraska-Lincoln; D. Snow, Water Science Laboratory; J. Gilley, USDA-ARS; D. Marx, University of Nebraska-Lincoln / Statistics Department. The Centre for Disease Control and Prevention says antimicrobial resistance (AMR) is one of the world's most pressing public health issues. One source of antimicrobials to the environment is animal agriculture. Antimicrobials are used for growth promotion, prophylaxis, and for disease treatment within concentrated animal feed operations (CAFOs). Antimicrobials allow resistant bacteria to proliferate in manure applied as fertilizer, and therefore antimicrobials and AMR genes can contaminate surface and ground water. This is studied through monitoring the quantities of antimicrobials and AMR genes in fresh and aged swine wastes. The fate and transport of antimicrobials, AMR genes in soil and surface runoff is quantified after land applications of surface applied and injected swine manure. Rainfall simulations were conducted after manure containing CTC was applied by broadcast, incorporated, or injected, and runoff samples were collected. Samples were analysed for CTC in liquid and solid phases through liquid chromatography tandem mass spectrometry or high pressure liquid chromatograph, respectively. Published qPCR protocols were followed to determine a concentration of AMR genes/ml of runoff for *tetQ*, *tetX*, and *16s*. Both CTC and AMR genes decreased in runoff throughout the three day experiment. Our results showed despite of some difference in the runoff profiles, the AMR gene runoff profiles were similar for the three application methods. Because the experiment only lasted three days, it is likely that the indigenous soil microbes had not developed substantial levels of resistance and the resistant bacteria in the runoffs were largely from the original manure. The concentrations of antimicrobials in the runoff indicate that manure injection may reduce transport of antimicrobials compared with broadcast and incorporation. The transport of antimicrobials is also affected by the timing of rainfall, as lower antimicrobial concentrations were observed in runoff after the third runoff event.

**124 Multiple factors govern the effects of pharmaceutical antibiotics in structured field soil** R. Reichel; S. Thiele-Bruhn, Soil Science, University of Trier. When pharmaceutical antibiotics reach soils via contaminated excreta, adverse effects on biota are expected. Numerous studies showed that soil microbial biomass, functions and structural diversity are affected after spiking soils with antibiotics. Effects on soil biota are routinely determined by standardized laboratory tests using homogenized soil, artificially spiked with the antibiotic compound in the presence of a nutrient substrate. In practice, manure exhibits varying molecular and microbial composition. Consequently, manure from medicated livestock represents a mixture of nutrient substrates, microbial inoculum and antibiotic chemicals including metabolites. This mixture is added to structured field soil that is characterized by heterogeneous microcompartments such as macroaggregates, earthworm channels and the rhizosphere. The consequences of such mixed influencing factors on the effects of antibiotic in microcompartments of structured soil are still poorly investigated. These aspects were evaluated by determining microbial measures, e.g. PLFA and DGGE patterns or enzyme activities, using topsoil from a Luvisol in several experiments from laboratory to field scale. Sulfadiazine and difloxacin were added to soil as test compounds ranging from artificial spiking to soil amendment with slurry from medicated pigs. Results showed that antibiotics already altered the molecular and microbial composition of the slurry, which blurred a precise differentiation between responses to slurry composition or to the excreted antibiotic compounds. Manure borne microorganisms were shown to survive for weeks in soil with likely consequences for the indigenous soil microflora. Moreover,

antibiotic-related effects in soil microcompartments commonly were different compared to the corresponding bulk soil. Hence, the evaluation of veterinary antibiotics' effects in soil has also to consider these mixed effects on manure and soil when assessing the environmental relevance of antibiotics.

**125 Effects of soil properties on the uptake of pharmaceuticals into earthworms** L. Carter, University of York / Environment; J.J. Ryan, GlaxoSmithKline; A. Boxall, University of York / Environment Department. Pharmaceuticals are being increasingly detected in soils. This is primarily due to the land application of sewage sludge and reclaimed wastewater containing high levels of pharmaceuticals which are then transferred to soil. Once in soil, there is the potential for pharmaceuticals to be taken up by soil dwelling organisms however relatively little is known regarding terrestrial invertebrates. This study was therefore performed to better understand pharmaceutical uptake into earthworms (*Eisenia fetida*) and to evaluate effects of soil properties on the uptake of pharmaceuticals. Earthworms were exposed to soils spiked with either  $^{14}\text{C}$  labelled fluoxetine, carbamazepine or orlistat (studies with an additional drug, diclofenac, are in progress) for 21 days (uptake phase) followed by a 21 day depuration phase. Soil and pore water was also sampled during the uptake period. Samples were then extracted and analysed by liquid scintillation counting. Internal worm, soil and pore water pH measurements were made to observe any pH changes that occurred during the exposure. All pharmaceuticals were taken up by *E. fetida*. Fluoxetine and carbamazepine were completely eliminated from the worm in the depuration phase whilst traces of orlistat remained. Slight pH changes were observed in the pore water and soil in the orlistat study and for fluoxetine the internal pH of the worm was higher during the uptake phase then decreased to pH values comparable to control in the depuration phase. Between the five different soil types Bioconcentration factors (BCFs) were very similar for carbamazepine whereas large differences in BCFs were observed (30.3-114.9) for orlistat. For fluoxetine BCFs ranged between 16 and 21.5 in the different soils. As fluoxetine is a basic drug you would expect an increase in BCFs as the pH increases as there would be more nonionised species with higher hydrophobicity than ionised species at pH values closer to the pKa. However this relationship is not shown; suggesting soil properties other than pH can influence uptake into worms. This research shows earthworms can accumulate pharmaceuticals if they are present in soils at environmentally relevant concentrations and that for some drugs soil properties can influence the degree of uptake into the worm and hence BCFs. Soil, pore water and earthworm data will be combined; with the ultimate aim to develop models to improve the assessment of the risks posed by pharmaceuticals in the terrestrial environment.

**126 Veterinary Antibiotics in Terrestrial Plant Tests – Effects of a more realistic exposure way via manure** M. Simon, Fraunhofer IME; M. Herrchen, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME); B. Foerster, ECT Oekotoxikologie GmbH (ECT); N. Graf, J. Roembke, ECT Oekotoxikologie GmbH; U. Kuehnen, I. Ebert, Federal Environment Agency. In the frame of a research project initiated and funded by the German Federal Environmental Agency (UBA), a special terrestrial plant test for veterinary pharmaceuticals – especially antibiotics - with a more realistic exposure scenario via manure application was developed. The research project comprehends: i) development of methods of preparation, acclimatization, incubation, and application of manure in a plant test, ii) tests investigating necessary technical background (e.g. suitable plant species, suitable manure concentration), iii) tests according to the OECD 208 standard test design and modified test designs, considering an application of the test substance via manure. To ensure a significant evidence of the studies regarding universal validity, main tests were conducted with six plant species and eight replicates. The studies were conducted with pig and cattle manure and two representative veterinary antibiotics. The test design considers additional effects of manure to the test substance (e.g. adsorption) as well as transformation/metabolization of the test substance in manure by investigating the influence of i) the duration of anaerobic incubation in manure, ii) the way of application,

and iii) the incubation conditions (anaerobic/aerobic) on the effects of the antibiotics in plant tests. The platform presentation presents the experimental results of the research project with a special focus on the methodology and the influence of the test design on the variability of the results.

**127 Biotransformation products of ibuprofen in soil – a new view on the relevance of non-extractable residues** K.M. Nowak, RWTH Aachen University / Institute for Environmental Research Biology V; C. Girardi, A. Miltner, UFZ - Helmholtz-Centre for Environmental Research / Environmental Biotechnology; A. Schaeffer, RWTH Aachen University / Institute for Environmental Research; M. Kaestner, UFZ - Helmholtz-Centre for Environmental Research / Environmental Biotechnology. Ibuprofen was reported to degrade quickly in soils and sediments with the significant formation of mineralisation products and non-extractable residues (NER). NER formed during biodegradation of organic contaminants in soil are considered to be a result of various physical and chemical interactions of a parent contaminant and / or its primary metabolites with soil organic matter (SOM). To date, their chemical composition is still unknown, therefore it is speculated that these compounds immobilised as NER may pose a risk for environmental and human health after their release from SOM. However, in the case of biodegradable organic compounds, NER may be biogenic and can contain microbial biomass components, for example fatty acids (FA) and amino acids (AA). After cell death, these biotransformation products are subsequently incorporated into non-living SOM, where they are stabilised ultimately forming hardly extractable residues of biogenic origin. We investigated biodegradation of  $^{13}\text{C}$ -ibuprofen, in particular the formation of  $^{13}\text{C}$ -labelled FA and AA and their fate in soil over 90 days.  $^{13}\text{C}$ -FA and  $^{13}\text{C}$ -AA in the living microbial biomass fraction initially increased, thereafter their contents decreased and a continuous incorporation of these biomolecules into the non-living SOM pool was observed. At the end, nearly all NER were biogenic as they contained only natural microbial biomass compounds. This can be relevant also for other biodegradable contaminants; therefore, the possible biogenic NER formation needs to be considered in the assessment of the potential risks of the readily biodegradable contaminants in soil for the environment.

**128 Genetic variation of transcriptomic expression in *Lymnaea stagnalis*** A. Bouetard, INRA; C. Hoede, INRA / BIA and Genotoul Bioinformatics; A. Besnard, INRA / UMR ESE 0985; T. Pecot, Ohio State University / Comprehensive Cancer Centre; M. Collinet, INRA; L.L. Lagadic, INRA / UMR INRAAgrocampus Ouest Ecology and Ecosystem Health; M. Coutellec, INRA / Aquatic Ecotoxicology, UMR ESE. Population response to stress may have a genetic component which, if additive, is the basis for adaptive evolution to local conditions. Apart from monogenic resistance, adaptive processes have been traditionally investigated through phenotypes at quantitative traits. However, more elementary responses may also entail a heritable component. This is true for gene expression, which results from various molecular interactions. We investigated the evolutionary potential of transcriptomic expression induced by a pro-oxidant herbicide, diquat, using lines from four natural populations of the pond snail *Lymnaea stagnalis*. Populations stemmed from contrasted environments (close to vs distant from agricultural zones), which allowed testing the influence of historical exposure to putative environmental stressors. These populations were significantly differentiated at neutral genetic markers and showed significant genetic divergence at several life history traits. Previous results on diquat molecular effects on *L. stagnalis* suggested (1) the occurrence of various responsive pathways, and (2) that induced transcriptional overexpression may not translate into detectable functional changes (enzyme activity). In the laboratory, F1 individuals were exposed during five hours to diquat vs control conditions. RNA was extracted from hepatic tissue. RNAseq analysis was based on high-throughput sequencing (Illumina HiSeq2000) of 16 cDNA libraries, i.e., two replicates (each based on three different families)  $\times$  four populations  $\times$  two exposure conditions (diquat, control). Read assembly strategy was based on a meta-assembly using Abyss and MIRA, after a preprocessing

to extend short-read length by overlapping paired-end reads using FLASH. Then all initial reads were mapped against all contigs using bwa. Filtering contigs on which less than 1/1000000 reads mapped led to 48321 contigs, 26958 of which had a blast hit against Swissprot, Refseq-Prot or Refseq-RNA. Diquat-induced differential expression is currently compared across genetic origins using DESeq package. Functional annotation is used to identify and compare responsive molecular pathways (KEGG). Results will be discussed in the light of diquat early toxicological impact and of evolutionary potential of transcriptomic responses. Family and population variance in life history traits will be linked to constitutive transcriptomic expression, to draw a more integrative interpretation.

**129 Micro-evolutionary response in a natural *Daphnia magna* population under Cu and Zn stress.** J. Hochmuth, Environmental Toxicology and Aquatic Ecology; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; K. De Schampelaere, Ghent University UGent / Environmental Toxicology and Aquatic Ecology. A 10 week experimental evolution study was carried out under semi-field conditions to test for micro-evolutionary effects in a natural *Daphnia magna* population exposed to a control, 2 Cu, and 3 Zn concentrations. We investigated if the long-term exposures to Cu or Zn resulted in a higher organism fitness compared to that in the control exposures and in the original (= start) population. At the end of the microevolution experiment a life-table experiment was initiated with clones from the start population, a control population and metal exposed populations to assess if metal acclimation had occurred in the exposed populations. The populations experimentally evolved at 180µg Cu/L and 760µg Zn /L had significantly higher reproduction at the corresponding concentrations than the lower metal or control treatments, i.e. evidence that metal acclimation had occurred. After 4 months of culturing under control conditions, thus eliminating any acclimation history, an additional life-table experiment was conducted to determine whether metal adaptation, measured as an increase in mean population fitness occurred. We observed a significantly higher total reproduction at 760µg Zn /L and at 180µg Cu/L in the respective long-term exposed populations compared to the long-term control exposed population and the start population. In long-term exposure to 760µg Zn/L acclimation and adaptation to the metal had enabled the population density to recover, matching that of the control, despite an initial reduction of 75% of the clones. Under long-term exposure to 180µg Cu/L, however, despite lesser initial mortality (50%), acclimation and adaptation effects were not sufficient to lead to a full recovery of the population density. Our results confirm that micro-evolution can occur after only a few generations but that adaptation in itself is not a guarantee for a complete recovery of the population density.

**130 Adaptation to pollutants through modifications of sensitivities and life-history traits: a case study based on nine field *Gammarus* populations.** a. vigneron; O. Geffard, a. chaumot, Irstea. The question of evolutionary processes in ecotoxicology becomes substantial in order to propose relevant ecological risk assessment. Actually, it is well admitted that pollutants can be strong selective agents which can induced adaptation in exposed populations. Our study analyses two possible ways of adaptation: first, the evolution of resistance and second, the evolution of life-history patterns. This second point is rarely tackled in ecotoxicological issues, contrary to other fields of ecology dealing with stress adaptation. To answer these questions we adopted a retrospective approach based on the comparison of sensitivities and traits of exposed and unexposed field populations. To provide representative results, the choice has been made to work on an ecologically and ecotoxicologically relevant species, the freshwater amphipod, *Gammarus fossarum*. Particular attention was also paid to the design of the study through the sampling effort with the selection of nine field populations spread out at a regional scale. The exposition of organisms from these populations to cadmium, and the measure of their life-history traits in the field and in common garden in the lab, show that organisms are not only able to respond to pollutants by increased tolerance, but also by life-history adaptation. Nevertheless, our results

also demonstrate that adaptation is not automatic and seems to depend on contamination strength. In addition, these phenomenon are probably not out of unexpected outcomes since they can induced fitness costs, and changes in population dynamics can affect higher ecological scales.

**131 Population genomics reveals adaptive variation and a potential path to environmental forecasting** J.R. Shaw, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; J.K. Colbourne, University of Birmingham / School of Biosciences. *Daphnia*, or the water flea, is a sentinel species of freshwater ecosystems. Their populations are defined by the boundaries of ponds and lakes, are sensitive to modern toxicants in the environment, and thus are used to assess the ecological impact of environmental change. Their short generation time, large brood sizes, and ease of laboratory and field manipulation have assured *Daphnia*'s importance for setting regulatory standards by environmental protection agencies, testing chemical safety, monitoring water quality, and as a model for environmental genomics research. A hallmark of the genome sequence is a large number of duplicated genes that are most responsive to ecological challenges and are specific to the *Daphnia* lineage. In this study, we take advantage of maturing genomics tools to understand the molecular basis for evolved tolerance to toxic levels of certain metals. We also test the adaptive significance of *Daphnia*'s genome structure. Natural populations that have faced severe chemical challenges for over a century of industrial iron/ore smelting demonstrate evolved tolerance to cadmium. Other reference populations that have no history of chemical stress are clearly harmed by metal exposure, showing slower growth rates, lower fecundity and higher mortality. By measuring the distribution of copy number variants (CNV) and interrogating differential expression of 31,000 annotated genes from sampled populations across chemical conditions and through time, this study provides new insights into the functional interactions between genome structure and environment. We observe allele specific copy number increases in both extant and extinct populations living along a steep metal cline. We identify a large number of CNV, including the metal detoxication protein metallothionein that strongly correlate and are predictive of phenotypic differences between populations. These studies begin to quantitatively link genomic variation with individual fitness and population-level outcomes, and both benefit from and contribute to the *Daphnia* Genomics Consortium.

**132 What genomic mechanisms cause a cadmium-adapted *Daphnia pulex* isolate to be more tolerant to cyanobacterial stress than a non-adapted isolate?** D. De Coninck, Ghent University / Laboratory of Environmental Toxicology & Aquatic Ecology; J. Asselman, Ghent University / Laboratory of Environmental Toxicology; S. Glaholt, Indiana University; J.K. Colbourne, University of Birmingham / School of Biosciences; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; J.R. Shaw, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; K. De Schampelaere, Ghent University UGent / Environmental Toxicology and Aquatic Ecology. Natural populations are characterized by genetic variability which may allow populations to adapt to a stressor. As a consequence of this genetic adaptation, populations may become more (co-tolerant) or less tolerant (cost-of-tolerance) to other stressors. In aquatic ecosystems, stressors rarely occur isolated and the number of stressors are predicted to increase with global climate change (e.g. cyanobacteria). The aim of this study is to unravel the genomic basis of the observed differences in *Microcystis aeruginosa* (MC, a cyanobacterium) tolerance among cadmium (Cd) adapted and Cd non-adapted isolates of *Daphnia pulex* to gain insight into the genomic basis of the co-tolerance of Cd-adapted *D. pulex* to cyanobacterial stress. Gene-expression profiles of a Cd adapted and a Cd non-adapted isolate in response to MC were characterized using a micro-array platform. Functional enrichment analysis of pathways, based on KEGG reference pathway maps was performed. In addition, gene copy numbers for different isolates from Cd adapted populations and Cd non-adapted populations were determined by hybridizing gDNA to the microarray. In total, 9 pathways and 14

paralogous gene families were found to be significantly enriched. Among the top most significantly enriched pathways that were regulated by MC in different ways in both isolates were the oxidative phosphorylation, ribosomes, proteasome, sucrose and starch metabolism and linoleic acid metabolism pathways. All pathways were mostly only differentially regulated in the Cd non-adapted isolate, but not in the Cd adapted isolate which suggest an overall lower stress experience in the Cd-adapted isolate. We could attribute this to (i) lower accumulation of toxins than in the Cd non-adapted isolate and (ii) higher naïve basal expression of genes related to these pathways as a consequence of its adaptation to Cd. We could not confirm that copy number variation correlated with the higher naïve basal expression. This study showed possible genomic mechanisms of co-tolerance of Cd-adapted *D. pulex* populations to cyanobacterial stress. As micro-evolutionary changes in populations and their consequences (such as co-tolerance) for the toxicity of unrelated stressors gain more and more interest in risk assessment, it is beneficial to understand the mechanisms that drive these consequences. E.g., if we understand mechanisms of co-tolerance we can perhaps ultimately predict which combinations of stressors may show co-tolerance.

### 133 Epigenetic programming of disease and the role of developmental exposure to environmental contaminants

Kamstra, VU University Amsterdam; L. Bastos Sales, P. Cenijn, T. Hamers, J. Legler, VU University Amsterdam / Institute for Environmental Studies. Epigenetic programming of disease and the role of developmental exposure to environmental contaminants Jorke Kamstra, Liana Bastos Sales, Peter Cenijn, Timo Hamers and Juliette Legler, Institute for Environmental Studies, VU University Amsterdam, The Netherlands, juliette.legler@vu.nl A growing body of literature has demonstrated the crucial importance of epigenetics in gene regulation. Research shows that environmental factors, including exposure to contaminants, can alter epigenetic control of gene expression, with important implications for development and susceptibility to disease. In our laboratory, we study the effects of developmental exposure to endocrine disrupting chemicals (EDCs) on the latent onset of diseases such as obesity, using in vitro mouse and zebrafish models. In this study, we focus on the effects of EDC exposure on in vitro adipocyte differentiation, and investigate underlying changes in global and gene-specific DNA methylation. To this end, murine 3T3-L1 pre-adipocyte cells were exposed to EDCs during differentiation, and multiple gene targets involved in the adipocyte differentiation pathway were assessed with QPCR. Global DNA methylation was analyzed with HPLC and specific DNA methylation was analyzed on promoter regions of PPAR $\gamma$ 2 and Leptin with Methylation Sensitive High Resolution Melting Analysis (MS-HRM). Our results show that exposure to EDCs can alter adipocyte differentiation in vitro, which is accompanied by changes in (global) DNA methylation. Novel effects of the brominated flame retardant BDE-47 on adipocyte differentiation and methylation of key genes involved in adipogenesis have been found.

**134 A modelling approach to characterize sub-lethal responses of *Daphnia magna* populations to chemical exposure in the presence of environmental stressors** F. Gabsi, RWTH Aachen University / Institute for Environmental Research; A. Schaeffer, RWTH Aachen University / Chair of Environmental Biology and Chemodynamics; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research. Changes in population responses to chemicals in the presence of environmental stressors are reported in the literature for several organisms. Yet, the current risk assessment (RA) methodology is based on laboratory tests where chemicals' effects are assessed independently of natural stressors. It is a challenging task to account for these factors experimentally because of the testing efforts required and the difficulty in simulating real scenarios at a laboratory scale. Also, joint chemical and environmental stresses cannot be assessed based on knowledge on their separate effects since interactions don't always lead to additive effects. The 'Virtual Ecologist' approach has been suggested as a method to circumvent data limitations. It relies on the use of powerful models that allow testing complex, realistic scenarios. Individual-based models (IBMs) relate the measured toxicity on individuals to populations. They

also integrate various environmental factors, allowing for a mechanistic understanding of ecological impacts on populations. These features make them powerful 'virtual laboratories' for testing diverse hypotheses on population properties. In this study, we use a developed and validated IBM for *Daphnia magna* as a virtual laboratory to explore potential interactive effects of chemical and non-chemical stressors on populations and determine how the ability of populations to cope with sub-lethal effects is affected by the presence of environmental stressors. In the model simulations, constant exposure to toxicants affects solely or in combination, the daphniid's reproduction, growth, filtration rate or survival. Environmental factors include predation (*Chaoborus crystallinus*), competition or food level. Model results revealed a strong implication of environmental stressors in determining population sensitivity to chemicals. Equal toxicity levels had different impacts on population size when integrating environmental stressors. Interactions between chemical and non-chemical stressors manifested through additive, synergetic or antagonistic effects. We conclude that population resilience cannot be only attributed to chemicals' effects. Ignoring environmental stressors might lead to non-realistic estimations of chemicals' risks to populations. Integrating validated models into the current RA procedure is urgently needed.

### 135 Impacts of imidacloprid on individual performance and population dynamics of *Daphnia magna*

A. Agatz, T.A. Cole, University of York; E. Zimmer, Vrije Universiteit Amsterdam; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research; C.D. Brown, University of York / Environment Department. Various effects of xenobiotics on aquatic organisms might not be caused directly by the compound, but rather arise from adaptation of the organism to stress invoked by feeding inhibition during exposure. We demonstrate how multiple lines of evidence linked by understanding the ecology of the organism are necessary to elucidate xenobiotic impacts along the effect cascade (feeding, growth, maturation, reproduction and survival) and implications of these effects for population dynamics. Experiments were conducted to identify effects of a one-week pulse of imidacloprid on all endpoints of the effect cascade for *Daphnia magna* under surplus and reduced food availability. Concentrations inhibiting feeding by 5, 50 and 95% after one day of exposure were 0.19, 1.83 and 8.70 mg/L, respectively. Surplus food availability after inhibition allowed recovery following growth inhibition of up to 53 $\pm$ 11%. Limited food availability provokes a loss of recovery potential even when feeding inhibition did not exceed 5%. A shift in the distribution of energy reserves towards reproduction rather than growth was the driving factor, resulting in changed reproduction after exposure to all concentrations tested; increased or decreased reproduction occurred depending on the intensity of effect on feeding. We determined that all effects beyond feeding depression were secondary using the individual based *Daphnia magna* population model IDamP as a virtual laboratory. Additionally, we assessed the effect of similar pulses to populations, applying imidacloprid at different developmental stages (different food supply due to intra-specific competition); and investigated the implications for response to subsequent stress comprising a one-day exposure to carbaryl. Inhibition of feeding by 3% switched the population from negative to positive growth resulting in significantly increased population abundance. Inhibition by 97% reduced the total abundance by 56 $\pm$ 7% within three days and thus transferred the populations from a phase of food limitation for individuals to a phase of high food availability. This transfer occurred from feeding inhibition not causing mortality when tested at the individual level and increased the sensitivity to carbaryl; resulting in a reduction of the population by 53 $\pm$ 14%; this impact was four times stronger than that to populations not inhibited in their feeding.

### 136 Effects of pesticide exposure on zooplankton dormant egg bank dynamics: from laboratory to mesocosm studies

S. Navis, Laboratory of Aquatic Ecology Evolutionary Biology KU Leuven / Laboratory of Aquatic Ecology, Evolution and Conservation; A. Waterkeyn, L. De Meester, L. Brendonck, KU Leuven. Many aquatic invertebrate species produce dormant eggs to survive unfavourable

environmental conditions. These dormant eggs accumulate in the sediment to form a dormant egg bank, from which only a fraction hatches during each growing season. When environmental conditions fluctuate, egg banks can function as a reservoir of species and genetic diversity. Through this benthic-pelagic coupling, events in the dormant phase can affect the active, aquatic phase and vice versa. Despite its importance in ecological and evolutionary processes, dormant egg bank dynamics are rarely included in zooplankton population and community studies. *Daphnia magna*, a well established model organism and standard test species in ecotoxicology, reproduces by cyclical parthenogenesis, where environmental cues associated with unfavourable conditions trigger the sexual production of dormant eggs. Standard ecotoxicity tests with *Daphnia* (OECD TG 202, 211) generally focus on the effects of chemicals on the asexual part of the reproduction cycle. However, there is almost no information available on the effects of pollution on dormant eggs of *D. magna*, or on dormant egg bank dynamics in general. To get more insight into the acute and chronic effects of pesticides on *D. magna* dormant eggs (ephippia), we have conducted two series of laboratory experiments. In a first series we focused on the effects of pesticides on embryonic development and hatching characteristics of the dormant eggs. In the second experiment we tested whether exposure to pesticides, during the time the eggs were also exposed to hatching cues, could have long term effects on survival and life history characteristics of the hatched neonates. In addition, to better understand the environmental relevance of these findings, we have conducted a two-year outdoor mesocosm experiment, in which the long-term effects of repeated pesticide exposure on both the active and dormant component of zooplankton communities were studied. Our results show that, depending on their mode of action, pesticides can have severe negative effects on hatching characteristics of *D. magna* dormant eggs (ephippia), as well as on survival, growth and reproduction of the hatched neonates. This indicates that, in addition to inducing mortality of active individuals, pesticides can affect zooplankton communities by altering hatching dynamics and life history traits of hatched individuals.

**137 Feeding activity and oxidative stress in *Daphnia magna*** S.M. Furuhaugen, B. Liewenborg, Stockholm University / Department of applied environmental science; M. Breitholtz, Department of applied environmental science; E. Gorokhova, Stockholm University / Department of applied environmental science. Caloric intake is an important factor influencing oxidative status, as increased caloric intake leads to enhanced metabolic rates and generation of reactive oxygen species. As biomarkers of oxidative stress are frequently used as indicators of exposure and toxicological effects, it is important to establish the connection between these biomarkers and basic physiological rates, such as feeding and metabolism. The aim of this study was to delineate the effects of feeding on anti-oxidative capacity and oxidative damage, measured as lipid peroxidation, in *Daphnia magna* from the effects attributed to toxicity. The pesticide lindane was used to test whether toxic exposure affects feeding rate and the relationships between feeding activity and oxidative biomarkers. Results show that feeding rate has a significant positive effect on protein content, which in turn is positively correlated to both anti-oxidative capacity, measured as ORAC (oxygen radical absorbance capacity), and lipid peroxidation, assayed as TBARS (thiobarbituric acid reactive substances). Lindane exposure had a significant negative effect on feeding rate, however did not alter the relationship between feeding rate and protein content. Moreover, lindane negatively affected the relationship between ORAC and protein content, whereas the relationship between TBARS and protein was unaffected by the toxicant. The ratio ORAC/protein can thus be used as a biomarker of anti-oxidant response to toxic stress. In contrast, TBARS/protein ratio is rather an indicator of altered feeding rates than of toxic effects. The results show that oxidative response to toxic substances is largely mediated via alterations in protein synthesis, which, in turn, is positively related to feeding activity. Therefore, feeding activity and protein content have to be considered when the effects of toxic substances on these biomarkers are evaluated. Otherwise, biomarkers normalized to protein content may be erroneously interpreted as toxicity effects

whereas in fact they reflect only alterations in feeding.

**138 The effect of temperature on cadmium kinetics in *Folsomia candida* (Collembola)** D.M. Jevtic, Institute of Environmental Sciences Jagiellonian University / Ecotoxicology and Stress Ecology; J.B. Schmidt, Roskilde University / Department of Environmental, Social and Spatial Change; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; R. Laskowski, Jagiellonian University / Ecotoxicology Stress Ecology Group. The importance of temperature in (eco)toxicological tests has been long recognized. Still, ecological risk assessment (ERA) protocols are based on tests conducted in one constant temperature. This approach lacks ecological realism and possibly leads to erroneous conclusions on effects of temperature in natural environments. Understanding toxicokinetic processes and applying toxicokinetic models to the analysis of (eco)toxicity data is one of the most useful approaches in linking exposure concentrations to the effects of toxicants. In this study individuals of the springtail *Folsomia candida* were exposed to cadmium-spiked OECD soil (100 mg/kg dry wt) under five different temperature regimes – two fluctuating and three constant temperatures. Fluctuating regimes represented daily fluctuations – high (from 9°C to 26.5°C) and moderate (from 15°C to 25°C). Constant temperatures corresponded to 15°C, 19.5°C and 25°C. The constant temperature of 19.5°C was chosen based on equalizing organisms' daily energy budgets with fluctuating regimes, taking into account that metabolic activity is not linearly related to temperature. The time-dependent internal concentration of cadmium was measured at ten time points during the exposure phase (28 days) and at four time points upon transfer to an uncontaminated soil (14 days). Accumulation ( $k_1$ ) and elimination rate constants ( $k_2$ ) were estimated by fitting the time course of internal concentrations to a one compartment first-order toxicokinetic model. Our results indicate that cadmium kinetics in fluctuating environments follows a different pattern compared to the corresponding constant temperature. Accumulation and elimination rates were lower in fluctuating regimes compared to constant temperatures. In contrast, the body burden was higher in fluctuating temperatures, suggesting that ecotoxicological tests conducted at a constant temperature may not adequately predict bioaccumulation. In order to reduce the uncertainty in current ERA approaches, methods have to be revised – either by measuring toxic effects under naturally varying temperature conditions, or by developing and applying appropriate mechanistic effect models able to accurately predict toxic effects in different temperature regimes.

**139 Plastic as a carrier of POPs to aquatic organisms. A model analysis.** A.A. Koelmans, Wageningen University / Environment; E. Besseling, A. Wegner, Wageningen University; E. Foekema, Wageningen IMARES. It has been hypothesised that persistent organic pollutants (POPs) in microplastic may pose a risk to aquatic organisms. Here, we present a conceptual model for bioaccumulation of POPs including uptake from water, food and ingested plastic. The model accounts for dilution of exposure concentration by sorption of POPs to plastic (POP 'dilution'), increased bioaccumulation by ingestion of plastic containing POPs ('carrier'), and decreased bioaccumulation by ingestion of clean plastic ('cleaning'). A dynamically modelled absorption efficiency from plastic is proposed, which is calculated from particle size, POP polymer diffusivities, the time variable gradient between plastic and organism POP concentrations, and gut retention time. The model is parameterised for the lugworm *Arenicola marina* and evaluated against recently published polychlorinated biphenyl (PCB) bioaccumulation data for this species from laboratory bioassays with polystyrene microplastic. Further scenarios include polyethylene microplastic, nano-sized plastic and open marine systems. Implications for species with longer food retention times are discussed. Model analysis shows that plastic with low affinity for POPs like polystyrene will have small negative effects on bioaccumulation, governed by dilution. However, for stronger sorbents like polyethylene, the dilution, carrier and cleaning mechanism are relevant. In closed systems as in laboratory bioassays, dilution and cleaning dominate, leading to decreased bioaccumulation. However, in open marine systems, dilution

will be marginal and increased uptake of plastic bound POPs is predicted.

**140 Characteristic of spatiotemporal distribution of microplastics in surface microlayer in southern coast of South Korea** Song, Oil and POPs research group; N. Heo, M. Jang, Korea Institute of Ocean Science and Technology; S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; G. Han, Korea Institute of Ocean Science and Technology; W. Shim, Korea Institute of Ocean Science and Technology / Oil and POP Research Group. Microplastics, less than 1 mm in size, have been recently recognized as marine pollutants of significant concern due to their persistence, ubiquity, toxic potential, and their ability to act as vectors for transfer of absorbed and additive toxic chemicals to marine organisms. Floating of plastics on sea surface is affected by the density of the plastic materials. For example, polyethylene, polypropylene and expanded polystyrene which are generally less dense than sea water is likely to become floating debris. There is a microlayer in the sea surface formed by the surface tension of water with thickness of 1 mm. Sea surface microlayer is a habitat of a variety of life and accumulates light particles as well as pollutants deposited from the atmosphere and buoyant from water column. So the place is used for researching of pollutants. Considering the size and specific gravity of the microplastics, they are also expected to be accumulated within the microlayer. In this study, microplastic debris was quantitatively determined in surface microlayer at 21 stations in southern coast of South Korea in May (dry season) and July (rainy season), 2012, which is receiving Nakdong River discharge. The microplastics in the microlayer samples were extracted using the surface tension. The specially fitted 2 mm mesh sieve was dipped into the sea surface for 100 times, then the trapped water within mesh space was collected in the range of 2.2-2.8 L per site in the stainless steel tray and transferred to the 1 L polyethylene bottle. In the laboratory, the microlayer sample was filtered, using a glass fiber filter and counted using a dissecting microscope. Microplastic abundances in microlayer are in the range of 50-110 particles/L in May and 55-132 particles/L in July. Even if the average of microplastics ( $110 \pm 45$  particles/L) in May was higher than those ( $132 \pm 106$  particles/L) in July, there is not a statistical significance. Among four categories of microplastics (fragment, fiber, sheet and spherule), fragment type accounted for 80% in May and 98% in July. Small size microplastics down to 0-100  $\mu$ m class are dominant in all the samples. Microplastics are relatively abundant at stations near shore in comparison with offshore stations. Abundances of microplastics in microlayer were fold or orders of magnitude higher than those in top 20 cm surface water collected by filtering (0.7  $\mu$ m pore), a hand net (50 mm mesh) and a manta trawl net (330 mm).

**141 Sorption, desorption and bioavailability of persistent organic pollutants by microplastics in the marine environment** A. Bakir, University of Plymouth Enterprise Ltd / Science and engineering; S. Wright, Biosciences; S.J. Rowland, University of Plymouth / SoGEES; T.S. Galloway, University of Exeter / Biosciences Department; R.C. Thompson, The Marine Institute, University of Plymouth / School of Biological Sciences. Microplastics are small fragments of marine debris. Such fragments now appear to be widespread in the marine environment and have been reported at the sea surface, on shorelines and on the sea bed. Microplastics have been identified as particles less than 5 mm in diameter, but fragments much smaller ( $< 20 \mu$ m) than this are widely reported including pieces of nylon, polystyrene, polyethylene and PVC. It has been suggested that microplastics present potential mechanisms for the transport of persistent organic pollutants (POPs) and the release of chemical additives from plastics, to organisms. Unplasticised PVC (uPVC) and ultra high molecular weight polyethylene (UHMW PE), in the size range 200 to 250  $\mu$ m, were investigated for their potential to sorb DDT, phenanthrene (Phe), PFOA and DEHP in seawater. Sorption equilibrium times were determined over 15 days and most contaminants reached equilibrium onto plastic in 24-48 hours. Equilibrium distribution coefficients ( $K_d$ ) were used to represent sorption capacity of plastic for the pollutants under

investigation. Desorption kinetics were investigated in seawater and using a gut surfactant to represent gut conditions of marine organisms. Desorption of contaminants from plastic was faster in sodium taurocholate than in seawater but is slower than desorption of organic contaminants from natural sediments. A bioavailability model was also proposed to predict contaminants concentration in the tissues of a range of marine organisms.

**142 Exploring the effects of microscopic plastic particles in ecologically-important benthic invertebrates** S. Wright, Biosciences; A. Bakir, University of Plymouth Enterprise Ltd / Science and engineering; S.J. Rowland, University of Plymouth / School of Geography; R.C. Thompson, University of Plymouth / Science and engineering; T.S. Galloway, University of Exeter / Biosciences Department. Plastic debris at the micro-scale is a widespread element of marine litter. Microplastics have accumulated in oceans and sediments worldwide from low densities to localized 'hotspots'. Since they occupy the same size fraction as sediment grains and some plankton, microplastics may be ingested by low trophic fauna adopting indiscriminate feeding strategies, with uncertain consequences for the health of the organism. The current work aims to determine the extent to which microplastics affect the behaviour and health of ecologically important invertebrate species. We are using an integrated approach combining sublethal toxicological measurements at the cellular and physiological level alongside behavioural responses to assess the short- and long-term implications of microplastics *in vivo*. We are also considering the effects on growth to assess population-level impacts. At present, we have attained data regarding the low and high density impacts of virgin microplastics on the sediment-dwelling polychaete *Arenicola marina*, representative of environmental and worst-case scenarios. Significant effects have been found at the cellular level. We now hope to quantify the impacts of different types of plastics, varying in shape and polymer-type. In addition, we are assessing the capacity for microplastics to facilitate contaminant transfer to organisms and whether this occurs at levels capable of causing harm. The results will offer insight into the potential impacts of marine litter on the fundamental physiological processes in these important components of marine food webs, indicating the level of risk microplastic debris poses to the marine environment.

**143 Micro-CT as a novel and innovative technique in microplastic research** L. Van Cauwenberghe, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; M. Dierick, Ghent University / Department of Physics and Astronomy; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology. Microplastics, the degradation product of larger plastic debris, are accumulating in marine habitats worldwide. Given the small dimensions of microplastics ( $< 1 \text{ mm}$ ) these particles can potentially be ingested by a wide array of marine animals. Especially marine invertebrates, commonly not affected by the larger marine debris, are threatened by these microplastics. Data on the ingestion, and subsequent translocation to the tissues, of ingested microplastics in invertebrates is, however, scarce. This is, amongst others, due to methodological challenges associated with the detection of these minuscule plastic particles. Traditional microscopic techniques, such as light microscopy of histological sections, are commonly confronted with distortions due to intensive specimen preparation and difficulties in reconstructing samples. This invasive technique will consequently lead to the loss of key information. These issues can be overcome with the help of CT-scanning. Here, X-rays are used to create cross-sections of a sample from which a virtual model can then be recreated. Especially micro-CT provides a very high resolution (up to  $1 \mu\text{m}$  for small samples) and hence high accuracy. Additionally, this high spatial resolution can be obtained without destruction of the sample and without invasive sample preparation. In this study, we explored the use of micro-CT as a novel technique in microplastic research. In particular, we looked into micro-CT techniques to identify the transport of ingested microplastics. Using radiopaque microspheres, i.e. microspheres containing barium sulphate (polyethylene 1.5 g/cc  $10\text{-}20 \mu\text{m}$ ), we visualised transport in the

intestine after ingestion of the microspheres. Using this technique we assessed the possible translocation of ingested micropastics through the gut wall and into the tissues of exposed animals. This research is currently ongoing.

**144 “F53B” an overlooked PFC produced in China for 40 years** C. harman, NIVA / Environmental Contaminants; J. Huang, S. Wang, Y. Yang, Y. Ge, Tsinghua University; T. Larssen, NIVA. Perfluorooctane sulfonate (PFOS) is a well studied compound due to concerns about its toxicity and persistence in the environment which have led to various national and international controls, for example inclusion in the Stockholm Convention. Whilst PFOS has largely been replaced by less toxic alternatives, concern has focussed towards China where production persists. However another very similar compound, which is almost unreported in the scientific literature has been produced and used as a mist suppressant in the chromeplating industry in China for over 40 years. Synthesized in 1970s this compound, locally called F-53B is the potassium salt of a one-chlorinated polyfluoroethanesulfonic acid. Its chemical formula is  $C_{10}H_{17}ClF_{11}KO_2S$  and it has only this year received a CAS No. (73606-19-6). The amount of F-53B produced and used in China is quite comparable with PFOS, and may be increasing since international controls placed on PFOS are a problem that China must face in the coming future. However, to our knowledge there are no reports concerning the existence of F-53B, its presence in the environment or its toxicity. Thus the aim of this study was to provide the first data in this regard. Acute toxicity was determined using Zebrafish (*Brachydanio rerio*) according to OCED guidelines. The 72h-LC50 was found to be 17.72 mg/L, and 96h-LC50 was 12.18 mg/L. Grab samples were taken at several points above and below a municipal wastewater treatment plant situated on the Oujiang River, Wenzhou city, which receives (treated) wastewater from the electroplating industry and samples were also taken at treatment plant directly associated with the industrial site. Levels in the treatment plant at the industrial site were unsurprisingly very high, in the order of 10s of  $\mu g L^{-1}$  and both PFOS and F-53B were found in the receiving waters near the treatment plant in similar amounts to each other (10s of  $ng L^{-1}$ ). In conclusion F-53B shows similar properties to PFOS in terms of toxicity, it is present in the environment and has likely been there for some decades. Results will be further discussed in terms of on-going degradation and bio-accumulation studies. There is an urgent need for more information concerning this PFC.

**145 Stockholm Arlanda Airport as a Source of Per- and Polyfluoroalkyl Substances to Water, Sediment and Fish** L. Ahrens, Swedish University of Agricultural Sciences SLU / Dept of Aquatic Sciences and Assessment; K. Norstrom, T. Viktor, IVL Swedish Environmental Research Institute; S. Josefsson, SLU / Dept. of Aquatic Sciences and Assessment. Per- and polyfluoroalkyl substances (PFASs) have been used in a variety of consumer and industrial applications such as aqueous fire fighting foams (AFFFs). AFFFs have been used at fire training facilities at airports and oil refineries since the 1970s and are a potential source of PFASs in the nearby environment. In this study, PFASs were measured in water, sediment and fish near a fire training facility at Stockholm Arlanda Airport in Sweden. Samples were analyzed for 11 PFASs including  $C_{10}-C_{11}$  perfluoroalkyl carboxylates (PFCAs),  $C_{10}-C_{11}$  perfluoroalkyl sulfonates (PFSAs), perfluorooctanesulfonamide (PFOSA) and 6:2 fluorotelomer sulfonate (FTS). The concentration levels and pattern of PFASs showed a high variety depending on their spatial distribution and matrices (i.e., water, sediment, fish). However, perfluorooctane sulfonate (PFOS) was the dominant compound in all compartments with a contribution of 40% for surface water, 86% for sediment and 98% for European perch (*Perca fluviatilis*). Highest PFAS concentrations were found in water at a ditch close to the fire training facility with about 4000 ng/L for PFASs. The PFAS concentrations decreased continuously from the fire training facility to lake Mälaren. During the transport, PFASs can partition to sediment or bioaccumulate in the food chain. The highest sediment/water partition coefficients ( $\log K_{ow}$ ) were found for PFOS (2.13) and PFOSA (2.99). Similarly, PFOS and PFOSA had also the

highest bioconcentration factors (BCF) with, on average, 3320 and 2310 for muscle tissue, respectively. PFAS concentrations in the water phase at a nearby lake did not show a decreasing trend over the last years which indicate that Stockholm Arlanda Airport may be an important source for long term contamination of the nearby environment with PFASs.

**146 Occurrence and Concentrations of Contaminants of Emerging Concern in Wastewater Matrices across United States** K. Dasu, US Environmental Protection Agency National Risk Management Research Laboratory / Department of Agronomy, Crop, Soil and Environmental Sciences; M.A. Mills, US EPA; L. Zintek, US Environmental Protection Agency / Department of Chemistry; W. Brashear, PTS; K. Tadele, B. Crone, Student services, USEPA. Contaminants of emerging concern (CECs) are detected globally in wastewater treatment plant (WWTP) matrices such as effluents and biosolids. Wastewater matrices may play an important role in the transport of these contaminants into the environment. Some of the CEC are environmentally persistent, and are shown to have endocrine disrupting effects and other harmful effects to the aquatic ecosystems and human health. Hence, there is a growing concern about the potential risks associated with their presence during water reuse and the land-application of biosolids. Once land-applied, the emerging contaminants present in biosolids can enter the surface and ground waters or carry over to the plants grown on such soils and this increases the potential exposure of humans and aquatic ecosystems to these compounds. The main objective of the current study is to quantify the occurrence and concentrations of CECs and their transformation products and/or precursors in treated wastewater effluents and biosolids. Differences in treatment performance will be evaluated based on the concentrations of contaminants detected in biosolids from different origin (i.e. aerobic or anaerobic digestion, presence of additives and other factors). Classes of chemicals to be monitored include perfluoroalkylated substances and their precursors, alkylphenol ethoxylates and alkylphenols, steroid hormones and pharmaceuticals and personal care products. Effluents and biosolid grab samples were collected from 9 wastewater treatment plants around the United States. Biosolids from 3 plants have undergone aerobic digestion and 6 plants have undergone anaerobic digestion treatment process. The effluent and biosolid samples are extracted separately for different classes of contaminants and are analyzed on UPLC/MS/MS and GC/MS/MS for different classes of analytes. The data will aid in understanding the fate of CECs in wastewater matrices and the results will provide insightful information for the risk management of these chemicals in the environment. From the current study, the data on the occurrence and concentrations of different CECs and their precursors or metabolites in effluents and biosolids from 9 WWTPs will be discussed in detail.

**147 Uptake of perfluorinated alkyl acids in crops via land applied biosolids: Field and greenhouse studies** A.C. Blaine, Colorado School of Mines / Civil & Environmental Engineering; C.D. Rich, L. Kudryk, Colorado School of Mines; L.S. Hundal, Metropolitan Water Reclamation District of Greater Chicago; C. Lau, M.A. Mills, U.S. EPA / Office of Research and Development; K.M. Harris, U.S. EPA / Region 5; C.P. Higgins, Colorado School of Mines / Civil & Environmental Engineering. Perfluoroalkyl acids (PFAAs) consist of a carbon backbone saturated with fluorine atoms in place of hydrogen. Of particular concern are PFAAs that contain either a carboxylate or sulfonate group, resulting in strong surfactant behavior. Their dual lipophobic and hydrophobic nature makes them both oil and water repellent, giving them a myriad of applications in both industrial and consumer settings. Many PFAAs are also environmentally persistent, bioaccumulative, and toxic making them a high priority contaminant of emerging concern. The presence of PFAAs in municipal biosolids has been well documented. Biosolids, like animal manures, are rich in both plant nutrients and organic matter and are commonly used as a fertilizer in crop production. As a consequence, concerns have arisen about the potential uptake and subsequent bioaccumulation of PFAAs into crops grown in biosolids-amended soils. Previous studies have documented



the potential for bioaccumulation of PFAAs, particularly perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) into food crops, while other field studies have documented the transfer of these and other PFAAs from industrially-contaminated biosolids-amended soils into grass. Additional literature has documented the transfer of PFAAs from spiked hydroponic systems into lettuce. The present study included both field and greenhouse experiments to evaluate the potential for PFAA uptake and bioaccumulation into crops grown in biosolids-amended soils. Results from this study demonstrate that lettuce, tomato, zucchini, and corn plants can uptake PFAAs, and that bioaccumulation is dependent on chain length, crop type, and soil properties. These results may have important implications with respect to the potential routes of PFAA exposure in humans. Disclaimer: This abstract does not necessarily reflect U.S. EPA policy.

#### **148 Uptake of perfluorinated alkyl acids by hydroponically and field grown crops**

**S. Felizeter**, University of Amsterdam; M.S. McLachlan, Stockholm University; H. Juerling, J. Mueller, Fraunhofer Institute; P. de Voogt, University of Amsterdam / IBED. Perfluorinated alkyl acids (PFAAs) are bioaccumulative persistent, organic pollutants (POPs), which can be detected ubiquitously in the environment. PFAAs pose a risk to human health due to accumulation in the food chain. The occurrence of PFAAs in animals, such as fish, birds and mammals including humans is fairly well documented, but little can be found in the literature about crops or plants in general. Also, most studies focus just on the two main compounds perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). Humans are possibly exposed to PFAAs through consumption of vegetables and other plant-related food items. The objective of this study is to understand the accumulation process of PFAAs in crops. In greenhouse experiments the uptake of Cabbage and Lettuce as leafy vegetables were tested to prove or disprove this hypothesis. Furthermore Tomato and Zucchini were analyzed to test for accumulation in fruits. These four plants will be tested using hydroponical culturing conditions and employing varying concentrations of PFAAs. Uptake factors were calculated for 14 different PFAAs. While short-chained PFAAs show higher Uptake factors in the Foliage than in the roots, longer-chained PFAAs predominantly accumulate in the roots. Furthermore, the uptake behavior of PFAAs cannot be described with existing uptake models. In a field experiment lettuce, radish, peas and corn were grown in lysimeters in 4 different concentrations of spiked soil to have a comparison to the greenhouse experiment. The results of the concentrations in the different parts of the plants show a different pattern than in the greenhouse experiment with higher concentrations in the foliage part for most of the compounds.

#### **149 Polyfluorinated chemical exposure in blood from mothers and infants of the Canadian Healthy Infant Longitudinal Development Study**

**C.S. Wong**, University of Winnipeg / Richardson College for the Environment; C. McConkey, M. Loewen, A. Becker, University of Manitoba. Concentrations of 17 perfluorinated compounds (PFCs) were measured in the blood of volunteers from Winnipeg, Manitoba, Canada, of the Canadian Healthy Infant Longitudinal Development (CHILD) study, to assess exposure levels and chemical distributions, and potential correlations of these with maternal and home characteristics from CHILD. Chemical measurements of PFCs were done for plasma from 414 women during pregnancy, 247 women one year after delivery, and 50 infants at the time of birth via cord blood. Mean levels of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were 1.1 and 2.6 ng/mL, respectively, in prenatal plasma, and 0.7 and 2.2 ng/mL, respectively, in postnatal plasma. These were similar to that observed in other human monitoring studies done elsewhere in the world, and were generally lower than those studies done earlier in the last decade or so, indicating possible decreases in PFOA and PFOS from decreasing primary use. Perfluoropentanoic acid (PFPA) had median concentrations of 0.5 ng/mL. This chemical was not detected in earlier studies, indicating increased exposure to this short-chain PFC used in recent manufacturing. Most perfluorocarboxylic and perfluorosulfonic acids were lower in matched maternal postnatal samples compared to that in prenatal blood (n = 161), except for PFPA for which the opposite

was observed. Cord plasma concentrations of PFCs were generally lower than that of maternal plasma (e.g., mean 0.7 and 1.0 ng/mL for PFOA and PFOS, respectively), with increasing maternal transfer for perfluorocarboxylic acids with shorter chain length. Principal components analysis indicated that there was a correlation between known PFC half-lives and the age of the mothers and the number of babies they had delivered, as well as for greater maternal transfer for postnatal samples and for mothers that had breastfed their infants for longer periods of time. There was no correlation between incidence of wheezing in infants and PFC levels, except for PFOA, nor evidence for linkages between plasma levels of PFCs and various developmental measures (e.g., birth length, ponderal index, head circumference, gestational age). No significant correlations were observed between PFC plasma levels and home characteristics (e.g., carpeting, home renovations, new furniture), suggesting exposure to cohort participants was ubiquitous.

#### **150 The necessity of disinfected toilet brushes – Information policy of companies on disinfectants in private homes**

**S. Wieck**, . The use of disinfectants in private homes is intensely debated among scientists. It is widely accepted that disinfectants should only be used by private persons if there is a medical indication (Bockmühl, 2011). These might be infectious diseases of family members or if people in need of care are living in the household. However, companies are promoting disinfectants for several areas of use apart from medical indications, e.g. disinfection of toilet brushes or hair rollers (Colgate-Palmolive, 2012). These uses have no beneficial effects for consumers and lead to a needless exposure of consumers to the active substances and unnecessary releases of chemicals to the environment. This causes several problems for the human health and the environment. In the project websites of 14 companies were analyzed with content analyses with regard to the information provided for consumers and whether they abode by the regulations concerning the advertisement of biocides. The results were compared to results of similar analyses of websites of German authorities engaged in the authorization of biocides. The analyses show that the information provided by the company websites is not enough to fulfill the information needs of consumers. Details on risks caused by disinfectants are missing and not all companies abide the regulations on advertisement. In the following prosecutions by the responsible federal state authorities even more violations were detected. The websites of the German authorities informed the consumer comprehensively. However, the texts often seemed hard to understand for laymen. The company websites do not educate the consumers properly about the use and the risks of disinfectants; other stakeholders involved in consumer education have to undertake this task. The German authorities provide a wide range of relevant information but the comprehensibility has to be improved to be understandable for interested consumers. Eventually, stricter regulations on the advertisement of disinfectants should be considered to support consumers in their decisions of buying and using disinfectants. This poster does not necessarily reflect the opinion or the policies of the German Federal Environment Agency. Bockmühl, Dirk: Welche Zukunft haben antimikrobielle Produkte im Haushalt? In: Hygiene + Medizin Bd. 36 (2011), Nr. 1&2, S. 12–18 Colgate-Palmolive. 2012. Tipps für Verbraucher. *Danklorix*. [Citation from: 23. 11 2012.] [http://www.danklorix.de/tipps\\_desinfektion.php](http://www.danklorix.de/tipps_desinfektion.php).

#### **151 Experience with Risk Communication in different Estuaries and Lagoons**

**L. Stresius**, Fachhochschule Lübeck / Laboratory of Urban Water and Waste Management; S. Heise, Hamburg University of Applied Sciences; M. Grottker, University of Applied Science Lübeck. Management and communication of environmental risks in complex natural systems like estuaries and lagoons implicates an enormous challenge for decisionmaker, stakeholder, experts and the general public. Stakeholders and decisionmakers often have to contend with multidisciplinary, multidimensional processes in systems with long time delays, positive feedbacks, non linear cause-and effect relations and uncertainties. For effective management and communication of complex tasks a deep understanding of the system and the mental models of all



participants of the process is required. For sustainable planning and management of complex natural systems a integration of existing scientific knowledge is of vital importance. To communicate scientific results and information about risks to non-scientists in a way that brings benefits for everybody is the ultimate challenge. In the Interreg IVB-project diPol the communication and management tool SIMACLIM was developed and used in four case sides in Oslo, Gothenburg, Copenhagen and Hamburg in several stakeholder workshops. SIMACLIM provides assistance to regional and national decision makers to illustrate the multiple impacts on ecological quality, to evaluate consequences of potential measures and to adapt their management to the changing climate. In the project ARCH, a framework 7<sup>th</sup> programme, SIMACLIM is used to employ a true participatory process with stakeholders including policy makers, authorities, NGO's and businesses to formulate realistic strategies towards sustainable lagoon management. Communication between science and stakeholder, policy and the general public as the beneficiary of scientific results needs to be a multi-way process. Scientists must listen to the public or other "consumers" of their scientific results like for example risk messages to get to know the knowledge level of their audience, to find the right language and to find out what kind of information is needed. An evaluation of the effectiveness of communication should guide the process.

**152 How does the public react when scientists disagree? Scientific consensus and risk communication** A. Hunka, A. Palmqvist, Roskilde University / Department of Environmental Social and Spatial Change; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences. The way people perceive risks is an important element of risk communication, that has to be considered by media professionals and food safety authorities alike. Non-experts in risk assessment have to rely on the expertise of others. Therefore, the role of scientific consensus and trust in the judgement of experts seem to lie at the core of risk perception and successful risk communication with the general public. Yet, studies on the role of scientific consensus in perceiving risk are scarce. On the 19<sup>th</sup> of September, a study on long term toxicity of herbicide-tolerant, genetically modified maize NK603 diet in Sprague Dawley rats was published online by the journal of Food and Chemical Toxicology. The results, illustrated by pictures of malformed rat females quickly found their way into leading popular internet media and triggered a heated debate within the scientific community, among European food risk authorities, and anti-GMO campaigners. So far the majority of the scientific critics have agreed that Seralini et al's study displayed some serious methodological flaws, while the first EFSA review of the study showed that Seralini et al.'s methods did not meet OECD standards. In our study we focus on public reactions to scientific controversy surrounding the study of Seralini's team, using this example as a case study. Our aim is to link perceived risk from GM organisms with the role of scientific consensus in risk communication. We employed a mixed-method approach, combining thematic analysis with a survey. We have collected materials from web pages on English-speaking internet sites and launched a web-based survey to study the general public's response to the GMO controversy. Our preliminary results indicate that the lack of scientific consensus is the main and very serious factor undermining trust both in food safety authorities and the scientific community in general. On the other hand, trust does not only apply to the information source, but to communication content as well. Many studies on risk perception show a very strong bias towards negative information occurring regardless of trust in the information source. The different tone of media reports in the U.S. and Europe indicate that initial attitudes towards GMO play a crucial role in risk communication. Finally, media also fine-tune their messages to readers' reactions, reinforcing initial beliefs of the public. Scientific consensus appears to be a promising way to break this vicious circle.

**153 Discussing environmental issues from different disciplinary perspectives. The first step for public communication on science.** F. Lafaye, Université de Lyon / ENTPE; P. Garrigues, University of Bordeaux / Institute for Molecular Sciences; N. Léca, Université

Montesquieu Bordeaux 4 / CRDEI. Environmental questions require the commitment of various research fields and disciplines (chemistry, toxicology, anthropology, laws). Most of the times, these disciplines remain within their own research field and when put together, the results look like split up. But dialog with the public needs to answer the questions globally and in a practical way. What are the needs for an integrated approach which allows everybody to understand scientific procedure and results? Interdisciplinarity implies to take time and to get committed both the disciplines and the researchers. Beyond the risks taken to spread their research fields, the researchers must also undertake a deep dialog based on a strong willing to understand and to respect each other.

**154 Plenary discussion: How can we communicate to improve our outreach?** P. Calow, University of Nebraska Lincoln / ORED. Environmental sciences are at the heart of what people affect in their daily lifes: environmental quality, safe food, clean air, fresh water - and hence crucial for sound public health. Why aren't we in their daily minds? How can we get there? Why should we want to? Moderated by Peter Calow and together with the presenters and external guests we want to discuss the current and future state of science and risk communication in environmental sciences. Panel members are: - Valery Forbes, University of Nebraska Lincoln - Ursula Klaschka, Hochschule Ulm - John Redshaw, SEPA - Birgit Sokull-Kluettgen, European Commission JRC - Ken MacDonald, BBC Science Correspondent

**155 Biomonitoring of an iron mine in the Arctic Circle using field transplanted mussels** S. Brooks, NIVA / Ecotoxicology and Risk Assessment; C. Harman, Norwegian Institute for Water Research (NIVA); M. Hultman, Norwegian Institute for Water Research; K. Macrae; J. Berg, NIVA. The blue mussel (*Mytilus* spp.) has been used to assess the potential biological effects of the discharge from the Sydvaranger mine, which releases its tailings into Bøkfjorden at Kirkenes in the north of Norway. Sensitive health biomarkers and metal bioaccumulation were measured in mussels caged at varying distances from the discharge outlet in Bøkfjorden. The biomarkers used include: Stress on stress; condition index; cellular energy allocation; micronuclei formation; lysosomal membrane stability by neutral red retention; basophilic cell volume; and neutral lipid accumulation. The individual biomarkers were integrated using the integrated biological response index (IBR/n). The accumulation of Fe was significantly higher in mussels located closer to the discharge outlet, indicating that these mussels had been exposed to the mine effluent. The IBR/n results were in good agreement with the location of the mussels in relation to the distance from the discharge outlet. The biomarker responses were not severe but did exhibit effects for some of the biomarkers resulting in a higher IBR/n in the mussels closest to the outlet. However, based on the individual biomarker responses the overall biological effects measured in the exposed mussels were considered low.

**157 Using WHAM-FTOX to assess the toxicity of mining-impacted freshwater systems** S. Lofts, Centre for Ecology Hydrology / Shore Section; E. Tipping, NERC Centre for Ecology and Hydrology; A. Stockdale, University of Mancheser; S. Ormerod, Cardiff University; W. Clements, Colorado State University; R. Blust, University of Antwerp. Freshwater systems impacted by metal mining are predominantly characterized by contamination with multiple metals and in many cases low pH. Tools to predict the toxicity of such systems therefore need to account for the toxicity of metals and acidity, and should also account for bioavailability of metals. The WHAM-*F*<sup>tox</sup> model, described here, is designed to perform this task. The model assumes that toxicity results from the binding of metals and protons to receptor sites on freshwater organisms, and that the binding of these ions to humic acid correlates to the organismal binding. Effects on field communities are quantified by fitting the model to data using piecewise quantile regression. The model has been parameterised using datasets from the UK, Japan and the United States. The parameterised model may be used to predict how water chemistry changes over time (resulting, for example, from remediation actions) may be expected to affect field communities.

### 158 Bioavailability and toxicity of metals to *Crassostrea gigas* larvae in an estuary affected by mining

**C.B. Braungardt**, Plymouth University / School of Geography, Earth and Environmental Sciences; C. Money, AstraZeneca / Brixham Environmental Laboratory; P. Worsfold, Plymouth University / School of Geography, Earth and Environmental Sciences; E. Achterberg, National Oceanographic Center. Proposed environmental quality standards (EQS) for metals in estuarine waters require renewed attention to two concepts: (i) bioavailability and (ii) the dynamic nature of the water body. Detailed understanding of estuarine processes that affect metal speciation and hence bioavailability, at appropriate spatial and temporal resolution, is required in order to set up appropriate monitoring strategies for EQS compliance. In this study, we deployed the Voltammetric In situ Profiler (VIP) for 10 h in the Fal estuary (SW England) to determine the truly dissolved (The OEL bioassay response to samples taken in Percuil Creek, located near the mouth of the estuary, was low (up to 11% PNR,  $n=3$ ) and varied little between samples taken at different times during the survey and indicated that the concentrations of dynamic Cu ( $3.8\text{--}7.0\text{ nmol L}^{-1}$ ), Cd ( $0.13\text{--}0.34\text{ nmol L}^{-1}$ ) and other contaminants had limited toxic effects. In contrast, total metal concentrations of Cu ( $5.0\text{--}88\text{ nmol L}^{-1}$ ) and Cd ( $0.68\text{--}2.3\text{ nmol L}^{-1}$ ) at Restronguet Point (RP) were elevated, and maxima of both metals were observed just after low water. The increase of Cu around low water occurred mainly in the colloidal phase (68%), while over 88% of Cd was potentially bioavailable at low water. The bioassay response to waters from Restronguet Point (RP) ranged from a minimum of -4% PNR for a sample taken near the time of high water to +93% PNR at low water. Dynamic Cu ( $6.1\text{--}28\text{ nmol L}^{-1}$ ) and Cd ( $0.24\text{--}2.0\text{ nmol L}^{-1}$ ) concentrations for the corresponding survey show a similar pattern as the bioassay, indicating that the increase in both, sources of metals and toxicity to the OEL were located upstream of RP, probably in the Carnon River, which receives acid drainage from an extensive historic mining area. This study shows the spatial and temporal variability of contaminant concentrations, speciation and combined toxicity in estuaries, thus emphasising the need for careful consideration of when, where and how often samples are to be taken for compliance monitoring of the new EU Water Framework Directive EQS.

### 159 Utilizing *Eisenia andrei* to assess the ecotoxicity of platinum mine tailings disposal facilities

**M. Maboeta**, North West University. South Africa is an important platinum mining country which results in environmental impacts due to the construction of tailing disposal facilities (TDFs). It is unclear what the effects of ageing are on the ecotoxicity of TDFs and whether it increases or decreases over time. The aim of this study was to determine the ecotoxicity of differently aged TDFs by investigating earthworm (*Eisenia andrei*) responses viz. growth, reproduction, neutral red retention times (NRRT) and tissue metal concentrations. Further, to evaluate the status of these in terms of a geoaccumulation index ( $I$ ), pollution index (PI) and integrated pollution index (IPI). Results indicated that earthworms showed reduced reproductive success (hatchlings per cocoon) and decreased NRRT in all the sites. Juveniles per cocoon between all of the different treatment groups were; control ( $2.83 \pm 0.54$ ) > Site 2 (20 years old;  $1.83 \pm 0.27$ ) > Sites 1 and 3 (40 years old;  $1.06 \pm 0.15$  and 6 years old;  $0.88 \pm 0.39$ ). This might be ascribed to the elevated levels of Cr ( $\pm 200\text{--}1166\text{ }\mu\text{g g}^{-1}$ ) and Ni ( $\pm 100\text{--}316\text{ }\mu\text{g g}^{-1}$ ) in all of the sites. Earthworms did not bioaccumulate metals with bioconcentration factors for all the different treatments < 0.01. Studies like these could be useful when establishing a ranking of TDFs in the future to provide legislative institutions with an indication of the environmental liabilities of platinum mines.

### 160 Sphalerite in agricultural soils: implications for Cd contamination of crops

**T. Robson**, C. Braungardt, Plymouth University / Biogeochemistry Research Centre; J. Rieuwerts, P. Worsfold, Plymouth University. Aerially and fluvially distributed fine mineral particles produced by mining operations can behave as vectors for toxic trace elements (e.g. Cd) in surface environments. Limited data exists on the short-term stability of these particles in agricultural soils

and the associated risk of producing contaminated crops. Sphalerite (ZnS) is abundant, widely distributed and generally contains 0.2-1 wt. % Cd. The principal intake route for non-smokers is dietary and Cd is readily taken up by plants. This study investigated whether ZnS weathering rates are relevant to crop growth and evaluated the bioavailability of the weathering products to wheat. Acid-neutral soil (Tamar Valley, UK) spiked with ground ZnS (< 63  $\mu\text{m}$ , 0.92 wt. % Cd) at 0.1 wt. % was incubated under laboratory conditions for 7, 30, 90, 180, 270 and 365 days, after which samples were freeze-dried prior to aqueous extraction: 0.1 M EDTA ( $\text{Cd}^{\text{EDTA}}$ ) and 0.01 M CaCl<sub>2</sub> ( $\text{Cd}^{\text{CaCl}_2}$ ). Extracts were filtered (0.45  $\mu\text{m}$ ) and analysed for dissolved Cd/Zn (ICP-MS/OES). Analogous incubations were planted with spring wheat seedlings after 180 days incubation. At maturity, plant tissues were freeze-dried, ground and digested (50% v/v HNO<sub>3</sub>) before tissue Cd concentrations were determined (ICP-MS). Extractable Cd in spiked soils deviated from those in control soils. A linear relationship between  $\text{Cd}^{\text{EDTA}}$  and incubation duration indicated steady-state dissolution occurred ( $2.81 \pm 0.30\text{ nmol Cd kg}^{-1}\text{ soil day}^{-1}$ ), resulting in  $1.23 \pm 0.26\text{ }\mu\text{g ZnS}$  dissolution after 365 days. Tissue Cd concentrations of wheat from spiked soil ( $24.8\text{ }\mu\text{mol kg}^{-1}$ ) were a factor of ? 70 of plants grown in the control soil ( $0.35\text{ }\mu\text{mol kg}^{-1}$ ). Flux calculations estimate  $3.7 \pm 0.6\%$  and  $35 \pm 5\%$  of the  $\text{Cd}^{\text{EDTA}}$  was taken up into seeds and stems, respectively. Total uptake was an order of magnitude greater than the predicted  $\text{Cd}^{\text{CaCl}_2}$  pool, suggesting it was readily replenished as Cd was absorbed by the plants. A Cd ingestion rate of  $1.25\text{ }\mu\text{mol Cd kg}^{-1}\text{ BW month}^{-1}$  was estimated for consumers of the contaminated wheat seed, 5.6 times current WHO guideline. ZnS undergoes slow, steady dissolution in acid-neutral soils and releases highly bioavailable Cd. Consequently the impact of ZnS soil contamination is long-lived. Wheat grown in ZnS-contaminated soils can accumulate hazardous Cd concentrations in the edible tissues; therefore populations subsiding on crops grown in ZnS-affected soils may be at risk of chronic Cd poisoning.

### 161 Ecosystem services in risk assessment and -management of pesticides

**J. Van Wensem**, TCB. The Ecosystem services (ES) concept can be used to place pesticide use and the concomitant agricultural management practices in a sustainability context, to evaluate the ecological, economical and societal aspects. Ecosystem services are the benefits people obtain from ecosystems. The ES concept is mainly used to strengthen the position of natural resources in decision-making. ES are highly relevant for agriculture as provision of food, feed and fibre is a major ecosystem service, both in term of wellbeing as in economical terms, and is supported by many other ES. Land is a limited resource, and productive land even more. There is scarcity of land for other uses too, and competition with for instance recreation, water regulation and biodiversity. Especially in densely populated countries there is pressure on agricultural (and urban) land to also deliver other ES. Changes in the EU common agricultural policy may lead to a focus on land sharing, e.g. multiple ES provision by agricultural land. At present the ES concept is in use in risk assessment and risk management of pesticides. The European Food Safety Authority published in 2010 a scientific opinion in which it was proposed to use the ES concept to specify general protection goals mentioned in the new Regulation No 1107/2009 and other relevant regulation(2010). The derivation of these specific protection goals by means of the ES concept allowed a more systematic way of inventorying the taxonomic groups that are important for delivering a suite of ES by agricultural landscapes, and was a one-time exercise. EFSA is currently working on a number of guidance documents (revisions) that build on this opinion. Many diagnostic risk assessment studies describe effects of pesticides on ES in the field, often by using indicators for ES. However, the link with ES is not always made clear, even when there is one. A promising development is the use of the ES concept in agricultural management scenarios, in which pesticide application is part of the management. Some studies use ES valuation (in monetary terms) to determine the optimal scenario. The results of the diagnostic risk assessment studies and the management scenario studies with ES might be of great use in pesticide regulation, despite the present lack of guidance how to apply the ES concept in

these areas.

**162 Examining new environmental data requirements under Regulation 1107/2009 – Anses opinion** V. Poulsen, ANSES. Current data requirements for pesticides, for active substances and plant protection products, are based on Regulations 544/2011 and 545/2011, respectively. Discussions at EU level occurred during the last few years, and final drafts were discussed in July 2012. For environmental fate and ecotoxicology, a couple of new data requirements were added to the previous one. These new requirements and additional tests are intended to increase environmental safety in the framework of pesticide use. Some of them are also intended to have a better understanding on pesticide properties, but to Anses' opinion, not all of them could be used for risk assessment purposes. Moreover, new guidance documents or update of the existing ones are necessary to develop risk assessment schemes including the new studies in a couple of areas, such as the risk assessment for vertebrates other than birds and mammals, or long range transport.

**163 Selection of an additional test species besides *Daphnia magna* for the effect evaluation of insecticides to invertebrates under Regulation 1107/2009** M. Daam, DPPF/CEER; L. Santos, M.J. Cerejeira, Technical University of Lisbon; T. Brock, Alterra. Lower-tier risk assessments of pesticides for aquatic invertebrates in the EU have been based on routine testing of the crustacean *Daphnia magna* and (in certain cases) the insect larvae *Chironomus* spp. An important change in the proposed update under the new Regulation 1107/2009 for the aquatic testing is the inclusion of an acute test for a second aquatic invertebrate (i.e., besides *Daphnia*) as a basic data requirement for insecticides and substances with insecticidal activity. Recently, Brock and Van Wijngaarden [2012. Environ. Sci. Pollut. Res. 19:3610-3618] evaluated these new requirements by comparing the threshold concentrations for treatment related effects of 31 insecticides, as derived from aquatic micro-/mesocosm tests with the predictive value of the European Tier-1 acute effect assessment on basis of laboratory toxicity tests with *Daphnia magna*, *Chironomus* spp., *Americamysis bahia* and *Gammarus pulex*. They concluded that the Tier-1 procedure on basis of acute toxicity data (EC50/100) for the combination of *Daphnia* and *A. bahia* and/or *Chironomus* overall is protective to pulsed insecticide exposures in micro-/mesocosms. The number of compounds that can be evaluated with model ecosystem studies and the number of populations of (potentially sensitive) species that are present in these test systems is by definition limited (e.g. species for a specific lentic or lotic community only). To obtain another line of evidence, a different approach was followed in the present study to evaluate the predictive value of the Tier 1 approach on basis the core data-set. To this end, we compiled toxicity data from single species tests evaluating insecticides with aquatic invertebrates and subsequently compared these with that of *Daphnia magna*, with or without an additional invertebrate species (*C. riparius* or *A. bahia*), by applying the assessment factors as set in the first-tier risk assessment in the EU. Implications and remaining uncertainties for the risk assessment of aquatic invertebrates under the new data requirements are discussed.

**164 OECD harmonized international guidance and ecoregion crosswalk for pesticide terrestrial field dissipation studies (Part 1)** M. Mitchell, Health Canada / Pest Management Regulatory Agency; R. Gangaraju, Health Canada; M. Shamim, Office of Pesticide Programs, US-EPA; I. Nicholson, Health Canada / Pest Management Regulatory Agency; M. Egsmose, EFSA European Food Safety Authority; B. Grenier, OECD. Data on pesticide terrestrial field dissipation/accumulation (TFD) are required by regulatory agencies worldwide for the registration of pest control products. Use of studies conducted at foreign sites can strengthen regulatory decisions and reduce burdens for both the regulated and regulatory communities. Studies conducted at foreign sites are generally acceptable if the study objectives, protocol and methodology are harmonized and the study sites represent local use conditions. A chemical is expected to behave similarly in ecoregions that are based on similar soils and climate.

Neither internationally harmonized TFD guidance or Europe-North American ecoregion comparison maps are currently available. Harmonized TFD guidance (led by the US Environmental Protection Agency) and an ecoregion crosswalk (led by Health Canada) are therefore, being developed under OECD project. **Guidance for TFD Studies:** In North America, the objective of a TFD study is to determine the fate and behaviour of a chemical when it is used according to label directions in representative use areas and all factors of transformation and transport are acting together. In the European Union (EU), the objective is to determine the DegT<sub>50</sub> and persistence under conditions where surface losses such as runoff, photolysis and volatilization are minimised. The proposed methodology is based on a conceptual model and modular approach. Guidance is provided on a basic study to determine persistence, residue carryover, transformation, leaching, formation and decline of transformation products, DegT<sub>50</sub>, and routes of dissipation, with additional guidance on modules to determine volatilization, surface runoff, leaching to ground water, etc. **Ecoregion crosswalk:** The second component of the project is the development of ENASGIS (Europe North America Soil Geographic Information for Pesticide Studies). This model identifies similar ecoregions between Europe and North America which will facilitate a study conducted in Europe to be considered by the North American regulators and vice versa. In Europe, FOCUS guidance is used to facilitate use of overseas TFD studies for regulatory purposes in EU. In addition the model permits selection of field sites based on concerns identified by the conceptual model. The use of field studies conducted at foreign sites for national and global joint reviews would strengthen regulatory decisions and reduce regulatory burdens for both registrants and regulators.

**165 OECD Harmonized International Guidance and ecoregion Crosswalk for Pesticide Terrestrial Field Dissipation Studies (Part2)** m.t. shamim, USEPA / OCSPPOPPUSEPA; R. Gangaraju, Health Canada / EAD PMRA; M. Egsmose, EFSA European Food Safety Authority. The OECD ecoregion crosswalk project was initiated to develop harmonized guidance for conducting terrestrial field dissipation (TFD) studies and to develop an ecoregion crosswalk across North America and Europe. The TFD studies assess the transformation, transport, and fate of pesticides under representative actual use conditions. They also validate and/or refine the relative importance of selected modules of dissipation such as soil abiotic/biotic processes and the processes of leaching, volatilization, run-off, plant uptakes, DegT<sub>50</sub> and others. The major objective of this project is to determine if studies conducted at a specific site in North America or Europe can be used across international borders based on the similarity of the ecoregion in North America and Europe. US-EPA has the lead for harmonization of the guidance for terrestrial field dissipation studies while PMRA-Canada has the lead for developing the ecoregion crosswalk component. EFSA is co-lead for both projects. This includes development of the GIS-based ENASGIS (Europe North America Soil Geographic Information for Pesticide Studies) model that is based on the understanding that site-specific conditions such as soil and climate play major role in determining the fate and behaviour of a pesticide in addition to vegetation, pesticide intrinsic properties and the use pattern. These environmental variables can be categorized into "ecoregions" and the fate and behaviour of a chemical is expected to be similar in ecoregions with similar environmental variables. This presentation will focus on the recommendations from the OECD ecoregion crosswalk workshop held in Ottawa, Canada in March 2011 to solicit input from experts on issues related to harmonization of terrestrial field dissipation guidance and construction of ecoregion crosswalk model, ENASGIS. It will also provide the status of the OECD project and the progress made in finalizing the draft guidance document and updating the ecoregion crosswalk model. *Note: The content of this presentation does not necessarily represent the official views of the OECD or of the governments of its member countries and EFSA.*

**166 Quantifying Soil Surface Photolysis under Conditions Simulating Water Movement in the Field: A New Laboratory Test Design** L.H. Hand, Syngenta Limited / Product Metabolism; C.

Nichols, S. Kuet, R. Oliver, Syngenta / Product Metabolism; C.M. Harbourn, Waterborne Environmental, Inc.. *New guidance on the conduct of terrestrial field dissipation studies in Europe require that surface processes, such as photolysis, are excluded by incorporation of the active ingredient into the bulk soil. The role of surface processes must now be addressed separately, through higher tier studies are required to better understand the contribution of surface processes under field conditions. Since minimal light penetration occurs below the top 1-2 mm of the soil surface, it is often assumed that, once a compound has moved out of this "photolytic zone" following a rainfall event, no further photolysis can occur. However, as both downward and upward water movement occurs under field conditions, there is the potential for relatively mobile compounds to return to the surface, prolonging effective exposure to UV light and increasing the potential contribution of photolysis. To test this hypothesis, a novel test system was designed to assess the contribution of photolysis, under more realistic conditions, to the dissipation of a new herbicide, bicyclopyrone. This compound dissipates rapidly in field dissipation studies but microbial soil metabolism is relatively slow. Thin layer soil photolysis studies have shown susceptibility to photolysis; however its contribution to dissipation in the field could not be established from these studies. Soil cores were taken from three US field study sites and treated with <sup>14</sup>C-bicyclopyrone. The radioactivity was moved away from the surface using a simulated rainfall event and the cores were incubated under a Xenon-arc lamp with continuous provision of moisture from below. The light intensity, light/dark cycle and soil surface temperature were set to mimic the US field study conditions as closely as possible. Cores were removed at intervals, sectioned to different depths and analysed for total radioactivity and remaining bicyclopyrone and its photodegradates. After only 2 days, most of the radioactivity had returned to the soil surface. Significantly more degradation was observed in the irradiated samples than in a parallel dark control sample. The degradation rates in the new core study were very similar to those observed in both the thin layer photolysis study and the field studies and was significantly faster than the regulatory soil metabolism studies, indicating that photolysis can be a significant process under field conditions, even after initial movement out of the photolytic zone by rainfall.*

**167 Development of country specific weighting factors and external cost of environment** K. Murakami, N. Itsubo, Tokyo City University; K. Kuriyama, Kyoto University; K. Yoshida, Nagasaki University; K. Tokimatsu, Advanced Industrial Science and Technology. LIME, an advanced life cycle impact assessment method based on endpoint modeling, has been developed as part of the LCA national project of Japan. One of the aims of LIME is to develop the weighting methodology, which enable us to integrate various environmental impacts that are used for life-cycle impact assessment (LCIA) and facilitates the interpretation of environmental information, such as in the selection of products. This project has now reached to the third phase with the additional aims, one of which is to update the weighting factors from the national average (LIME2) to the global scale (LIME3). We report our preliminary results obtained by pilot survey at five countries: Japan; China; Vietnam; South Africa; Kenya. The calculated results can be used to develop integration factors in LIME3, enabling us to express LCIA results as a single index, such as external cost.

**168 Economic valuation of environmental impacts under REACH - possibilities and limitations** N.M. Deleebeeck, Arcadis Belgium / REACH & Product Stewardship Services; H. Descamps, Arcadis Belgium; J.K. Verhoeven, M. Beekman, D. Sijm, National Institute for Public Health and the Environment (RIVM); S. Bogaert, Arcadis Belgium. The European REACH Regulation foresees procedures for Restriction and Authorisation of chemicals that (may) give rise to unacceptable risks to human health and/or the environment. Under both procedures, a socio-economic analysis must/may be performed to weigh the costs and benefits to society of different scenarios (e.g., discontinued use versus continuation of use, continuation of use versus use of an alternative substance or technology) in terms of economic, social, human health and environmental impacts. The outcome of the socio-

economic analysis is taken into account when the European Commission takes its decision on the continuity of use. Ideally, all costs and benefits are compared in a cost-benefit analysis using the same denominator (e.g., Euros). Therefore, it needs to be investigated to what extent human health and environmental impacts resulting from production and use of a REACH substance can be monetised. Monetisation of environmental impacts seems to be more challenging compared to monetisation of human health impacts, as generally, much more uncertainties need to be dealt with. Currently, no guidance documents have been issued by the European Chemicals Agency on this specific topic. Therefore, RIVM asked Arcadis Belgium to investigate the applicability of existing methods for economic valuation (revealed preference, cost-based, and stated preference methods) for monetisation of environmental impacts under REACH. A thorough evaluation was done of pros and cons, data needs, data availability (e.g., for benefit transfer), compatibility of the different methods with typical outcomes of an environmental impact assessment, etc. This resulted in a conceptual framework that should assist the user in 1) deciding whether or not it is possible/useful to monetise the environmental impacts, and 2) selecting the most suitable method when it is decided to proceed to the step of monetisation. Consequently, several illustrations were elaborated for further testing the applicability, possibilities, and limitations of the preferred methods. Based on the outcome of this exercise, data gaps were identified and recommendations were made for future research.

**169 Impact-pathway approach and metals: monetisation of external costs of cadmium emissions to soil from agricultural fertilizer** M. Pizzol, Aalborg University / Development and Planning; J. Smart, Griffith University / Griffith School of Environment; M. Thomsen, Aarhus University. We applied the Impact-Pathway Approach (IPA) for the monetary evaluation of health-related impacts of Cadmium (Cd) emitted to soil via agricultural application of phosphorous-rich fertilizers in Denmark. Due to the high persistency of Cd in soil, and high soil-to-plant transfer rates, humans may be exposed to Cd through their diet, with adverse health impacts. We used the Simplified Fate and Speciation model (SFSM) to calculate Cd increase in soil for different future scenarios of agricultural application of fertilizer to Danish soil. Based on soil-plant bio-concentration factors and Danish dietary intake rates, we determined human exposure. We used updated dose-response functions (DRF) linking lifetime Cd intake with the probability of developing Cd-induced renal disease and osteoporosis. These impacts are converted into monetary values by using the EU standard value of VOLY (40000€) adjusted for quality of life experience. Total costs over 100 years are then annualized and discounted at 3% to present value to obtain the external costs. Results show that discounted external costs of Cd are of ?3800 [€/kg Cd] for sludge application, and ?4100 [€/kg Cd] for mineral fertilizer, due to the lower Cd content of sludge in kg dry matter. When P content is considered, costs are of ?0.092 [€/kg P] for sludge and ?0.089 [€/kg P] for mineral fertilizer, due to the higher content of P in the latter. Since the method is under testing, such values are considered as only preliminary. The pilot study is a unique example of monetization for a previously uninvestigated impact pathway. It allows exploring the IPA approach beyond its classic applications (e.g. airborne non-persistent contaminants) and to address critical issues such as uncertainty assessment, long-term modeling perspective and discounting. The study provides useful insights to Life Cycle Impact Assessment (LCIA). Most LCIA methods apply a similar emission-to-impact causal chain approach, and the same models can be used in IPA/LCIA. Although IPA is applied to a single contaminant, IPA is more detailed than LCIA concerning the definition of temporal and spatial conditions. The innovative modeling of Cd fate in soil and the identified DRF could also be applied, for example, to improve LCIA of metals.

**170 Spatially explicit characterization factors for damage costs of soil erosion due to agricultural land occupation on a global scale** R. Van Zelm, Radboud University; M. van der Velde, IIASA; T. Koellner, University of Bayreuth; M. Nunez, IRTA; M. Obersteiner, IIASA; E. Schmid, University of Natural Resources and Life Sciences; M.A. Huijbregts, Department of Environmental Science. A main cause

of increased soil erosion is agricultural land use, as the cultivation (irrigation, ploughing, soil processing etc.) of crops contributes to increased soil loss, which on its turn degrades arable land. The objective of this work is to provide spatially explicit characterization factors (CFs) for erosion regulation due to crop cultivation for the world. CFs are provided for land occupation by cassava, rapeseed, sunflower, sugarcane, soybean, corn, and wheat for three levels of agricultural intensification defined by fertilizer and irrigation uses. Because different management of the crops results in different crop yields, the characterization factors express the damage caused per kg of crop and not per m<sup>2</sup> of land use, which has been more common up to now. The damage is in terms of extra onsite (water and nutrient) and offsite (problems caused by the soil particles) costs (in US \$/kg crop) due to soil loss caused by crop production. To simulate erosion rates, a global implementation of the biophysically based agro-environmental simulation model EPIC (Environmental Policy Integrated Climate) was employed. EPIC is driven by spatially explicit information on climate, weather, topography, soil and agricultural management practices and operates on a 30 arcmin scale. Rangeland and trees were simulated with EPIC around the world as an estimation of the potential natural vegetation when there would be no crop cultivation. On- and offsite erosion costs in 2012 were estimated to be 13 US \$ per ton of eroded soil. Largest CFs were found around and below the equator. For some grids, the potential natural vegetation showed larger erosion rates than crop cultivation, hence negative characterization factors were derived. When aggregating to country level (yield-weighted), the negative factors showed no influence due to the insignificant yields in these grids compared to the overall country yield. Larger CFs are obtained when management without input of nutrients or irrigation is performed. Small yields obtained without fertilizer and irrigation generally lead to higher costs per kg crop due to erosion than when fertilizer and irrigation are applied. CFs for the 7 crop types vary up to 6 orders of magnitude from each other, with soybean cultivation generally leading to the lowest CFs per country and rapeseed to the largest CFs. The high variability in CFs per crop, grid, and input scenario show the importance of the applied method.

**171 Assessing the impacts of abiotic resource use: application to electric vehicles batteries** V. De Bruille, CIRAIG École Polytechnique de Montréal / Chemical Engineering; C. Bulle, CIRAIG Polytechnique Montreal / Chemical Engineering; T. Dandres, CIRAIG; C. Gaudreault, NCASI; O. Jolliet, University of Michigan / School of Public Health; R. Samson, Ecole Polytechnique de Montreal / Department of Chemical Engineering. In this study, a new assessment method is proposed to assess the impacts from resources use, as none of the previously existing methods is considered mature enough to be recommended in the ILCD handbook. This new approach allows impact monetisation based on resource functionality, future abundance and the financial consequences of the use of resources. This allows considering an effect factor expressing the extent to which the extraction of a certain amount of abiotic resource forces a future user to pay more to preserve the function provided by the resource as it becomes scarcer. It is translated by the difference between the cost for a resource to fulfil a given functionality today and that of the back-up alternative. In specific, this project aims at including the main affected users and at defining both the increase in cost to avoid functionality loss and substitution costs. The environmental impact is obtained by multiplying the effect factor by a competition factor, which is a material competition scarcity index (MACSI) varying between 0% and 100% and defined to assess the competition. The MACSI is based on the “remaining years of availability”: a ratio between resource consumption and available known stock, representing the years left before total dissipation of a resource at current dissipation rate. Resource dissipation is the amount used from which recycling and reuse is subtracted. This choice was made considering that resource will remain (partly-) functionally equivalent, even after the end of a product life through reuse or recycling. The approach was illustrated by applying it to metallic resources used in electrical vehicles batteries to calculate the impact on natural resources depletion. Results have been compared with the impacts obtained with

other LCIA methodologies to put in perspective advantages and drawbacks of each of them and see to what extend results obtained are consistent when applying different approaches showing different degrees of sophistication. Inconsistencies between methodologies are put into light as the ranking between contributors to the impact differ from one methodology to the other. This indicates the need for clarification on how resource depletion should be assessed in LCA and for the development a coherent framework for that assessment.

**172 Monitoring herbicide toxicity in rivers of the Great Barrier Reef catchments using benthic diatom community changes** R.J. Wood, P. Depresle, S. Mitrovic, University of Technology, Sydney; R. Prasad, S. Choy, Department of Science, Information Technology, Innovation and the Arts; B.J. Kefford, University of Technology Sydney / Department of Environmental Science. The extensive use of herbicides in agricultural regions of the Great Barrier Reef (GBR) catchment area in North-east Australia, has resulted in the widespread contamination of its waterways. Routine chemical monitoring within GBR catchments has regularly detected concentrations of herbicides over the recommended trigger values for ecological protection. Freshwater ecosystems are at greatest risk of herbicide toxicity due to their proximity to the source of herbicide application in the environment. Sensitive monitoring tools are needed to be able to determine the ecological impacts of herbicide toxicity. This study aims to develop a new method for monitoring herbicide toxicity in rivers using benthic diatom community changes. Despite the importance of benthic diatoms as primary producers and as potential bioindicators, there have been few studies on their sensitivity to herbicides. This study will address the current knowledge gap, by examining the sensitivity of freshwater diatoms to herbicide exposure through rapid toxicity testing. The effect of pre-exposure and light intensity on the relative sensitivity of diatoms genera was examined for the herbicides; Atrazine, Simazine, Hexazinone, Tebuthiuron, Diuron, MCPA, Glyphosate, 2,4-D. Preliminary results indicate the following diatom genera sensitivity ranking: Navicula > Achnanthes > Synedra > Cymbella > Gomphonema. Diatom sensitivity rankings remained consistent between the herbicides tested. The sensitivity data presented was used to establish a new biomonitoring index, the SPEcies At Risk (SPEAR) index, which uses this toxicity data to determine the fraction of the abundance of sensitive taxa in a community at particular sites. The new index will be used to determine if the abundance of benthic diatom genera sensitive to herbicides declines across a herbicide exposure gradient consisting of 14 sites within the GBR catchment. The results of this project to date will be assessed in terms of developing a new SPEAR index that uses benthic diatoms as an indicator to provide a cost effective and ecologically relevant method for the detection and assessment of herbicide impacts in rivers flowing into the GBR.

**173 Zoning and spatiotemporal evolution of PAHs, described combining sediment concentration, baseline toxicity, bioaccumulation in fish and TEC for fish** E. Rojo-Nieto, Cactymar University of Cadiz / Department of Environmental Technologies; J. Perales, CACYTMAR-University of Cadiz / Department of Environmental Technologies. In this work, fate and effects of PAHs in the Bay of Algeciras, a semi-enclosed coastal zone subject to an intense industrialization, have been studied. This Bay has suffered a chronic anthropogenic pressure, due to urban (five urban areas with more than 250,000 inhabitants) industrial activities (petrochemical and metallurgical industry), and to the intense maritime traffic (Algeciras Harbor is ranked among the most important ports of the world). In previous studies occurrence and levels of PAHs in sediment were studied, and a zoning of the bay according to contamination level and source was proposed. Additionally, sources, transport and fate of these compounds in sediments were studied obtaining a spatiotemporal evolution. The aim of this work was to study if different parameters related to biota, as Bioaccumulation in feral fish, Baseline Toxicity for marine organisms (described by the chemical activity) and Toxicity Equivalent Concentration for fish of PAHs found in sediments (TECs), could provide a similar zoning and spatiotemporal

evolution that produced by sediment concentrations, to define PAHs pollution in the study area. The results obtained show that the study of the total concentration, the interstitial concentration and the chemical activity of PAHs, in marine sediments from chronically polluted environments (and the translation of them to other parameters, through well-defined factors), provides fairly accurate knowledge about the distribution and spatiotemporal evolution of the environmental risks associated to their presence. However, since in natural environments rarely the environmental compartments are in total equilibrium and since the biological effects and bioaccumulation are influenced by several factors such as the metabolization of the compounds under study, the analysis of biotic compartment can not be completely ignored, being this compartment essential to define, among others, site/species-specific BSAs.

**174 Toward a tailored ERA tool for mosquito control practices in France** M. Roucaute, INRA / UMR ESE; L.L. Lagadic, INRA / UMR INRAAgrocampus Ouest Ecology and Ecosystem Health; T. Caquet, INRA / UMR ESE 0985. In metropolitan France, mosquito control mainly targets larval stages and, in most cases, this means spraying insecticides directly into natural aquatic ecosystems. This obviously raises the question of the risk of such practices for non-target organisms especially in Natura 2000 protected areas where the European legislation now requires the implementation of incidence studies of human activities. Therefore, there is a need for tools that are able to detect potential environmental effects of biocides used for mosquito control. Indices such as SPEAR (SPECies At Risk) aim at detecting effects of the exposure to chemicals (e.g., pesticides) on invertebrate communities in streams. They do not make assumption about the pattern of exposure and rely on a generic vulnerability profile of species toward chemicals. In the context of properly managed mosquito control, the nature, rates and strategies of application of authorised products are known. It is therefore possible to assess the real exposure pattern to larvicides. Based on such knowledge, it is feasible to develop an ERA tool specifically designed for detecting the impacts of mosquito control on the most vulnerable part of aquatic biota. We applied this approach to the case of two insecticides used for larval mosquito control: *Bti* (*Bacillus thuringiensis* var. *israelensis*), the only currently authorized larvicide in France, and S-methoprene, a candidate compound. Based on published laboratory acute toxicity data for both compounds, we ranked the species found in the samples from a past environmental survey according to their level of sensitivity. The assessment of the potential effect of treatments was then performed by comparing toxicity thresholds to predicted larvicide environmental concentrations based on currently used application rates. Such an approach may allow the a priori assessment of the risk of mosquito control practices on the invertebrate community of a given ecosystem. It may also be used to focus long term surveys on the most vulnerable part of this community. The rationale of the method, the results of species sensitivity ranking and a comparison of the outcomes of this approach with the results of field invertebrate community studies are presented.

**175 Using observational field data for risk assessment of nutrients in freshwaters** L.B. Azevedo, Radboud University Nijmegen / Department of Environmental Science; R. Van Zelm, Radboud University; R.S. Leuven, Radboud University Nijmegen / Department of Environmental Science; J.A. Hendriks, Radboud University Nijmegen / Department of Environmental Sciences; M.A. Huijbregts, Department of Environmental Science. We present a framework in which observational field data can be used to derive concentration-response relationships of the effects of nitrogen (N) and phosphorus (P) on species richness. To attain that, we first gathered data on individual species occurrences and N and P levels in temperate lakes and streams. We included autotrophs and heterotrophs as the two main species groups in our analysis. Second, we determined the species richness along TP and NO<sub>3</sub> levels separately and used log-logistic regressions to calculate the potentially not occurring fraction (PNOF) of freshwater species along the two stressors. Finally, we tested if the species richness effects could be explained by concentration addition of combined N and P

stressors. At SETAC, we will (1) illustrate how logistic regressions illustrating the effects of nutrient stress to species richness can be obtained from observational field data; (2) test if concentration addition of N and P explains our empirical data; and (3) provide ecological grounds for the difference between effects of N and P, in lakes and streams, and for autotrophs and heterotrophs.

**176 Competition between an indigenous and invasive snail (Physidae) under impact of additional stressors (temperature, fungicide)** R. Mueller, LOEWE Biodiversity and Climate Research Centre / Aquatic Organisms and Ecosystems; A. Seeland, Goethe University Frankfurt Main; J. Albrand, Helmholtz Centre for Environmental Research GmbH - UFZ; D. Jilani, Goethe University Frankfurt; J. Oehlmann, Johann Wolfgang Goethe-Universität Frankfurt am / Aquatic Ecotoxicology. Aquatic species are highly influenced by abiotic and biotic factors such as temperature and pollution (e.g. pesticides) or intra- and interspecific competition. Moreover, the ongoing process of climate change enforces these impacts and leads to far-reaching shifts in the aquatic environment. Beyond, these disturbed environments may favour invasive species. Within this context, the aim of the present study was to investigate the potential of the invasive snail *Physella acuta* to displace the indigenous *Physa fontinalis* out of their natural habitat under a multistressor situation. To investigate the temperature-dependent sensitivity of these pulmonates towards the fungicide pyrimethanil, a juvenile growth test (dose-response-design) at optimal and suboptimal temperatures (15°C, 20°C and 25°C) was conducted. Furthermore, the competitive relationship between both species was studied directly with juveniles at a sublethal concentration (0.1 mg pyrimethanil L<sup>-1</sup>) and 20°C using a response-surface-design. Both stressors, temperature and the fungicide pyrimethanil, caused singular and interactive effects on both species. However, the growth of the indigenous *P. fontinalis* was more severely influenced by pyrimethanil compared to the invasive *P. acuta*, especially at higher temperatures. The direct competitive situation depicts that *P. acuta* points to a lower mortality rate and faster development than *P. fontinalis*. Our results clearly show that *P. acuta* has a clear competitive advantage compared to *P. fontinalis*, in particular with regard to progressive climate and environmental changes. Therefore it can be concluded that *P. fontinalis* will be most likely displaced from its habitats by *P. acuta* with increasing frequency.

**177 Combined exposure of *Chlamydomonas reinhardtii* to copper and solar-simulated radiation** G. Cheloni, Aquatic Biogeochemistry and Ecotoxicology, Institute F.-A. Forel, Earth and Environmental Sciences, Faculty of Sciences; E. Marti, V.I. Slaveykova, University of Geneva / Aquatic Biogeochemistry and Ecotoxicology, Institute F.-A. Forel, Earth and Environmental Sciences, Faculty of Sciences. In natural aquatic environments photosynthetic organisms experience variable levels of light composed of photosynthetically active radiation (PAR) and UV radiation. High PAR intensities have a direct effect on algal photosynthesis, while high UV radiations, besides being harmful for the organisms, may alter the biogeochemistry of redox sensitive metals and thus their chemical speciation and bioavailability. However in laboratory toxicity tests these organisms are grown under artificial light which consist of only PAR at very low intensities, variations in light intensity and their effect on both organism physiology and metal bioavailability are not taken into account. Considering the paucity of information concerning the metal toxicity in complex aquatic environments, in this work we explored the combined effect of solar-simulated light and copper on the green alga *Chlamydomonas reinhardtii*. *C. reinhardtii* cells were exposed to two different free copper ions concentrations (6x10<sup>-8</sup> and 6x10<sup>-6</sup> M) representative for slightly and heavily contaminated water bodies. Three different light conditions were tested: 1) a low PAR typical for laboratory tests, 2) a solar-simulated light (PAR+UV) with spectral distribution and intensity comparable to what measurable at midday in a winter clear day at mid latitude in Europe and 3) a solar-simulated light (PAR+UV) with increased UV radiation, aimed to simulate future changes in UV scenarios. Short term effects of copper on algal cells under different

light conditions were determined using flow cytometry in order to evaluate changes in algal population features at single cell level and, using different fluorescent dyes, changes in membrane permeability, oxidative stress and lipid peroxidation. Combined exposure to copper and solar-simulated light revealed a synergic effect with increased damages compared to those obtained in cultures exposed to copper or solar simulated radiation alone. Cells irradiated with increased UV radiation presented an enhanced membrane permeability and experienced stronger oxidative stress than cultures exposed to laboratory light conditions. The membrane alteration potentially resulted in an increased copper bioavailability explaining in part the synergic effect observed.

#### **178 Validation of the iSQ (invertebrate soil quality) Chip for Ecological Effects Assessment of Soil Quality Management G.**

**Chen;** T. De Boer, Vrije Universiteit / Ecological Sciences; A. Vakhrusheva, Vrije University Amsterdam; N.M. van Straalen, Vrije Universiteit Amsterdam / Inst. of Ecological Science; D. Roelofs, Vrije University Amsterdam. Environmental pollution is a worldwide problem, and the ability of soil to function properly is threatened by soil contamination. Transcriptomic tools in ecologically relevant organisms, such as springtails, enhance classical ecotoxicological research by presenting more descriptive mode of action of soil pollutant. To study ecological responses to soil conditions, an invertebrate Soil Quality (iSQ) Chip (74k gene clusters) for the springtail *Folsomia candida* were developed and applied. Animals were exposed field samples collected from river Dommel area in Netherlands, then reproduction rate of juveniles and gene expression profiles were measured. We identified different transcription profiles in different field soil samples, and acquired specific responded genes and metabolic pathways for each soil samples. Also, according to diverse expression patterns among different soil samples, some genes (e.g. abc transporter genes) may potentially be a good indicator to screen potentially polluted sites, in order to identify the ones that need further ecotoxicological investigation. Combining with soil chemical analysis, we discovered some biotic stress factors may disturb traditional ecotoxicological test. This study shows that in order to apply transcriptomic analysis tool to soil quality management, more insight is needed to understand the complexity of field samples and better utilize classification method to predict contamination in soil samples. Key words: soil; ecotoxicogenomics; microarray; springtails.

#### **179 Unraveling mode of actions and toxin profiles with high throughput microarrays: a case study in *Daphnia* exposed to different cyanobacterial stressors.**

**J. Asselman,** Ghent University / Laboratory of Environmental Toxicology; M. Pfrender, J. Lopez, Notre Dame University; J.R. Shaw, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; K. De Schamphelaere, Ghent University UGent / Environmental Toxicology and Aquatic Ecology. Over the last decade, molecular technologies have evolved into robust high throughput platforms available for many scientists in a wide variety of disciplines. Yet, knowledge of stress responses and mechanisms of toxicity across stressors remains limited. Therefore, we have studied stress response to 6 distinct cyanobacterial species in *Daphnia pulex*. By studying effects on gene level with high throughput microarray technology and effects on organism level through standard ecotoxicology methods for a group of stressors simultaneously, this study aimed at identifying and unraveling stress response patterns and mechanisms of toxicity. Furthermore, by first considering all 6 species as cyanobacterial stress in general without distinction, it was possible to identify mechanisms involved in general cyanobacterial stress response. Four day old Juveniles of *Daphnia pulex* were exposed to a diet contaminated with cyanobacteria for a period of ten days. The experiment consisted of six different treatments, representing six different species of cyanobacteria. Afterwards, RNA was extracted, reverse transcribed to cDNA and hybridized to a two color whole transcriptome microarray. Image data was processed and normalized in R using Limma. Data analysis revealed six pathways involved in a general cyanobacterial stress response. These could be divided in three groups of mechanisms. First, pathways

involving detoxification (Cytochrome P450 and Glutathione) were significantly induced in organisms exposed to cyanobacterial stress. This could potentially be a response to the toxins produced by the cyanobacteria as these pathways are known detoxification steps for the cyanobacterial toxins. Second, pathways involved in steroid and poly unsaturated fatty acid synthesis, were also significantly induced. This may potentially be linked to the lack of steroid and PUFA's in cyanobacteria in contrast to green algae as a nutrient source. Third, we observed a significant inducement of genes in pathways related to the energy metabolism (ubiquinone cofactor and carbohydrate metabolism). By combining expression of exposures to different cyanobacteria, it was possible to gain insights in mechanisms involved in cyanobacterial stress response that could be potentially related to effects at the organism level. Finally, species specific stress response for each cyanobacteria can be identified by contrasting the expression profiles with these mechanisms of general cyanobacterial stress.

#### **180 Dose-dependent effects induced in *Arabidopsis thaliana* after uranium (U) exposure at pH 7.5**

**E. Saenen,** SCKCEN / Biosphere Impact Studies; n. Horemans, Belgian Nuclear Research Centre SCKCEN; N. Vanhoudt, Belgian Nuclear Research Centre (SCK-CEN); H. Vandenhove, Belgian Nuclear Research Centre, SCK-CEN / Biosphere Impact Studies; G. Biermans, SCKCEN; M. Van Hees, Belgian Nuclear research centre (SCK-CEN); J. Wannijn, Belgian Nuclear Research Centre (SCK-CEN); J. Vangronsveld, A. Cuypers, Universiteit Hasselt. To evaluate the environmental impact of U contamination, it is important to unravel the mechanisms by which plants respond to U stress. It was already shown that U exposure at pH 5.5 can disrupt the cellular redox balance and induce oxidative stress related responses in *Arabidopsis thaliana* plants. However, U speciation strongly depends on environmental pH. Since U uptake and hence its toxicity is influenced by U speciation, it is important to investigate the effects of U at different pHs. This study aimed to investigate dose-dependent effects of U at pH 7.5. *A. thaliana* plants were exposed to a U concentration range at pH 7.5 during 3 days. U concentration and fresh weight were analysed. Enzymes and metabolites of the antioxidative defence system were analysed on protein level. Effects at molecular level were analysed by real-time PCR. A significant decrease in root and shoot fresh weight was observed after U exposure. Together with an increased dry weight, this can be an indication that the plants are stressed. In the leaves, the antioxidative capacity is increased by an increased concentration of total and reduced ascorbate (Asc), a metabolite important in the antioxidative defence. In the roots however, the redox status could not be maintained. Here, a decrease in reduced Asc and an increase in dehydroascorbate was found, indicating that the roots are damaged after exposure to U. By analysing enzyme activities in the roots, increased activities in Asc peroxidase (APX) and guaiacol peroxidase (GPX) were found. Together with the changes in Asc concentrations, the increased APX activity can be an indication for the importance of Asc under U-stress. GPX in turn is important in cell wall lignification. The increased GPX activity can indicate an enhanced lignification of the root endodermis. This can be a response to increase cell wall binding sites in the roots and hence limit the transport of U to the shoot. By analysing the U responses at molecular level, the involvement of microRNA398b/c was observed. MIR398b/c binds to mRNA of *CSD1/2* and hence, a decreased expression of the latter genes was observed. However, CSDs are indispensable, and an increase in root *FSD1* expression was observed, that probably guaranteed sufficient ROS-detoxification capacity. In conclusion, elevated U-concentrations at pH 7.5 can cause important morphological, physiological and biochemical effects in *A. thaliana* seedlings.

#### **181 The informative value of transcriptomics in combination with the fish embryo test**

**M. Fenske,** Fraunhofer Gesellschaft IME / Institute for Molecular Biology and Appl. Ecology; E. Muth-Koehne, Fraunhofer Institute for Molecular Biology and Applied Ecology IME / Department of Ecotoxicology; V. Schiller, Fraunhofer IME / Institute for Molecular Biotechnology (Biology VII); V. Delov, RWTH Aachen University / Institute for Molecular Biotechnology (Biology VII); A.



Wichmann, H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V); R. Kriehuber, Jülich Research Centre / Radiation Biology; M. Hecker, University of Saskatchewan / School of the Environment & Sustainability; C. Schaefer, FraunhoferInstitut / Department of Ecotoxicology. In aquatic ecotoxicology, the fish embryo toxicity test FET can be considered as one of the most promising animal alternative methods. Internationally, considerable endeavours have been made to bring the FET to acceptance as a valid alternative method to the fish acute toxicity test (OECD203). However, numerous studies have repeatedly shown that the FET can deliver more than just acute toxicity data, in particular when molecular endpoints are included. Notably postgenomic approaches, like transcriptomics using microarrays or qPCR, and fluorescent microscopy-based morphometrical analyses, are well suited to evaluate subacute or teratogenic effects mechanistically due to their high sensitivity and information rich data. In a proof-of-principle approach, we conducted gene expression studies with zebrafish and medaka embryos after exposure (for 48 hpf to 120 hpf and 7 days, respectively) to different groups of chemicals. For each chemical, we conducted a standard FET (48h), and for the insecticides also extended FETs (120h) in 96-well plates. Embryos exposed to a selection of the tested substances were subjected to whole-genome expression analysis (using the Agilent® Zebrafish Gene Expression Microarrays) or quantitative PCR. To exclude non-specific stress responses, embryos were exposed to concentrations at EC<sub>10</sub>, EC<sub>50</sub>/2 and in some cases at EC<sub>100</sub> level for the gene expression experiments. Estrogenic and anti-androgenic compounds (ethinylestradiol-EE<sub>2</sub>, flutamide, genistein, bisphenol A-BPA, linuron, prochloraz) were studied in both species. Thirteen insecticides of known acute adult fish toxicity, representing four different modes of action were tested only in zebrafish. Insecticides classified as 'energy metabolism modulators' by IRAC displayed a higher number of differentially expressed genes than those classified by 'nerve action'. Intersections revealed that insecticides with functionally similar MoAs share a higher number of differentially expressed genes than those with dissimilar ones. Functional analysis showed that regulated genes relate to molecular functions, which are linked to the morphological effects and the given MoA of an insecticide. For the EDCs, responsive pathways comprising endocrine relevant pathways were found in both species and for all chemicals. An estrogenic mode of action was clearly discernible, and the transcriptome of all tested substances showed patterns indicating either a predominantly estrogenic or antiandrogenic activity.

**182 Assessment of adverse effects of environmental contaminants in complex mixture using physiological, transcriptional and metabolic endpoints in fish** H.R. Habibi, University of Calgary / Biological Sciences; A. Zare, Department of Biological Sciences; J. Jordan, University of Calgary / Biological Sciences; S. Henderson, Biological Sciences; A. Weljie, L.J. Jackson, University of Calgary / Biological Sciences. Environmental contaminants including natural and synthetic steroids, organic compounds and pharmaceuticals were detected at various sites sampled along the Southern Rivers in Canada. The nature of contaminants was different depending on the location and extent of agriculture activities, upstream and downstream of wastewater treatment plants. Trace amounts of pharmaceuticals, phthalate esters, and all nonylphenol ethoxylates were observed in receiving river waters of Southern Alberta. In field studies, we used longnose dace as an abundant local species to assess health impact of contaminants in Southern Alberta Rivers. A significant increase in female to male adult ratio was observed in longnose dace caught down stream of certain municipalities along the Oldman River correlating with high levels of vitellogenin expression in male longnose dace. These observations suggested severe endocrine disruption of gonadal development likely due to presence of compounds with estrogen-like activities. To investigate the effects of chemicals detected, we performed controlled laboratory experiments in which fish in aquaria were exposed to the same concentrations of a selected number of chemicals detected in the river system, individually and as mixtures. We used various biological end points, including morphological abnormalities, transcriptomics and metabolomics in

samples obtained from liver, gonads and brain of fish. The results demonstrate that different chemicals present in the Oldman River disrupt the gene expression profile and metabolism of the liver, ovary, testis, and brain. Significant changes were observed in gene expression profile as well as dysregulation of amino acid, lipid, energy, carbohydrate, nucleotide and cofactor/vitamin metabolism. The effects of mixture of contaminants were significantly different from the individual treatments in the liver and testis. The results provide novel information on the effect of contaminants individually and in mixture on global metabolism dysregulation in fish and a framework for better understanding of the metabolic pathways affected by environmental contaminants in fish. The results are important to assess biological impact of contaminants and more accurate risk assessment associated with complex mixture of contaminants in our environment. Funded by grants from NSERC of Canada.

**184 A study of the effects of Cr(VI) on the earthworm *Eisenia andrei*: from molecular/cellular changes to whole organism responses** S. Sforzini, Università Del Piemonte Orientale Amadeo Avogadro / Department of Science and Technological Innovation; M. Boeri, S. Olivieri, A. Viarengo, University of Piemonte Orientale. The aim of this investigation was the evaluation of the quality of Cr(VI)-contaminated soils using the earthworm *Eisenia andrei*. A set of physiological parameters at different levels of functional complexity was assessed in diverse cells and tissues, and in the whole organism. Field soil contaminated by a relatively high concentration of Cr(VI) (175 mg/kg) was investigated. Relevant biological changes were observed in worms exposed for 28 days to contaminated soil, without resulting in mortality. No effect was found in the number of juveniles hatched from each cocoon, although the number of cocoons and total offspring was reduced. The exposure to polluted soil caused significant changes in lysosomal biomarkers evaluated in the cells of the chloragogenous tissue i.e. an increase in lipofuscin and neutral lipid content as well as in the lysosome/cytoplasm volume ratio, a biomarker of tissue damage. A drastic decrease of lysosomal membrane stability was measured in the immunocompetent coelomocytes and significant genotoxic effects (DNA damage and micronuclei frequency) were observed in the same cells. The perturbation of the worm immune system was also demonstrated by an increase in lysozyme activity in the coelomic fluid. Based on these results, we investigated the potential toxicity/genotoxicity of soils contaminated by lower environmental levels of Cr(VI). *E. andrei* was exposed for 28 days to Cr(VI) at concentrations of 1, 2, 15 mg/kg spiked in standard soil. The selected Cr(VI) concentrations fall within the limits for soils set by Italian law. In addition to biomarkers employed in the first part of this study, we utilized a novel method to assess the activity of Ca<sup>2+</sup>-ATPase on frozen tissue sections. Moreover, in the coelomocytes, the oxidative DNA damage was also determined by the use of Fpg enzyme. Significant changes in different biomarkers were observed in earthworms exposed to Cr(VI) spiked soils, with greater effects at the highest concentrations. There was a significant decrease in the activity of Ca<sup>2+</sup>-ATPase in the intestinal epithelium, as well as a relevant increase of oxidative DNA damage in worms exposed to soils containing the lowest level of Cr(VI). Overall, the data demonstrated that *E. andrei* is a suitable model organism for ecological risk assessment and that the use of sensitive sublethal parameters is important for demonstrating the vulnerability level reached by the biotic resources in terrestrial ecosystems.

**185 Assessing chemical effects on earthworm physiology: an energy budget-driven agent based modelling approach** A.S. Johnston, University of Reading / Biological Sciences; M. Hodson, University of York; P. Thorbek, Syngenta / Environmental Safety; T. Alvarez, EcoRisk Solutions Ltd / Dept. of Ecological Sciences; R. Sibby, University of Reading / Biological Sciences. Earthworms are significant contributors to the ecosystem services provided by soils. Consequently, they are established model organisms for environmental risk assessments of plant protection products (PPPs). If PPPs are deemed to pose an unacceptable risk to earthworm populations, based on laboratory studies for acute mortality or chronic effects on growth or reproduction in lower tier risk

assessments, risk has to be further investigated at a higher tier using field trials. Field trials are expensive, time-consuming and variable, providing coarse information about population-level effects. Mechanistic population modelling of organism responses to PPPs has the potential to act as a refinement option in risk assessment, using lower tier results to make ecological extrapolations to the population level. Here, we evaluate the ability of energy budget-driven agent based models (ABMs) to serve as methods in current risk assessments for soil invertebrates. As multiple physical and chemical stressors have biological effects on all organisms in nature, such methods combining biological detail with environmental exposure are useful in aiding ecotoxicological inferences. We develop an initial step towards generating a useful tool for predictive toxicology, by investigating the ability of the model to replicate sublethal effects of different chemicals on individual physiology (e.g. maintenance, growth, reproduction). Energy budget models offer a robust method for predicting physiology by relating individual life cycle processes to one another through energy and mass conservation. Organisms uptake resources from their environment and expend assimilated energy on maintenance, growth and reproduction. The allocation of energy to these different subsystems depends on a combination of environment- and organism-specific conditions. Using typical lower tier risk assessment results from the literature, we incorporate toxicity data by a simple curve-fitting exercise to translate individual endpoints into metabolic modes of action. We predict the effects of two chemicals, copper oxychloride and chlorpyrifos, on the earthworm *Eisenia fetida*. Good model fits to the data show the simple energy budget ABM to be capable of making detailed predictions of interacting chemical and environmental stress at the individual level. Limiting food conditions had a particularly antagonistic impact on toxicity effects at the sub-individual level.

**186 Hazards of anthropic contaminated soils: what could we learn from environmental genotoxicity markers?** S. Lemiére, University of Lille / Nord; A. Deram, University of Lille / LSVF/ILIS University of Lille 2; F. Bernard, PRES Univ Lille Nord de France / LGCgE Univ Lille1 and LSVF Univ Lille2; S. Dumez, PRES Univ Lille Nord de France / LSVF Univ Lille2; F. Nessler, A. Platel, PRES Univ Lille Nord de France / Toxicology Lab - Pasteur Institute of Lille; M. Delattre, PRES Univ Lille Nord de France / Univ Lille1; D. Cuny, Univ Lille Nord de France / LSVF Univ Lille2; F. Vandebulcke, PRES Univ Lille Nord de France / LGCgE Univ Lille1. It is now well-accepted that hazard and risk assessment of anthropic contaminated soils may lead to erroneous diagnostics if only analytical chemical results are taken in account. Biological responses should be considered in decision-making regarding risk evaluation and strategies of remediation or management of contaminated soils and wastelands. Among these biological responses, environmental genotoxicity has to be regarded particularly. Study of formerly or recently contaminated soil genotoxicity is complex, since we have to consider: (1) the environmental availability, environmental and toxicological bioavailabilities of present contaminants, influenced by soil factors, ageing phenomena..., their concomitant presences (2) at low concentrations and then (3) their potential toxic interactions. For such purpose, methods of classical genetic toxicology do not seem fully satisfactory and we decide to implement environmental genotoxicity markers. The comet assay, a technique allowing the evaluation of DNA damage (single and double strand breaks, alkali-labile sites) in a cellular population, was developed for example on cœlomocytes of the biological model largely used in soil ecotoxicology, *Eisenia fetida*. In a first experiment, we conduct *in vivo* short-term exposures using eight well-characterised soils originating from two different areas in the North of France, (1) from the vicinity of a former smelter and (2) from a suburb zone close to a battery plant still in activity. In both contexts, soils were sampled along a contamination gradient. Little DNA breaks are observed after exposures to these anthropic contaminated soils. The rare positive responses are not obtained with the most contaminated soil. These results raise the question of the suitability of this environmental marker to assess genotoxic hazard in soils. So, we conduct *in vivo* short to mid-term exposures (3, 10 and 56 days) using an urban-surrounding soil spiked

with cadmium or/and lead at concentrations found in soils sampled from the former smelter impacted area. Observed genotoxicity results, as well as metal bioaccumulation, after these exposures will be detailed and discussed. The main conclusion of this work is that our marker of environmental genotoxicity is useful and relevant for environmental hazard assessment of anthropic contaminated soil.

**187 Soil Phototrophic microorganisms as sensitive indicators for the assessment of herbicide stress** O. CROUZET, INRA / soil ecotoxicology; J. Wiszniowski, Silesian University of Technology / Environmental Biotechnology Department; F. Bonnemoy, C. Mallet, LMGE, Université Blaise Pascal (UMR-CNRS). This study aims to investigate dose-response effects of an herbicide on the soil phototrophic microbial communities, and more particularly cyanobacteria and microalgae, by a microcosm approach. Pure mesotrione (active ingredient), belonging to triketone herbicides, and Callisto® (commercial formulation) were spread at different rates on soil microcosm surfaces. Chlorophylls in soils were quantified to assess photosynthetic biomass and genetic structure and diversity of the cyanobacterial community was investigated by a group-specific polymerase chain reaction followed by denaturing gradient gel electrophoresis (PCR-DGGE). Dose-dependent responses were evidenced for both functional and structural parameters. No effect was detected in the 1×AR (one fold recommended application rate) treated soils, irrespective of the herbicide formulation. At 10×AR (ten folds recommended application rate), only Callisto® treatment induced significant decreases of photosynthetic biomass and shifts on structural parameters. At 100×AR (one hundred folds recommended application rate) both pure mesotrione and Callisto® had strong negative impacts on soil chlorophyll concentrations and cyanobacterial genetic structure and diversity. At both 10×AR and 100×AR, Callisto® induced significant stronger effects than pure mesotrione. Also, the indicators of photosynthetic biomass responded (within 7 days) more quickly to herbicide stress, than structural parameters of cyanobacterial communities (within 14 days). This study underlined the relevance of soil phototrophic microbial communities to develop indicators for herbicide risk assessment. It also showed, the usefulness of molecular tool to improve the knowledge about the biodiversity of these unrecognized microorganisms in terrestrial ecosystems.

**188 The enzymatic functional stability: a suitable approach to detect terrestrial ecotoxicity of metals on tolerant microbial communities** L. Lessard; S. Sauve, Environmental Chemistry Laboratory / Chemistry; L. Deschenes, CIRAIT Polytechnique Montreal. A soil microbial community exposed to metals over a long period can acclimate or adapt and some of its biogeochemical functions may become metal-tolerant. This tolerance can increase the vulnerability of the microbial community to additional soil disturbances and in turn, decrease the soil microbial functional stability and ecological resilience. It is hypothesized that the functional stability assessment can detect an ecotoxic effect that would not be found using common bioindicators. This concept is timely and well-suited in the current context of multi-stressed ecosystems and global assessment of soil health. The objective of this study is to determine whether the functional stability of soil enzymes assessed by the RSSI, Relative Soil Stability Index, provides complementary information to the routine enzymatic assessment of metal-contaminated soils. The RSSI and the activity of four enzymes (arylsulfatase, phosphatase, protease and urease) were obtained for 20 Zn-contaminated field soils after a heat disturbance simulating severe drought (desiccation at 60°C for 24hrs). Among the most meaningful results obtained, the RSSIs of two enzymes were highlighted: they were negatively correlated to the labile Zn concentrations of the field soils (partial inhibition model: phosphatase  $R^2=0.39$  and  $EC_{50}=1160 \mu\text{g/kg}$  and protease  $R^2=0.42$  and  $EC_{50}=365 \mu\text{g/kg}$ ), while their enzymatic activities were not correlated to it (same model: phosphatase  $R^2=0.05$  and protease  $R^2=0.02$ ). These findings suggest that assessing functional stability by RSSI leads to different ecotoxicological information than enzymatic activity assessment and may be used as a complementary approach to assess metal toxicity on a metal-tolerant soil community.

**189 Relative bioavailability of tropical volcanic soil-bound chlordecone in farm animals: laying hens, piglets and lambs**

**C. Jondreville**, URAFFPA; S. Jurjanz, C. Bouveret, S. Lerch, Université de Lorraine / URAFFPA; M. Lesueur-Jannoyer, CIRAD / PRAM; H. Archimède, M. Mahieu, INRA / URZ; C. Feidt, G. Rychen, Université de Lorraine / URAFFPA. Chlordecone (CLD) is a chlorinated polycyclic ketone pesticide used from 1971 to 1993 in French West Indies to fight against banana black weevil. The former use of this organochlorine insecticide has resulted in long-term pollution of soils, sediments and of related food chains. CLD may be transferred into products from farm animals reared outdoors, through involuntary polluted soil ingestion. However, due to different properties of clays, tropical volcanic soils display variable capacities of pollutant retention: CLD is less available to plants and more persistent in andosol than in nitisol. In order to assess the risk of animal products' contamination by soil-bound CLD, the impact of soil type on CLD bioavailability to animals has to be established. It was assessed in three farm animal species differing for their digestive tract properties (laying hens, piglets and lambs), through relative bioavailability (RBA) studies. Thus, the response of CLD ingestion through andosol and through nitisol was compared to the response obtained with CLD ingestion through oil, taken as a reference matrix. Our hypotheses were that i) CLD would be less available in soils than in oil, ii) CLD would be less available in andosol than in nitisol and iii) RBA in soils may differ between animal species. The deposition of CLD in egg yolk (hens), in liver (piglets) and in serum (lambs) was measured in individually housed animals fed graded levels of CLD from polluted andosol, nitisol or spiked oil. Hens, piglets and lambs were exposed to CLD during 28, 14 and 15 days, respectively. For each animal species, the concentration of CLD in target tissue linearly increased with the amount of ingested CLD within each ingested matrix ( $P < 0.001$ ). However, the responses to andosol-diets, nitisol-diets and oil-diets could not be differentiated ( $P > 0.1$ ), indicating that CLD was equally bioavailable, irrespective of the matrix. The current experiments clearly show that, for the three animal species, soil does not modulate CLD availability. Therefore, involuntary ingestion of polluted soils by farm animals must be considered as potential contributor to the risk of contamination of animal products. In addition, accounting for the higher CLD concentration in andosol compared to nitisol, raising animals on andosol would result in a higher risk of products' contamination with CLD than on nitisol.

**190 Genotoxic impact of oil sands process-affected water to rainbow trout: what is the role of naphthenic acids?**

**E. Lacaze**, A. Devaux, Université de Lyon-ENTPE; F. Gagne, Emerging Methods Environment Canada / Emerging Methods. Oil sands exploitation has raised major environmental concerns, particularly regarding the presence of naphthenic acids found at high concentration in the oil sands process-affected water (OSPW). Naphthenic acids have been proved to have toxic effects on a variety of organisms including plants, fish, and mammals. However, their role in the toxicity of OSPW is not well known. Along the Athabasca River in Alberta, Canada, extraction of oil sands occupies almost 600 km<sup>2</sup> of boreal forest and generates a huge quantity of wastewater, released in tailing ponds. In a previous study, Athabasca water extracts in the vicinity of an oil sand extraction site were able to induce DNA damage in trout hepatocytes. Whereas naphthenic acids are suspected to be responsible for the largest part of toxicity in tailing ponds, nothing is known concerning their potential genotoxicity. The purpose of this study was to evaluate the genotoxic impact of oil sands process-affected water (OSPW) and OSPW-related contaminants to an indigenous fish species in order to investigate the role of naphthenic acids. For this purpose, rainbow trout hepatocytes were exposed *in vitro* to extracts of OSPW (oil sands processed water, obtained by a laboratory bitumen extraction), oil sands leachate water (OSLW, mimicking lixiviation of the shore) and environmentally relevant concentrations of naphthenic acids mixture, synthetic naphthenic acids found in tailing ponds, and PAHs. DNA damage was assessed by the Fpg-modified Comet assay. Genotoxic impact was observed in hepatocytes exposed to OSPW and OSLW extracts in a dose-dependant manner, and genotoxicity was more marked for OSPW

than for OSLW. Furthermore, exposure to a mixture of naphthenic acids at concentration lower than those measured in runoff water has resulted in higher genotoxic impact compared to benzo(a)pyrene exposure at concentration found in tailing ponds. These results proved that the genotoxicity of OSPW could also be due to naphthenic acids. High concentrations of naphthenic acids in runoff and ground waters in the vicinity of oil-sands extraction (24 and 50 mg/L respectively), could represent a clear ecotoxicological risk. Further research has to be conducted to understand the relationship between the structure of naphthenic acids and their genotoxicity, to finally gain knowledge about the potential reclamation of tailing ponds.

**191 Chemical characterization and treatment by enhanced coagulation of dissolved organic matter in biochemical-treated effluent of textile wastewater**

**Y. Lu**, Peking University / Department of Environmental Engineering, School of Environment and Energy, Peking University Shenzhen Graduate School; S. WU, Beijing Gaia Technology Center Co. Ltd.; L. Zhao, School of Environment and Energy, Peking University Shenzhen Graduate School; J. NI, Peking University / Department of Environmental Engineering. Performed with regular physicochemical-biochemical combined treatment process, textile wastewater effluent was treated by enhanced coagulation with a new kind of coagulant. Dissolved organic matter (DOM) in biochemical-treated effluent of textile wastewater as well as effluent treated by enhanced coagulation were isolated and enriched by extraction. Both DOM isolates were characterized by Gas Chromatography-Mass Spectrum (GC-MS) and both effluents by Excitation Emission Matrix (EEM). Results showed that biochemical-treated textile wastewater effluent mainly contained hydrophilic organic matters: triethylene glycol, tributyl phosphate and phthalates, and hydrophobic organic matters: amino acids and humic substances. And after enhanced coagulation treatment, most of the dissolved organic matter was removed from the biochemical-treated textile wastewater effluent.

**192 Removal of hexavalent chromium from aqueous matrices using plantain (*Musa paradisiaca* L.) peel biomass**

**B.O. Opeolu**, Cape Peninsula University of Technology / Faculty of Applied Sciences; O.S. Fatoki, Cape Peninsula University of Technology; O. Olatunji, Cape Peninsula University of Technology / Department of Chemistry. Chromium (VI) has found application in metallurgical, tanning, textile and paint industries. Hexavalent chromium ( $\text{Cr}^{6+}$ ) is very toxic and mutagenic when inhaled. It is also one of the substances whose use is restricted by the European Restriction of Hazardous substances Directive. The use of  $\text{Cr}^{6+}$  in various industrial processes has led to its presence in industrial wastewaters and consequently, surface waters. Abatement methods such as chemical precipitation and reverse osmosis are often expensive when available in the developing world. Furthermore, most conventional removal methods have been associated with the generation of secondary wastes such as sludge. This work therefore aimed at assessing the removal capacity of plantain peel for  $\text{Cr}^{6+}$  from aqueous solutions. Parameters studied include effects of contact time, pH, temperature, weight, particle size, and shaking period. Physico-chemical characterization of the biomass (plantain peel) was carried out and residual  $\text{Cr}^{6+}$  measured using atomic absorption spectrophotometry. The optimal contact time, concentration, temperature and weight for adsorption were 90 min, 100 mg/L, 50°C and 0.4g respectively.  $\text{Cr}^{6+}$  removal increased with shaking time and carboxyl and hydroxyl groups seem to be responsible for  $\text{Cr}^{6+}$  binding. Percentage removal from paint and textile effluents was over 90%. Plantain peel biomass may therefore be an innovative alternative to imported expensive synthetic resins in Africa.

**193 Nutrients, pharmaceuticals, and antibiotic resistance genes in municipal wastewater after wetland treatment: A case study at Grand Marais, MB, Canada**

**J. Anderson**, Department of Chemistry; J.C. Carlson, University of Winnipeg / Richardson College for the Environment; J.E. Low, University of Winnipeg / MSc student; J. Challis, University of Winnipeg; C.S. Wong, University of Winnipeg / Richardson College for the Environment; C.W. Knapp, University of

Strathclyde; M.L. Hanson, University of Manitoba / Department of Environment and Geography. The discharge of complex mixtures of nutrients, organic micropollutants and microbes bearing antibiotic resistance genes (ARGs) from treated municipal wastewater into freshwater systems are global concerns for human health and aquatic organisms. Use of lagoon treatment systems for managing rural wastewaters is common across the North America. In the rural community of Grand Marais, Manitoba, Canada, wastewater is treated passively in sewage lagoons followed by passage through a treatment wetland prior to release into surface waters. Using this facility as a model system, the aim of this study was to assess the presence of nutrients, micropollutants, and ARGs in lagoon outputs and their potential removal by the wetland. Nutrients, organic micropollutants (i.e., pesticides, pharmaceuticals), and standard water quality parameters in the system were characterized prior to and following lagoon discharge events into the treatment wetland over in 2012 and the quality of the water assessed by comparisons to regulatory guidelines where they exist and through hazard quotients. Quantitative PCR was used to measure the abundances of ten ARGs, as well as 16S-rRNA, in order to evaluate the effectiveness of the wetland in removing ARGs. As expected, concentrations of nitrogen and phosphorus species were greatest in the lagoon and declined with movement through the treatment system. Pharmaceutical and agricultural chemicals were detected at concentrations in the ng/L range and predictably, concentrations spiked downstream of the lagoon following discharge. Only atrazine and carbamazepine were shown to be significantly attenuated by processes within the wetland. Hazard quotients calculated for micropollutants of interest indicated minimal toxicological risk to aquatic biota. There was no significant removal of ARGs in the wetland and the results suggest that up to 2.7% of the bacterial population in this system may have genes imparting antibiotic resistance. The results of this study indicate that while the treatment wetland may effectively attenuate excess nutrients and remove some micropollutants and bacteria, it does not specifically target ARGs for removal. Additional studies would be beneficial to determine whether upgrades to extend retention time or alter plant community structure within the wetland would optimize removal of micropollutants and ARGs.

**194 First determination of diclofenac, sulfamethoxazole and their nitration transformation products in wastewaters and evaluation of their acute toxicity** V. Osorio; J. Sanchis, IDAEA-CSIC; J. Abad, IQAC-CSIC; A. Ginebreda, M. Farre, S. Perez, D. Barcelo, IDAEA-CSIC. Diclofenac (DCF) is a non-steroidal drug with anti-inflammatory and analgesic effects, widely used for treatment of rheumatic diseases and pain relief. Sulfamethoxazole (SMX) is a sulfonamide bacteriostatic antibiotic extensively used in both veterinary and human medicine. These pharmaceuticals are of environmental concern due to frequent detection in monitoring surveys on sewage-impacted surface waters associated with high consumption rates and low removal efficiencies during conventional activated sludge treatment in wastewater treatment plants (WWTPs). DCF is proposed to be included in the priority list of organic pollutants in surface waters. Despite this, the overall fate of these compounds has as of today been considered only marginally and little is known about the biotransformation processes taking place in the aeration tank of the activated sludge treatment. The formation of two transformation products (TPs), nitroso-DCF and nitro-DCF during conventional treatment, was observed. Nitration of DCF and SMX was also reported in aquifers under denitrifying conditions. Despite natural attenuation of these compounds, it is assumed that they can behave as pseudo-persistent compounds simply because of their continuous entry into the aquatic environment via wastewater (WW) effluents. The generation of nitrated derivatives in the WWTPs is a highly relevant matter of concern because of their potential toxicity. This research aimed at investigating still uncovered aspects of the fate of DCF in the aquatic environment by determining the occurrence of the TPs, formed in the WWTP, in influent, effluent and also in surface waters samples. In addition, the potential ecotoxicological effects of DCF, nitro-DCF, nitroso-DCF, SMX and nitro-SMX on the aquatic organisms *Daphnia magna* and *Vibrio fischeri* were assessed. For that purpose, one

objective was to develop a quantitative analytical methodology for trace determination of compounds targeted, based on off-line solid-phase extraction (SPE) followed by LC/ESI-MS/MS analysis on a hybrid quadrupole-linear ion trap (QqLIT) instrument.

**195 Continuous monitoring of turbidity and conductivity in wastewater network: an easy tool to assess the pollution load discharged into receiving water** T. Bersinger, LCABIE IPREM; G. Bareille, LCABIE IPREM UMR 5254; T. Pigot, IPREM ECP UMR 5254; I. Le Hecho, LCABIE IPREM UMR 5254. Sanitation represents a major threat for the aquatic environment since it is collecting a wide variety of sources from both domestic and industrial activities and it is continuously discharging various polluted effluents. Urban stormwater runoff which is considered as transient source may also contain high levels of pollutants discharged directly in receiving streams by Combined Sewer Overflow (CSO). Continuous monitoring of no treated wastewater via CSO becomes a key issue for management of sanitation and protection of receiving environment. Monitoring based only on samples being technically and economically unrealistic it is thus necessary to turn to an indirect measure of pollutant parameters. This can be done via the measurement of physical parameters such as turbidity and conductivity. In such a context we have studied the urban catchment of Pau urban area (south west France) which is about 50 km<sup>2</sup> with about 150 000 inhabitants. 4 rain gauges, 40 flowmeters, 3 turbidimeters and conductimeters were installed in all the study area to monitor the sanitation and particularly CSO discharges. After intensive and various samples periods it has been possible to correlate safely in one hand global parameters such as Chemical Oxygen Demand (COD) and Total Suspended Solid (TSS) to turbidity and in the other hand conductivity and total nitrogen. These correlations will be first presented. The sensors were placed at the entrance of the wastewater treatment plant (WWTP) and main CSOs. Continuous recording of turbidity and conductivity have been achieved during one year. Use of acquired data allows a real time evaluation of: - Pollutant fluxes directly discharge by CSO and treated by WWTP - Input of sanitation on receiving streams - Micro pollutant fluxes (metals and hydrocarbons aromatic polycyclic) mainly associated to suspended solids **This continuous monitoring is innovative for wastewater network management. This allows continuous estimation of discharged wastewater concentrations into the receiving environment. The sanitation manager can then resize and optimally manage the treatment and wastewater storage.**

**196 Is it possible to increase bioavailability but not environmental risk in PAH bioremediation?** J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiología / Agroquímica y Conservación del Suelo; M. Tejada-Agredano, Instituto de Recursos Naturales y Agrobiología de; C. Jimenez Sanchez, Inst de Recursos Naturales y Agrobiol de Sevilla; L. Congiu, European Food Safety Authority; R. Sunghong, Department of Agrochemistry and Soil Conservation; M. Cantos, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC). The current poor predictability of end points associated with the bioremediation of polycyclic aromatic hydrocarbons (PAHs) is a large limitation when evaluating its viability for treating contaminated soils and sediments. This fact is usually caused by a poor bioavailability of the chemicals for biological destruction by microorganisms. Due to their partitioning into sorbents and NAPLs, the chemicals may exhibit only weak chemical activity gradients that promote their uptake and transformation by active microbial cells. Hence, the biodegradation rates may reflect the dependencies of restricted phase exchanges, and, as a result, a priori biodegradable PAHs may present longer persistence. Biodegradation may be effective in reducing risks, but recalcitrant pollutants, left behind by bioremediation, may still follow exposure routes specific to humans and ecological targets. Therefore, innovative methods are needed to increase bioavailability of these pollutants for an enhanced bioremediation performance. This overview contribution will examine our recent research efforts in this issue, performed at IRNAS-CSIC, Spain, including surfactant applications to promote biodegradation in soils exhibiting a slow-desorption profile (*Environ. Sci. Technol.*

45:3019-3026, 2011), targeted fertilization of free-oil phases or NAPLs (*Environ. Sci. Technol.* 45:1074-1081, 2011), modulating deposition and motility of degraders in porous media (*Environ. Sci. Technol.* 46:6790-6797, 2012), and promoting bioavailability with plants (*Soil Biol. Biochem.* In press, 2012). The integration of these strategies into practical remediation protocols would be beneficial to the bioremediation industry, as well as improve the quality of the environment.

#### **197 Classification and modelling of non-extractable residue (NER) formation from xenobiotics in soil – a synthesis**

**M. Kästner**, Helmholtz Centre for Environmental Research UFZ / Department of Environmental Biotechnology; K. Nowak, RWTH Aachen University / Institute for Environmental Research; A. Miltner, Helmholtz-Centre for Environmental Research / Department of Environmental Biotechnology; A. Schaeffer, RWTH Aachen University / Chair of Environmental Biology and Chemodynamics; S. Trapp, Danmark Tekniske Universitet. This presentation provides a comprehensive overview about the formation of non-extractable residues (NER) from organic pesticides and contaminants in soil and tries classifying the different types. Anthropogenic organic chemicals are deliberately (e.g. pesticides) or unintentionally (e.g. polyaromatic hydrocarbons [PAH], chlorinated solvents, pharmaceuticals) released in major amounts to nearly all compartments of the environment. Soils and sediments as complex matrices provide a wide variety of binding sites and are the major sinks for these compounds. Many of the xenobiotics entering soil undergo turnover processes and can be volatilised, leached to the groundwater, degraded by microorganisms or taken up and enriched by living organisms. Xenobiotic NER may be derived from parent compounds and primary metabolites that are sequestered (sorbed or entrapped) within the soil organic matter (type I NER) or can be covalently bound (type II NER). Especially type I NER may pose a considerably environmental risk of potential release. However, NER resulting from productive biodegradation, which means the conversion of carbon (or nitrogen) from the compounds into microbial biomass molecules during microbial degradation (type III, bioNER), do not pose any risk. Experimental and analytical approaches to clearly distinguish between the types are provided and a model to prospectively estimate their fate in soil is proposed.

#### **198 Hexachlorobenzene-contamination of sediments of the Upper Rhine River – Impact of clay minerals on the deviation of KOC values**

**L. Boehm**, Justus Liebig University Giessen / Institute of Soil Science and Soil Conservation; T. Pohlert, Federal Institute of Hydrology (BfG); R. Duering, Justus Liebig University Giessen / Institute of Soil Science and Soil Conservation. Due to historical emissions of hexachlorobenzene (HCB), the sediments of the ponds of Upper Rhine River in southern Germany remain highly contaminated (up to 7000 µg kg<sup>-1</sup>). HCB is a bioaccumulative, toxic, and persistent hydrophobic organic pollutant (HOC), and is mainly bound to organic matter (OM) (quantified as total organic carbon, TOC) and its content is mostly considered to be the significant parameter controlling the extent of sorption. Therefore, sorption coefficients K<sub>d</sub> of samples are specified as K<sub>d</sub><sup>OC</sup> values, normalized to their TOC content. Recently, the influence of inorganic components on HOC sorption increasingly has been identified as relevant. Knowledge of the specific HCB-sorbents in the different fractions is of importance, because their characteristics control sorption strength, which is an important factor for remobilization as well as bioavailability of HCB. The mineral composition of sediments (measured by X-ray Diffraction, XRD) and its influence on the amount of sorption is presented, as well as composition of and sorption to sediment fractions. Sorption and desorption isotherms and partition coefficients (K<sub>d</sub>, K<sub>d</sub><sup>OC</sup>, K<sub>d</sub><sup>des</sup>) for sediments, sediment fractions, and reference minerals were determined in batch equilibrium studies by solid-phase microextraction (SPME) coupled to GC-MS. Sorption studies resulted in log K<sub>d</sub> values of sediments from 2.8 to 3.5 and log K<sub>d</sub><sup>OC</sup> values from 4.5 to 5.0. K<sub>d</sub> values of the sediments yield similar K<sub>d</sub><sup>OC</sup> values, except for two highly contaminated sites, where the amount of sorbed HCB could not be explained solely by TOC nor by

particle size distribution and specific surface area. However, this deviation can be explained by sorption to the mineral fractions. Sorption studies with clay suspensions resulted in very high log K<sub>d</sub> values from 2.9 to 3.5. The pattern of sorption intensity measured in clay suspensions matched the pattern of deviation for K<sub>d</sub><sup>OC</sup> values of total sediment fractions. Hence, sorption of HCB to minerals plays a significant role in sorption processes, in particular due to their high occurrence. Results on physicochemical properties of sorbents contribute to a better understanding of the environmental behaviour and pathways. Knowledge of sorption strength of specific adsorbents as well as desorption processes and hysteresis facilitates the location of contaminated sites which function as key sources of HCB remobilization and should therefore be prioritised in remediation purpose.

#### **199 Sorption capacity of agricultural hormones to different particle size fractions of a natural aquatic sediment**

**J. Sangster**, University of Nebraska / Civil Engineering; D.D. Snow, University of Nebraska / Associate Professor; S. Bartelt-Hunt, University of Nebraska-Lincoln / Civil Engineering. Increasing evidence indicates that agricultural production constitutes a significant source of steroidogenic compounds to aquatic systems. While several studies have explored the fate and transport of steroidogenic compounds in agricultural systems, relatively little is known about the effects of soil or sediment particle sizes on steroid fate in the environment. This study explores the partitioning of agricultural hormones, estradiol (E2), estrone (E1), progesterone (Pr), testosterone (T), and 17 $\beta$ -trenbolone (Tb), to different size fractions of a natural sediment and evaluates how competition between particle size fractions effect the distribution of hormones in the sorbed phase. Experiments were performed using a natural sediment fractionated using both sieve and deposition methods. Five fractions of the sediment were used in sorption experiments to determine the sorption capacity of the fractions for five common agricultural hormones. Based on the results of sorption capacity experiments, several trends are evident from the data. All hormones evaluated exhibit significantly higher sorption capacity in the clay and colloid fractions when compared to the sand and silt fractions. This may be at least partially attributed to an increased surface area within the smaller particle fractions. Hormone sorption to colloids is not significantly different from sorption capacity of the clay fraction for E2, E1, and Pr. However, in the case of T, colloids exhibited a lower sorption capacity than that of the clay fraction. The estrogens, E2 and E1, sorbed at comparable levels for both the sand and silt fractions. In contrast, Pr and T, in the sand and silt fractions behave significantly differently. Results for Tb have not been evaluated as of the time of this abstract submission, nor have the results of sorption with competition between particle size fractions. The data from this study may help in our understanding of steroid transport and bioavailability in aquatic systems.

#### **200 The uptake of PPCPs in curcubits and the influence of soil factors on bioavailability**

**M. Williams**, CSIRO / Land and Water; E. Harris, Cranfield University; S. Martin, J. Kirby, R. Kookana, CSIRO. Relatively few terrestrial studies have been undertaken to assess the potential impacts of PPCPs in the terrestrial environment, with those that have demonstrating that uptake of PPCPs in plants from either contaminated soil or irrigation water can occur. The aim of this study was to assess the uptake of four PPCPs, the human pharmaceuticals carbamazepine (CBZ), propranolol (PRL) and fluoxetine (FLX) and the personal care product triclosan (TCS), in the curcubit zucchini (*Curcubita pepo* spp. *pepo* var. Black Beauty) at nominal initial soil concentration of 5 mg/kg. *C. pepo* was grown in two different soils (Inman and Tepko) over a 28 day period within a growth chamber. Tepko soil was further modified through the addition of 0.5% w/w biochar derived from wheat and eucalyptus, respectively, to assess its impact on uptake. In all soil treatments, there was a large extent of uptake of CBZ in *C. pepo*, with bioconcentration factors (BCFs) ranging from 1.8±0.7 to 13.5±0.2. Conversely, there was comparatively little uptake of PRL or FLX with respective BCFs ranging from 0.008±0.001 to 0.1±0.01 (PRL) and 0.014±0.004 to 0.02±0.01 (FLX). TCS was not detected in plant residues from any of the soil treatments. Monitoring of soil concentrations over the 28 d period suggested that TCS was possibly

transformed, with a decrease in its soil concentrations from 20 to 43% of original values. Porewater concentrations of the PPCPs were also monitored over the 28 d *C. pepo* growing period, with TCS and FLX unable to be detected in any porewater samples extracted from the soil treatments. In contrast, high levels of CBZ were measured in porewater, corresponding with  $K_d$  values ranging from  $1.5 \pm 0.1$  to  $15 \pm 0.7$  L/kg. The  $K_d$  values measured for PRL were considerably higher and ranged from  $62 \pm 28$  to  $753 \pm 197$  L/kg. For both CBZ and PRL, addition of both wheat and eucalypt-derived biochar reduced the extent of uptake relative to the unamended Tepko soil, although uptake in the amended soils was still comparable with the Inman soil. Dissipation of TCS in biochar-amended soil occurred but was found to occur at approximately half the rate of that in the unamended soil. Work is currently being undertaken to adapt a stable isotope technique, commonly used in for the assessment of inorganic contaminants, to measure and predict the bioavailable fraction of the PPCPs present in the soil.

**201 Atrazine degradation in a Brazilian Acrisol under no-tillage** d. dick, Federal University of Rio Grande do Sul / Physical Chemistry; D. Barbosa, Federal University of Rio Grande do Sul / Soil Science Department; P. Burauel, Forschungszentrum Jülich / Institute of Chemistry and Dynamics of the Geosphere, IBG 3; A. Lelickens, Institute of Chemistry and Dynamics of the Geosphere, IBG 3 - FZJülich. The pattern of ATZ degradation (mineralization and extractable residues) in a Brazilian Acrisol cropped with maize under no-tillage was evaluated, focusing on the roles of the ATZ application history and of the cover straw on these processes. Incubation experiments were conducted for 85 days with soil samples from an Acrisol (0-10 cm) located in southern Brazil. Four treatments were used: Microcosm 1, cultivated soil since 1991 and with history of ATZ application; Microcosm 2, native soil from an adjacent area; Microcosm 3, same soil as Microcosm 1, covered with straw (*Avena sativa*); Microcosm 4, similar to Microcosm 1. A spiking solution was prepared using technical-grade and  $^{14}\text{C}$ -ATZ in ethanol and applied to the Microcosms. For monitoring of water and ASE extractable radioactivity soil samples were analyzed at 0, 9, 16, 30, and 85 days of incubation. The  $^{14}\text{C}$  activity was measured by liquid scintillation counter. Cultivated soil presented higher ATZ mineralization (86%) than native soil (10%) by the end of incubation. The water-extractable  $^{14}\text{C}$  activity decreased from 69 to 1% and from 62% to 20% in Microcosms 1 and 2, respectively. The amount of ASE-extractable  $^{14}\text{C}$  ranged from 8 to 17% in Microcosm 1 while in Microcosm 2 it increased from 15 to 51%. Besides indicating the lesser persistence of ATZ in the cultivated soil, these results suggest that ATZ was transferred to a soil compartment less available to its mobilization during the incubation. The amount of non-extractable  $^{14}\text{C}$ -residues were lower than 0.08%. In Microcosm 3, that included cover straw, the mineralization rate was slower than in Microcosm 4 (soil without straw) and at the 85<sup>th</sup> day the mineralized  $^{14}\text{C}$  ATZ amount reached 30% and 85 % respectively. The water extractable  $^{14}\text{C}$ -residues in Microcosm 3 were higher in the straw than in the soil until the 30<sup>th</sup> day of incubation and reached 9.2% in the soil and 7% in the straw at the 85<sup>th</sup> day. In the soil of Microcosm 3, the amount of ASE-extractable  $^{14}\text{C}$  increased from 2 to 13% and that of non-extractable  $^{14}\text{C}$ -residues from 0.9 to 8% along the incubation. The water extractable  $^{14}\text{C}$ -residues in the straw increased with time and were greater than in the respective soil. Our results corroborates the accelerated degradation of ATZ in cultivated soils. The cover straw, frequently found in Brazilian agricultural soils under no-tillage represents an important sorbent for ATZ, reducing its mineralization.

**202 First report of pyrethroid insecticides in birds: A case study on Doñana National Park (Spain)** C. Corcellas, IDAEA / Química Ambiental; A. Andreu, EBD-CSIC; F. Sergio, F. Hiraldo, EBD-CSIC / Applied Biology; E. Eljarrat, D. Barcelo, IDAEA-CSIC / Química Ambiental. In last decades the usage of pyrethroids has increased widely in the indoor as household insecticides, insect-control products, pet shampoos and lice treatments, and in the outdoor as agricultural pesticides and for pest control. Because of that, they are almost ubiquitous and, as long as they are dumped continuously, they will be

always present in the environment. Even when their toxicity was supposed low for non-target organisms, it is known the high toxicity in fish. Despite the assumption that these insecticides are converted to non-toxic metabolites by hydrolysis in mammals, recent studies showed their presence in dolphins or even in human breast milk. These studies point out a potential bioaccumulation of these insecticides. The aim of this work was studied the presence of 13 common pyrethroids in infertile bird eggs of different species from different trophic level, collected at the Doñana National Park, a protected reserve in the South of Spain, of high environmental interest because all migratory Afro-European birds cross through it. For the first time, pyrethroids were detected in all egg samples analysed, at concentration levels ranging between 0.75 and 25 ng/g lw. These results confirmed the bioaccumulation potential of these insecticides in biota samples, corroborating the previous findings in marine mammals and humans. Of the 13 investigated pyrethroids, resmethrin and fluvalinate were usually under the limit of quantification. However, cypermethrin and permethrin were the more ubiquitous pyrethroids, followed by bifenthrin and tetramethrin. As regards the contamination of each bird specie analysed, the less contaminated corresponded to glossy ibis, whereas white storks and black kite were the most contaminated ones. These results could indicate that pyrethroids bioconcentrate along the food chain, and that because the lower levels corresponded to the species of lower trophic level, while the highest concentrations were found in the higher trophic level species. Finally, enantiomeric characterization was carried out in order to determine potential enantiomeric-specific accumulation of pyrethroids. This study seems to point out some enantiomeric-selective accumulation. This is the case of enantiomeric-selective accumulation of the enantiomer II of *trans*-tetramethrin observed in egg bird samples analysed.

**203 Bird communities in chlorpyrifos-treated citrus in Spain: 3 years intensive field monitoring of species-diversity, abundance & reproduction 2010-12** R. Ditttrich, Tier Solutions GmbH / Wildlife Ecology; B. Giessing; S. Norman, Ridgeway Eco; B. Striffler, Tier3 Solutions GmbH; C. Wolf, Tier Solutions GmbH. The organophosphate insecticide chlorpyrifos (CP) has been the foundation of red scale control in citrus cultivation in Spain for decades. Chlorpyrifos has a relatively high acute toxicity to birds in lab studies so the regulators require intensive field evidence to demonstrate safety to birds. Therefore the long-term response of the bird community to CP has been studied in citrus for 3 yrs (2010-2012; 2.4 kg/ha) culminating in probably the largest bird study ever done on a pesticide. A comprehensive approach was followed which highlights the structure of the bird community over consecutive years. This allows measurement and ranking of any factors influencing the local bird community, including the application of CP itself. The bird communities of ten CP treated citrus orchards in the Xatíva valley (Valencia province, Spain) were studied during the breeding seasons from 2010 to 2012. Following common practice a spray of chlorpyrifos (2400 g a.i./ha) was done on all ten sites between end of May and mid-June. Methods applied were regular bird trapping with mist nets in all sites and years. Trapped birds were sexed, ringed and checked for signs of reproduction. Also systematic nest searching was conducted by periodical check of every tree over the breeding season. Active nests were monitored until the young birds had fledged or the nesting failed. The bird community found in CP treated citrus orchards was diverse and abundant with 13,511 birds of 82 species trapped in three years. The total number of birds trapped and the species composition varied between the ten sites. Also the nest density varied between sites, in particular between one site (site 4) and the other nine sites (due to difference in surrounding habitats). The average rate of successful nests was 32 % of all active nests in 2010-2012. The breeding success and density of nests depends on the presence of predators and pruning activities. The re-trapping of individuals year to year indicates long term survival of adults in CP-treated orchards. Overall, no clear effects at the population and community level could be attributed to CP.

**204 The use of monitoring results of plant protection products in surface water in Product Stewardship and PPP authorisation**

de Werd, WageningenUR Applied Plant Research / Applied Plant Research; R. Kruijne, WageningenUR - Alterra; W. Tamis, Leiden University - Institute of Environmental Sciences. Exceedances of environmental quality standards (EQS) for plant protection products (PPP) in Dutch surface water need to be further reduced. A procedure for the use of PPP monitoring results for ERPs and a feedback to PPP authorisation has been developed and tested. It is based on responsibility for the registration holder and obligatory measures if necessary. It consists of: 1) identification and ranking of problematic substances 2) analysis of plausible causes for the exceedances and the composition of an ERP 3) feedback to the board of PPP authorisation and ministries involved. Yearly substance selection and ranking is carried out, based on the last three years of monitoring results for PPP in the Dutch Pesticides Atlas. Substances exceeding the EQS in the Water Framework Directive (WFD) water bodies are selected. WFD priority substances are ranked highest. The remaining selected substances are given points based on water body type, exceedance degree and percentage of monitoring locations with exceedances. Registration holders can be requested a causal analysis and ERP, triggered by: 1) a prolongation request for an existing registration, 2) a new registration request for a substance already on the Dutch market, or 3) top position in the list. The causal analysis searches for *plausible* relations between specific applications and emission pathways on the one hand, and standard exceedances on the other hand. First a fact sheet is compiled, covering i) substance properties (ii) authorised use (iii) usage statistics; (iv) indicators for the emission to surface water & crop maps, and (v) monitoring results. Next, experts are consulted, using predefined questions referring to the factsheet. If after interpretation the plausible causes are identified, the protocol manager draws the final conclusions. Alternatively, additional fact finding and expert consultation is carried out for certain topics or regions. The final conclusions are the fundament for the ERP. Causal analysis and ERP are reported to the responsible authorities. If plausible that authorised use (applying Good Agricultural Practice) led to standard exceedances, and the ERP is expected to be insufficient, there may be obligatory consequences for the registration. Implementation of this methodology is expected to contribute to the reduction of standard exceedances, whilst respecting the registration holders' responsibility. The standardised methodology facilitates transparency and substantiated mitigation measures.

## **205 National Pesticide Screening for the Selection of Relevant Compounds for Improved Future Monitoring Strategies.**

Moschet, Eawag / Uchem; A. Piazzoli, ETH; I. Wittmer, H. Singer, C. Stamm, Eawag; M. Junghans, I. Werner, Oekotoxzentrum; J. Hollender, Eawag. Monitoring and risk assessment of agricultural and urban pesticides are critical issues. In most countries, pesticide monitoring focuses on herbicides and is not carried out systematically. The goal of this study was to define relevant compounds from all pesticide classes on a national scale which are recommended to be monitored in future activities. The study consisted of a systematic, step-wise procedure with a combined theoretical, analytical and ecotoxicological approach including the screening of nearly all pesticides registered in Switzerland. In a first step, all registered pesticides were ranked according to sales numbers, organic carbon to water partition coefficients and degradation rates in soil. The 156 substances with the highest ranking (occurrence in surface water likely) were selected for a quantitative target screening using high-resolution mass spectrometry. The pesticides with lower rankings were qualitatively screened in the samples (suspect screening). All substances were measured in 5 catchments across Switzerland with different cropping pattern between March and July 2012. The analytical method covered 88% of all registered organic pesticides at low detection levels. From the 156 target substances, 97 were detected in at least one sample; another 13 substances were detected in the suspect screening. Results showed that 95-percentile herbicide concentrations were on average 2.3 times higher than fungicide concentrations and even 6.9 times higher than insecticide concentrations. The ecotoxicological risk assessment showed that in a few samples some substances already exceeded their environmental quality standard (EQS). From these results, Switzerland-relevant pesticides were selected. It was shown that

besides substances already monitored (e.g., azoxystrobin, isoproturon), less known substances (e.g., thiamethoxam, piperonyl butoxid) were also found to be relevant. Whereas some substances were only important in catchments with a specific cropping pattern, others were found throughout the catchments. As expected, pesticides detected depended on land use e.g., highest insecticide concentrations were found in catchments with high vegetable or orchard density. Risk estimation of combined exposure allowed identification of critical catchments where risk mitigation measures are necessary. This integrated approach provides stakeholders with important information in the planning of future monitoring and risk assessment programs.

## **206 Monitoring effects of pesticides on pollinators – a review of methods and outcomes by the ICPPR working group.**

Alix, Dow Agrosiences / Risk Management; A. Bartels, AGES; C. Garrido, IBACON; C. Hart, PMRA; K. Knauer, Bundesamt für Landwirtschaft BL; J. LAPORTE, Syngenta; B. Maurizi, Ministry of Agriculture; C. MAUS, Bayer Ag; M. Miles, Dow Agrosiences; C. Schneider, BASF; H. Thompson, FERA; J. Wassenberg, CTBG. Monitoring studies, in the context of the environmental assessment of Plant Protection Products (PPP) or pesticides, aim at getting feedback regarding the fate and/or effects of active substances and/or their relevant degradation products in/on the environment, when PPP are used under realistic conditions for crop protection. These studies complement the risk assessment performed in application of Regulation 1107/2009/EC and previous texts, which aims at identifying the conditions of exposure of organisms in the environment, the conditions of occurrence of risks if necessary and propose appropriate risk mitigation measures. In this context, monitoring studies are required to complement the risk assessment in addressing possible uncertainties that cannot be fully addressed through field studies for time/space scale reasons. Monitoring studies are also a way to validate or adjust the risk mitigation measures that may have been recommended as a condition of approval of the product. Monitoring effects of pesticides on honey bees has been getting more importance over the last years and has been recommended along with approval decisions for some active substances. However there exists no harmonized guidance on monitoring methodology and this raises legitimate questions about how to use the generated data in support of decision making as well as on possibilities for extrapolations. This presentation illustrates the work undertaken by the International Commission on Plant-Pollinator Relationship to review existing monitoring of the effects of pesticides on managed and wild bees and propose guidance on good monitoring practices.

## **207 Guideline and practical tools for a sustainable use of Plant Protection Products within the framework of the Sustainable Use Directive**

M. Calliera; F. Berta; P. Meriggi; R. Rossi; T. Galassi; F. Mazzini; A. Bernard; R. Bassi; A. Di Guardo; A. Marchis; E. Capri, . In 2008-2009, a survey in the Emilia Romagna region of north Italy collected information on the farm use of Plant Protection Products (PPPs) and evaluated whether the provisions of the Directive 2009/128/EC for the Sustainable Use of Pesticides (SUD) are applicable. It was concluded that the provisions of the Directive can be implemented, even if some gaps need to be filled and also the behaviour of farmers needs to be improved. Moreover, it was observed that all stages in the use of PPPs on farms could generate risks for the operator and/or the environment. One of the Directive recommendations is to promote training for operators and to adopt good agronomic practices in order to improve sustainable use of PPPs. The findings were used to develop a Guideline for Sustainable Use of PPPs to help the user in identifying the flaws in current practices at farm level as well as their corresponding corrective actions. The Guidelines are accompanied by free on-line software ([www.agricoltura-responsabile.it](http://www.agricoltura-responsabile.it)) to be used as a diagnostic tool as well as to provide recommendations for improvements. The project had a stepwise approach: Step 1: Preliminary investigation and survey on a sample of 100 farms. The main objective was to identify the potential environmental and human health pressures of pesticide use at farm level, starting from pesticide delivery to the farm through to the final disposal of the packing materials. Step 2: Based on



the data collected in the survey and their analysis, the project participants decided to develop an operational, sustainable-use guideline to be used for training and raising awareness among professionals involved with pesticides. The objectives are to collect recommendations for responsible, safe and sustainable use of these products and at the same time to provide tools to evaluate how they are managed. Step 3: To fulfil the latter objective, the guidelines were equipped with a check list and a free on-line software to assist the user in the analysis of their agricultural practices and to identify the critical points in the pesticide management, the critical issues on the farm, taking into account both the structural aspects of the farm that the behavior of the farmer so as to prevent environmental contamination and to assure a high standard of safety for operators. The tools can be considered both as a support to train technicians and as an operational guide to provide an innovative assistance service with the purpose of improving the safe, responsible and sustainable use of pesticides. The approach adopted, taking into account the variability in farm structure, cropping pattern, risk attitude and economic availability, is not an instrument to identify the most suitable protection strategy for a given crop in a given period, but to help professional users to improve their practices in managing PPPs on farms and to make the most appropriate choices leading to reduced environmental and human risk, without compromising the profitability of agricultural production and food standards.

**208 Climate change related impacts and benefits from wood extraction for bioenergy on a global scale** P. Muchada, Radboud University Nijmegen; R. Van Zelm, Radboud University; M. van der Velde, IIASA; G. Kindermann, IIASA / Ecosystem Services and Management Program; M.A. Huijbregts, Department of Environmental Science. A current challenge in impact assessments of bioenergy is how to derive a scientifically valid greenhouse gas (GHG) life cycle balance as there are many processes that lead to GHG emissions during the life cycle of the biofuel. Common practice in life cycle impact assessment (LCIA) of forestry and wood has been to assume that any carbon dioxide ( $\text{CO}_2$ ) emission related to biomass combustion equals the amount of  $\text{CO}_2$  absorbed, thus assuming a carbon neutral system with no climate change impacts. This carbon neutrality assumption has been challenged because it ignores the damage caused by the  $\text{CO}_2$  during the time it spends in the atmosphere and when a change in forest management occurs. In a study that addresses some recurring issues in LCIA namely land use, spatial variability, implications of value choices, and endpoint damage, we developed and applied a method that estimates biogenic climate change related impacts of global wood extraction for biofuel. We modeled changes in global forest carbon stock due to an increase in wood extraction with the global forestry model G4M on a  $0.5^\circ \times 0.5^\circ$  scale. Resultant carbon emissions lead to climate change related damage to ecosystem quality and human health. Grid-specific and country-specific characterisation factors (CF) were derived as disappeared fraction of species over space and time ( $\text{PDF} \cdot \text{m}^2 \cdot \text{year}$ ) and disability-adjusted life years (DALYs) per unit of wood biomass harvested ( $\text{m}^3$  wood). CFs were derived for three scenarios, coinciding with three cultural perspectives, to show differences in time horizon, species protection, discount rate, and age weighting. Our results for the hierarchist scenario show that the wood biomass extraction for biofuel has an effect of between  $-8.5 \cdot 10^{-3}$  and  $1.5 \cdot 10^{-2}$   $\text{DALY} \cdot \text{m}^3$  wood on human health and between  $-9 \cdot 10^3$  and  $+2 \cdot 10^4$   $\text{PDF} \cdot \text{m}^2 \cdot \text{yr} \cdot \text{m}^3$  wood on ecosystem quality, depending on the extraction region. The negative values indicate climate change benefits obtained due to a change in rotation time of the forest ultimately leading to more carbon uptake. As a case study, we compared the life cycle impacts of energy production via wood with coal to determine the significance of including biogenic  $\text{CO}_2$  emissions due to changes in forest management in LCIA. Results for the bioenergy production case study show that current life cycle assessment (LCA) methods underestimate, and in some cases overestimate the (climate change) impacts of wood used as a biofuel.

**209 Inclusion of Climatic Tipping Potential in LCA** S. Jorgensen, M.Z. Hauschild, Technical University of Denmark / Department for Management Engineering; P.H. Nielsen, novozymes a/s. Much

attention is today given to the climate change driven by release of anthropogenic greenhouse gases (GHGs) to the atmosphere. Often this impact is only considered in terms of global warming potential (GWP) most commonly by applying the GWP<sub>100</sub>. However, this metric does not take into account the need for staying below certain climatic target levels, in order to avoid crossing critical and possibly irreversible climatic tipping points. Some suggestions have been made as to include a target level in climate change impact assessment, such as the global temperature potential, but with the consequence of disregarding impacts beyond that target level. The climatic tipping potential (CTP) presented here is suggested as a supplementary impact category to the GWP, treating the issue of climatic tipping potential relative to a specific target time. The CTP is expressed as the impact from a GHG emission compared to how much impact can still occur before reaching the given climatic target level. The latter is considered the 'capacity' of the climate system with respect to the target level. The capacity is diminished over time due to increasing atmospheric GHG levels, meaning that the CTP impact of GHGs increases as a function of closeness to the climatic target level at the time of emission. The relative impact of three GHGs,  $\text{CO}_2$ , CH<sub>4</sub> and N<sub>2</sub>O are explored and the results show that the relative impact of the gases is also affected by the time of GHG emission relative to the target time. The calculated CTP values of the different GHGs at release times from present until the target time enable direct application of this metric in LCA. The inclusion of the CTP as additional impact category in LCA could enable better damage modeling and improve the value of LCA as a tool for decision support for climate change mitigation. Furthermore, the CTP impact category will be very useful for representing the climate change mitigating impact of temporary carbon storage in an analysed product system.

**210 Endpoint characterisation modelling for marine eutrophication in LCIA** N. Cosme, Technical University of Denmark DTU / DTUMAN QSA; H. Larsen, Danish Road Directorate / Research and Development; M.Z. Hauschild, Technical University of Denmark (DTU) / Dept. of Management Engineering. Marine eutrophication processes include the excessive growth of phytoplankton biomass in response to increased availability of nitrogen (N) in the photic zone of marine coastal waters. The eventual degradation of this biomass results in oxygen consumption in bottom waters by bacterial respiration. The excessive depletion of dissolved oxygen (DO) presents a potential impact to ecology, economy, and water quality. Land based human interventions are increasing the N loadings to marine coastal systems and overrunning their natural capacity to absorb N. Marine eutrophication is an impact category of LCIA which is still lacking a sound methodology to link midpoint and endpoint indicators and an overall model to assess the potential impact of the over-enrichment of marine ecosystems by N. Within the EU FP7 project LC-IMPACT, a model was built for the estimation of endpoint CF for marine eutrophication: Fate Factors (FF) were estimated for both airborne and waterborne N emissions based on modelling of river-N and marine-N losses to deliver the midpoint category indicator (i.e. increase of N in the marine compartment); Exposure Factors (XF) were estimated based on the Redfield ratio and a stoichiometric conversion of N in the photic zone to DO consumption in the benthic habitat. Finally, Effect Factors (EF) were estimated by applying the statistical Distribution of the Species Sensitivity (SSD) to hypoxia delivering the endpoint category indicator (i.e. species diversity loss). The product of the three factors,  $\text{FF} \cdot \text{XF} \cdot \text{EF}$  delivers the CF with the desired damage dimension, i.e.  $(\text{PAF}) \cdot [\text{m}^3 \cdot \text{d} / \text{kg}]$ , to be applied to the emitted amount of N [kg]. The Large Marine Ecosystem (LME) biogeographical classification system was used to define the receiving spatial units. The model provides CFs for the "N to air" and "N to freshwater" inventory flows at a country-to-LME (214), country (143), region/continent (11), and global level. The proposed methodology covers all the processes of relevance to the marine eutrophication phenomenon and delivers CFs at a considerably high geographic applicability, good environmental relevance and reproducibility. The sensitivity and uncertainty analyses identified key issues for data quality improvement, namely PP datasets and N-export splitting rules for multiple receiving LME. Further improvements should

address the inclusion of spatial differentiation of N-losses within the fate models.

**211 Characterisation factors for the impact category human noise** S. Cucurachi, Institute of Environmental Sciences CML / Industrial Ecology; R. Heijungs, Institute of Environmental Sciences - CML. Leiden University. In 2010, the European Commission's LCA handbook tried to set the standard for the best practise in LCA. The handbook suggested methodologies to be applied for different impact categories. Even though the handbook recognised the importance of the impacts caused by noise and the necessity of increasing the modelling effort of this impact category, no recommended approach was presented. As part of the LC-IMPACT project, we have developed a framework for the assessment of noise impacts on humans. This contribution presents the results of the operationalization of the proposed methodology. Characterisation factors (CFs) for human noise are presented at different levels of spatial differentiation, as well as temporal and frequency differentiation. The example of sound emissions is useful to discuss the right level of differentiation that is needed both at the LCI, as well as at the LCIA modelling phases. The influence of the input factors to the variance of the output is discussed, and may provide a useful insight of the way the variance of the output can be tested using techniques of global sensitivity analysis. Uncertainty and data availability are also taken into account when the transition from midpoint human noise to endpoint human health is described. \n

**212 Land use in LCA: Global characterization factors based on regional and global species extinction** L. de Baan, ETH Zurich IED / NSSI; M. Curran, C. Mutel, Institute of Environmental Engineering, ETH Zurich; S. Hellweg, ETH Zurich / Institute of Environmental Engineering; T. Koellner, University of Bayreuth. Large areas of our land surface are strongly modified by humans for agriculture, industry and settlements. This has major impacts on ecosystems and biodiversity. However, no consensus exists on how these impacts could be quantified within Life Cycle Assessment (LCA). Here, we present a globally applicable approach how to assess the impacts of land use on biodiversity on regional and global scales. We model the potential regional species loss due to all accumulated land use activities within all global WWF ecoregions and use this as a basis for calculating characterization factors for life cycle impacts assessments. We distinguish between potentially reversible impacts (i.e. regionally extinct, non-endemic species) to calculate land occupation and transformation impacts and irreversible impacts (i.e. global extinction of endemic species) to calculate permanent impacts. Species loss is calculated based on the matrix calibrated species-area relationship. We calculate characterization factors based on multiple species groups, such as mammals, birds, amphibians, reptiles and plants. Finally, we analyze and quantify the uncertainties of the characterization factors applying Monte Carlo simulations. Characterization factors for four land use types were calculated: agricultural land, pasture, forestry and urban land. In the ecoregions which have been largely converted to human use, and thus only little undisturbed habitat remains, the impacts are highest. For all three impact types, the results range several orders of magnitude across ecoregions. In general, the results show high uncertainties. Application and testing of the characterization factors in case studies will show their robustness and applicability. Currently, data availability limits a more refined land use classification. However, in future better data availability might allow including a refined classification and which might reduce some of the uncertainty.

**213 Assessing impacts on biodiversity from land use in LCA with global datasets, exemplified with kiwifruit production in New Zealand** O. Michelsen, NTNU / Industrial Ecology Programme; C.R. Coelho, Auckland Council / Geospatial Services Delivery. The question on how to include impacts on biodiversity from land use and land use changes (LULUC) in LCA has been discussed for decades and at present there is still no widely accepted method for assessing changes in quality in terms of biodiversity, partly as a consequence of lack of data. Here a method making use of globally available datasets is presented. The idea

is to combine data on ecosystem vulnerability and ecosystem scarcity on ecoregion level, dividing the world in 825 ecoregions. Ecosystem scarcity is a measure on the rareness of an ecosystem and ecosystem vulnerability is a measure on the present condition of the ecosystem type on a global scale. These data are combined with data on naturalness which indicates how much of the biodiversity is still present in an area given the present land use activities. Impacts from land use is assessed as changes in quality  $\times$  ha  $\times$  year, so the value 1 indicates a complete removal of biodiversity from one ha for one year. The method is tested on a case study on kiwifruit production on 10 different localities in New Zealand. The sensitivity of a number of assumptions is also tested. The results show a range in the impact from 0.054 to 0.141 for the production of 1,000 tray equivalents (approximately 3600 kg green kiwifruit). The results are sensitive to some of the assumptions made. Since ecosystem scarcity and ecosystem vulnerability are given values in the range from 0 to 1, they must be normalized to a reference value. The choice of a global or national reference value highly influences the results. National data on percentage of natural vegetation left is also tested as an alternative to global data on ecosystem vulnerability. The range of the scores was not changed, but the rank of some of the areas changed. This shows that present data on conservation status on ecoregion level may be too coarse to give a realistic picture of the actual situation on site, but at the same time the method with using local data incorporated using GIS modelling shows how this can be overcome. As a conclusion the results obtained using globally available data show how this method is applicable to incorporate impacts on biodiversity from LULUC in LCA, capturing much of the essence in biodiversity valuation through vulnerability and scarcity. The only information needed to apply the method is geographic location to identify the relevant ecoregion and productivity to identify land requirement.

**214 Inhaled toluene from printed matter indoors: Complementary results from Life Cycle Assessment and Risk Assessment** T. Walser, R. Juraske, Institute of Environmental Engineering, ETH Zurich; E. Demou, College of Medical, Veterinary and Life Sciences, University of Glasgow; S. Hellweg, ETH Zurich / Institute of Environmental Engineering. Toluene is the most important solvent used in rotogravure printing. The pronounced presence of toluene indoors partly results from the high use frequency of printed matter together with lower air volumes and air exchange rates compared to outdoors. In homes, more people are exposed to toluene emissions from printed matter, albeit toluene concentrations are lower than in printing rooms. If inhaled, toluene can cause dizziness, mild throat, and eye irritation over short term, and impaired cognitive functions over long term. While several studies investigated indoor concentrations of toluene in printing halls, knowledge of the consequences to human health from toluene emissions from printed matter downstream in the life cycle of magazines is still scarce. Therefore, the aim of this study was to quantify negative impacts on human health caused by toluene emissions from rotogravure printed magazines over the entire life cycle, with a particular focus on indoor environments. We used a one-box indoor model for the estimation of toluene concentrations in printing facilities and newspaper stands with large ventilation rates and multiple emission sources as well as in the small residential rooms with low ventilation rates and single emission sources. We validated the model with measurements from literature. Effect factors were taken from the USEtox database. Health impacts resulting from indoor exposure were quantified and then compared to the total human health impacts of a magazine, calculated with Life Cycle Assessment (LCA). These results allowed a relative quantification of human health impacts on a population basis. Then, the absolute individual health risk in the indoor compartments was determined with Risk Assessment (RA). The results showed that one box models provide an accurate estimation of toluene concentrations indoors for the different indoor characteristics. While workers are exposed to significant toluene concentrations in printing facilities (3), the concentrations in homes are rather low ( $< 0.4$  mg/m<sup>3</sup>). However, inhaled toluene at home becomes the dominant contribution to the total impacts from toluene of printed matter if assessed with LCA, because of the large number of people exposed. With the significant contribution of

the toluene exposure indoors to the total life cycle impact of a magazine (44%), we demonstrated that the indoor compartment requires particular attention and that RA and LCA results provide complementary information.

#### **215 Volatile Chlorinated Solvents from Consumer Products:**

**Emissions to Exposures** W.J. Doucette, T. Wetzel, Utah State University / Utah Water Research Laboratory; E. Dettenmaier, K. Gorder, Hill Air Force Base. Volatile organic compounds (VOCs), including many with documented short- and long-term adverse health effects, can enter indoor environments through internal (i.e. consumer products, building materials) and external sources (i.e. vapor intrusion from contaminated groundwater). Indoor air concentrations of VOCs vary widely, but concentrations of most VOCs are consistently higher indoors than outdoors. Many consumer products such as adhesives and cleaning solvents contain volatile chlorinated organic compounds (cVOCs) such as trichloroethene (TCE) tetrachloroethene (PCE) that are also the focus of soil and groundwater cleanups in the USA and Europe. In this study, emissions rates of volatile chlorinated solvents from several consumer products (opened and unopened) including adhesives (PCE), cleaning solvents (TCE), household cleaning products (CCl<sub>4</sub>) and molded plastic objects (1,2-DCA) were measured using a flow<sup>4</sup> through laboratory chamber approach. Measured emission rates varied from 0.001 to 20 ug/min depending on the product and CVOC. Subsequent screening-level calculations suggested that the emissions from some these items could lead to indoor concentrations high enough to be of regulatory concern. To verify this, several controlled release experiments were conducted in an actual residence. Consumer products were introduced into a single room of the test residence and indoor air concentrations of CVOCs were measured throughout the house over time using sorbent tube sampling followed by thermal desorption gas chromatography/mass spectrometry (GC/MS) and/or a portable GC/MS. Measured indoor air concentrations of PCE and TCE and peaked within the "source" room and became relatively uniform throughout the residence within several hours after being circulated through the heating, ventilation, and air conditioning (HVAC) system. Both opened and unopened consumer products emitted significant amounts of chlorinated solvents that could confound VI investigations. Initial indoor air concentrations of chlorinated solvents were highest in the room where the consumer products were introduced but were quickly and uniformly distributed throughout the three level house when circulated with the HVAC system. The measured emission rates could be used along with a simple box model to estimate exposure concentrations after "mixing" with the HVAC system.

#### **216 The Fate of PBDEs in The Use and Waste Phases Following Changes of PBDE Levels in Products; An SFA Application**

A. Golnosh, University of Toronto / Geography; A. Buser, ETH Zurich; M.L. Diamond, University of Toronto / Department of Earth Sciences. Brominated flame retardants (BFRs) are synthetic additives which are widely used to reduce the flammability in consumer products. Due to their persistent, bioaccumulative and toxic (PBT) properties, some representative of BFRs such as polybrominated diphenyl ethers (PBDEs) were regulated or phased out in Europe and North America. Despite the global decline in the use of PBDEs, the large inventory of flame retarded products will continue to contribute to emissions of these compounds into indoor and then outdoor environments. Further, "old" PBDEs may return in new consumer products fabricated with recycled materials. In this study, a substance flow analysis (SFA) was used to characterize the flow of PBDEs from product inventory to the surrounding environment. This system is built upon a detailed dynamic inventory of flame retardant products for North America (US and Canada). The results show the changing PBDE inventory over time in the use and waste phases as a result of changes in PBDE levels in consumer products. The outcome of this study can be served as a rational basis for planning systematic action and measures to reduce of the use and human exposure to these substances.

#### **217 Evaluation of the Stockholm Multimedia URban Fate (SMURF)**

**model – application to BDE 209 and two phthalate esters** A.P. Palm Cousins, Natural Resources and Environmental Effects; T. Holmgren, Umea University / Department of Chemistry; M. Remberger, IVL Swedish Environmental Research Institute / Natural Resources and Environmental Effects. The indoor environment has been proposed to play an important role for release of SVOCs to urban air as a result of emissions from consumer products. Cousins (2012) recently studied the contribution of emissions indoors to outdoor pollution using an indoor-inclusive multimedia fate model (SMURF). The aim of this study was to evaluate the SMURF model using realistic emission estimates and real monitoring data and through uncertainty analysis. The SMURF model is a steady state, 8-compartment multimedia fate model parameterized for the municipality of Stockholm. The model incorporates a novel indoor module representing the indoor environment of Stockholm. Evaluation was conducted using emission estimates of DINP, DEHP and BDE 209 based on data from literature and from new emission tests studying the emissions of DINP from PVC materials. Measurements in urban air and ventilation outlets were performed and used in combination with literature data for comparison with model predicted concentrations. Predicted concentrations agreed well with monitoring data for phthalates, but BDE 209 concentrations were a factor of 100 too low, indicating that current emission estimates for BDE 209 do not capture all the important sources. Emission to indoor air, background concentration and ventilation rate were influential inputs for the variance in the target outputs for all substances. The wet removal rate contributed significantly to the variability in dust concentration. The indoor vertical deposition contributed to the variance in all target outputs for BDE 209, reflecting the capacity of BDE 209 to partition to particles, thus emphasizing that particle behaviour is central for the fate of BDE 209. Cousins, A.P. 2012 The effect of the indoor environment on the fate of organic chemicals in the urban landscape. Science of The Total Environment. 438(0): p. 233-241.

#### **218 Prioritizing consumer product categories based on exposure estimates**

A. Ernstoff, School of Public Health, University of Michigan; P. Fantke, Technical University of Denmark; O. Jolliet, University of Michigan / School of Public Health. Data on exposure to consumer products and the chemicals therein is limited and costly to obtain. To prioritize mitigation efforts on chemicals and consumer product categories of concern, we must systematically predict dominant consumer product exposure pathways for a range of chemicals even when empirical data are absent. In this study we present a risk assessment framework for modeling cumulative exposure to consumer products with the end goal of identifying the contribution of each exposure pathway. We investigate a case study of parabens (methyl-, ethyl-, butyl-, and propyl-) in dermally applied cosmetics. Dermal exposure to cosmetics is often not considered in risk assessment and evaluation of cumulative exposure with respect to population scale *in vivo* chemical concentrations. To validate our predictions of consumer exposure we compare modeled results with *in vivo* US National Health and Nutrition Examination Survey NHANES data, and provide insight to develop a consistent and accurate evaluation of chemical exposures through various consumer product uses. Results suggest our method may aid predicting consumer exposure (specifically the 95%-ile of consumer exposure for women) to chemicals, like parabens, found in cosmetics. Estimated doses for daily use range from 0.001 to 0.04 mg/kg/day, and urine loads, extrapolated from NHANES *in vivo* data, are at comparable magnitude. Paraben-specific predicted doses correlate best with NHANES 95%-ile for women especially when considering the maximum likely concentration of each respective paraben ( $R^2=0.60$ ). Most importantly, predicted doses best correlate with urinary loads (95%-ile for women) when adjusting the dose by the relative occurrence in paraben-containing cosmetics ( $R^2=0.97$ ) across ranges of likely concentrations within the products. Using an advanced skin permeability model to estimate systemic absorption through skin does not improve prediction of urinary excretion. As parabens are also present in some foods, oral exposure must also be considered; however, is likely negligible in comparison to dermal exposure to cosmetics. Urinary doses of CP-related chemicals are orders of magnitude higher than exposure to

many environmental contaminants, this considered with our findings suggests dermal exposure to cosmetics is an exposure route category which must be prioritized within the overall framework for calculating human exposure to chemicals in consumer products.

**219 Comparison of generic modelling of emissions from different material sources** T. Holmgren, Umea University / National CBRN Defence Centre; P. Andersson, Umea University; P. Haglund, Department of Chemistry. Building materials and consumer goods contain organic additives that may be released to indoor air and reach the environment. In this study, 2 generic models were developed and compared with experimental data. Three cases were studied: emissions of di-isononyl phthalate (DINP) from vinyl flooring to air, triphenyl phosphate (TPP) from LCD-TVs to air, and leaching of triisobutyl phosphate (TiBP) and tributyl phosphate (TBP) from concrete to water. Emissions to air were studied using a 1 m<sup>3</sup> emission chamber. Four vinyl floors and a 32-inch LCD-TV were tested; the TV at various conditions (standby, on, 40°C, and 60°C). Exhaust air was sampled on PUFs for 10 days, test object removed, and chamber heated to recover residues. Concrete leaching tests were made using 9 concrete cylinders, 3 with TiBP, 3 with TBP, and 3 without additive. Water was circulated around cylinders for 16 weeks. Samples (daily/weekly) were extracted with hexane and analysed with GC-MS and GC-FPD. The generic model used to predict emissions used an Abraham solubility model to estimate partition, the Piringer equation to predict diffusion and the Chilton-Colburn analogy to estimate convective mass transfer. Emission factors were calculated using a numeric solution and a simplified model that do not consider material diffusion resistance. When additive concentration is high and additive volatility low the depletion of the material surface is insignificant and diffusion in the material of low importance. This was clearly the case for the LCD, Figure 1, in which TPP are added at high concentration to the front layer of the LCD-screen. It was, however, not the case for the vinyl materials for which the simplified model overestimated the emissions. For such thick materials the material resistance to mass transfer had to be considered and the detailed model used. For concrete, the prediction models did not fit experimental data. In hardened cement, capillary pores and cracks are formed that disturbs the cement structure. Experiments showed an initial release of 4200 µg TiBP / m<sup>2</sup>h, which rapidly dropped to 10 µg / m<sup>2</sup> h. The initial release could be explained through a porosity model and the lower long time release through diffusion theory.

**220 An overview on emerging contaminants** J. Readman.

**221 The integration of chemistry with toxicological testing in European systems** M. Lamoree, Chemistry & Biology.

**222 Effects of multistressors and bioinvasions in estuaries and coastal areas: implications for ecological risk assessment**L. Guilhermino, CIIMAR University of Porto.

**223 Alternative flame retardants in the coastal and marine environment** R. Ebinghaus, Helmholtz-Zentrum Geesthacht.

**224 What Defines Nanomaterials? - An examination of the size dependence of physicochemical properties critical to environmental risk assessment** C.D. Hassinger, ARCADISUS; K. Sellers, ARCADIS; E.A. Bleeker, RIVM; W.J. Peijnenburg, RIVM / Laboratory for Ecological Risk Assessment; D. Sijm, National Institute for Public Health and the Environment (RIVM). Efforts to assess the risks from nanomaterials implicitly incorporate a definition of what it means to be "nano". Members of the scientific community and regulators around the world commonly use a definition of 1-100 nanometers (nm) in size. However, uncertainties about this definition persist, with some defining nanoparticles as sizes up to 2000 nm. This presentation will describe, based on a comprehensive literature review, what is known about the size dependence of the physical/chemical properties critical to environmental risk assessment (the known knowns) and current data gaps (known unknowns). As the size of a particle decreases into the

'nano' size range some physicochemical properties may significantly change. Therefore, within the context of the European Commission's (EC) definition of "nanomaterial" and the data needs under the Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH) regulation (EC 1907/2006), this research sought to determine which physicochemical properties make a nanomaterial different from a 'conventional' material and at what size these properties are changed to 'nanospecific' properties. The literature review focused on: surface morphology, crystalline structure, water solubility, reactivity, and photocatalytic reactivity. Relative few papers were identified investigating size-related changes in surface morphology. With respect to crystallinity, which can affect reactivity, several aspects of metals and metal oxides (e.g., lattice contraction/expansion, phase transformation) may vary with particle size although no simple conclusions can be drawn regarding size dependence. Some experimental data show that the solubility of metal and metal oxides and sulphides can increase at nanoscale; additionally, the increased rate of solubility is well known. Reactivity can also increase with decreasing particle size. Maximum catalytic activity of nanoparticles occurs at sizes well below 100 nm (15-20 nm), with a sharp change in reactivity below approximately 5 nm in some cases. Photocatalytic reactivity of certain metal oxides and sulphides also increases with decreasing particle size, in some cases changing at a particle size of approximately 5-10 nm. These studies reviewed primarily investigated nanoparticles

**225 Mechanistic interpretation of single-particle ICP-MS data** G. Cornelis, University of Gothenburg / Department of Chemistry and Molecular Biology; M. Hasselov, Goteborg University / Chemistry Dept. Progress in (Eco)toxicological research on fate and effects of engineered nanoparticles (ENP) is hampered by a lack of sensitive methods that can measure particle number-based size distributions (PSDs) of ENP in environmental matrices at realistically low concentrations. Single particle inductively coupled plasma mass spectrometry (spICP-MS) is a promising technique in this respect as it offers element specific measurements of ENP that generate ion bursts in the plasma, the magnitude of which corresponds to their size and the number of which corresponds to the frequency of particles, thus allowing to calculate PSDs. However, the lower size limit of ENP size measurements is limited mainly by lack of a process-based data analysis tools. The current study proposes an alternative approach, where precise knowledge of dissolved and background signals in ICP-MS is used to deconvolute it from spICP-MS. Well-characterised gold ENP of 10, 30 and 60 nm were analysed using spICP-MS and the data was analysed using known algorithm and the algorithm proposed in this study. It is proposed that much more accurate PSD can thus be obtained, especially for relatively small ENPs. A mechanistically based spICP-MS signal analysis algorithm was thus developed that was able to deconvolute dissolved signals from ENP signals in a combined dataset, but in the case of small ENP, there was no statistically significant difference between dissolved and ENP signals that could be used to distinguish the signals, effectively imposing a size limit to spICP-MS determinations of PSDs and concentrations of ENP in the environment.

**226 Fate of nanosilver in lake mesocosms** C.D. Metcalfe, L. Furtado, Trent University / Environmental & Resource Studies; J. Fischer; M. Hoque, H. Hintelmann, Trent University; D. Mitrano, J. Ranville, Colorado School of Mines; B. Cheever, M. Xenopoulos, P. Frost, Trent University. Silver nanoparticles (AgNPs) are widely used in textiles and in household products as antimicrobial additives. AgNPs may enter aquatic ecosystems via discharges of municipal and industrial wastewater. AgNPs undergo transformations in aquatic environments that can alter the toxicity to aquatic organisms. In a study conducted as part of the Lake Ecosystem Nanosilver (LENS) project, we studied the transformations and fate of AgNPs in 12 mesocosms (2 m diameter x 2 m deep) installed in a soft water lake in Ontario, Canada. AgNPs with a mean diameter of 50 nm and capped with either PVP or citrate were purchased as a suspension in deionized water from NanoComposix, CA, USA and this material was added to mesocosms either as incremental ("drip") additions every second day for a 70 day period, or as a single

("plug") addition followed by a monitoring period of 50 days. Total Ag in the drip mesocosms increased over time and reached about 80% of the target nominal concentrations of 4, 16 and 80 ppb by the end of the experiment. About 40% of Ag was retained on a 0.22 µm filter, indicating some hetero- and homo-aggregation. There was no significant difference between the fate of the PVP and citrate capped AgNP in the drip mesocosms. In the plug experiment with PVP capped AgNP at a nominal concentration of 80 ppb, the half life for total Ag in the two mesocosms was approximately 20 days. Analysis of the size of the AgNPs in the plug mesocosms using single particle ICP-MS indicated that the mean particle size declined over time from approximately 50 nm to approximately 35 nm. ICP-MS analysis of the filtrate after ultrafiltration indicated that the concentrations of dissolved silver (dAg) in the mesocosms were low, but the results were highly variable. Estimates of concentrations of dAg using passive sampling with diffusive gradient in thin film (DGT) devices deployed in the 80 ppb drip treatments indicated that dAg concentrations were in the range of 1-4 ppb. Overall, this study showed that the AgNPs were relatively stable in suspension in the mesocosms, which could be due to the low ionic strength and the moderate levels of dissolved organic material present in the lake water.

## 227 Release of TiO<sub>2</sub> Nanoparticles from Sunscreens into Surface Waters

A. Gondikas, University of Vienna / Environmental Geosciences; F. Von der Kammer, Vienna University / Department for Environmental Geosciences; R. Reed, Colorado School of Mines / Department of Chemistry and Geochemistry; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; T. Hofmann, University of Vienna / Environmental Geosciences. The natural cycle of information flow between risk assessment studies (RAS) and research and development (R&D), which is vital for the production of 'environmentally responsible' engineered nanomaterials (ENM) is currently lacking appropriate momentum. One of the major reasons is the scientific gap between ideal lab-scale studies and realistic environmental conditions (e.g. complex media, transformations of ENM, realistic concentrations, time-dependent exposure, and relation to background materials) that introduce uncertainties in RAS. In order to reduce these uncertainties, it is necessary to develop methods that detect and characterize ENM in natural environments. In this work, we attempted to address the issues of detecting ENM release (TiO<sub>2</sub> from sunscreens) in a complex matrix (lake water), at environmentally relevant concentrations, distinguish them from natural background, while taking into account the time-dependent nature of the release. By collecting samples of suspended matter and sediments we quantified TiO<sub>2</sub> concentrations in the *Alte Donau* Lake (Vienna, Austria) that is heavily used for bathing activities during the summer season. The Alte Donau Lake can serve as a 'low background' water body with high bathing activity that is prone to release of TiO<sub>2</sub> ENM from sunscreens. Indeed, an increase of Ti concentrations was monitored during the bathing season in the sediments, SPM, and the total water samples. However, distinguishing between engineered and naturally occurring TiO<sub>2</sub> nanoparticles is an additional major challenge. In an attempt to address this issue, we compared titanium to aluminium concentrations. The ratio of Ti/Al is heavily weighted towards the denominator for natural soils, while the opposite is true for the majority of sunscreens products, where the mineral particle consists of a TiO<sub>2</sub> core and only a thin layer of aluminium oxides on the surface. We measured total Ti and Al content in the sediment fractions and SPM collected at several time points spanning from June to October of 2012. The Ti/Al ratio appeared to increase during the bathing season of July and August, following changes of temperature during this time. Additionally, in SEM investigations of the filtered SPM some TiO<sub>2</sub> particles could be found with EDX signatures similar to particles from sunscreen products.

## 228 Filling the gaps in nanomaterial exposure assessment - heteroaggregation and fate modelling in surface waters

A. Praetorius, ETH Zurich / Institute for Chemical and Bioengineering; M. Scheringer, ETH Zurich / Institute for Chemical and Bioengineering; J. Labille, J. Bottero, CEREGE, Aix-Marseille Université, CNRS; K.

Hungerbuehler, ETH Zurich / Institute for Chemical and Bioengineering. Environmental fate models serve as an important tool to obtain predicted environmental concentration (PEC) values and enable a proactive risk assessment of engineered nanomaterials (ENMs). For reliable model predictions, a thorough understanding of the dominant processes affecting the fate of ENMs is of great importance. At currently expected environmental concentrations of ENMs, heteroaggregation of ENMs with naturally occurring suspended particulate matter (SPM) is probably much more important than homoaggregation as a factor determining the environmental fate of ENMs. However, to date, very little research has been done to elucidate and quantify the heteroaggregation of ENMs with SPM under realistic conditions. We here present a new method for measuring the heteroaggregation of TiO<sub>2</sub> nanoparticles (NPs) with SPM and for the determination of heteroaggregation attachment efficiencies (?<sub>het-agg</sub>). The heteroaggregation kinetics of SiO<sub>2</sub> particles (0.5 µm) and TiO<sub>2</sub> NPs (5-20 nm) were followed by laser diffraction and ?<sub>het-agg</sub> values<sup>2</sup> for determined for different environmentally relevant solution conditions by fitting the aggregation curves with a Smoluchowski-based model. Our results show that ?<sub>het-agg</sub> increases with increasing salt concentration (NaCl and CaCl<sub>2</sub>), whereas the presence of humic acid stabilizes the particles against heteroaggregation. The obtained ?<sub>het-agg</sub> values were implemented in an environmental fate model predicting the transformation and transport behaviour of TiO<sub>2</sub> NPs in a river system. The modelling results show that most TiO<sub>2</sub> NPs accumulate in the sediment compartment, due to heteroaggregation with SPM and subsequent sedimentation, but a small fraction of NPs remains in the water compartment. The combination of measured ?<sub>het-agg</sub> values and an environmental fate model enables predictions of the environmental fate of TiO<sub>2</sub> NPs and thereby fills important gaps for a realistic exposure assessment of ENMs.

## 229 Why testing stones in mesocosms?

C. Schaefer, FraunhoferInstitut / Department of Ecotoxicology; B. Knopf, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; H. Ruedel, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Molecular Biology & Applied Ecology; K. Ebke, MESOCOSM GmbH Institut für Gewässerschutz; U. Hommen, Fraunhofer IME. Within a large research activity on the ecological risk assessment of slag material from copper production used as armour stones, the potential leaching of metals and its effects on the aquatic community in outdoor mesocosms was monitored over one year. The study was conducted in 25 stainless steel enclosures of 2 m<sup>3</sup> volume, installed in a large artificial pond with an established aquatic community. Five enclosures served as controls including 25 g/L basanite crushed stone fines and 100 g/L basanite armour stones as natural reference material. In eight enclosures 3.25, 6.25, 12.5 and 25 g/L iron silicate crushed fine stones (two replicates each) and in 12 enclosures 12.5, 25, 50 and 100 g/L iron silicate armour stones (three replicates) were introduced. In each enclosure the appropriate amount of basanite stone fines and stones were added to achieve the same amount stone fines and stones in all enclosures including the controls. During the study the concentrations of metals in the water and the sediment was measured while metals in biota were measured at the end of the study after one year of exposure. For the effect assessment the development of the populations of algae, macrophytes, zooplankton and macroinvertebrates was monitored. Cu, Ni, Zn, Mn and Fe concentration in the water increased, related to the amount of introduced iron silicate. The maximum Cu concentrations found at the highest stone treatment level were 14 µg/L in March 2010 while the highest concentration in the crushed fines enclosures were found to be close to 13 µg/L 7 days after introduction of the test items. After one year of exposure, Cu concentration in the water decreased down to 3 and 5 µg/L in the highest crushed fines and stone treatment level, respectively. No dose related increase of metals could be found in the sediment. In biota Cu concentration increased up to a factor of 5 compared to the controls while other metals showed usually no or a smaller increase in biota. No indication of biomagnification in the food chain was found. Up to 12.5 g crushed stone fines / L or 50 g stones / L no long-term or pronounced effects on the bio-coenosis were observed.

At 25 g crushed stone fines / L or 100 g stones / L, effects on algae, macrophytes and insects over more than 8 weeks or at the end of the study could not be excluded. Thus, 12.5 g crushed stone fines / L (1:80) or 50 g armour stones / L (1:20) are considered ecologically acceptable amounts of iron silicate slags in this study.

### 230 Linking structural, functional and behavioural endpoints to detect effects on *Gammarus roeseli* after repeated insecticide pulses

**S. Mohr**, Umweltbundesamt / IV 2.5; R. Berghahn, Federal Environmental Agency / Field Station Marienfelde; R. Boettger, M. Feibicke, Umweltbundesamt. Gammarids play a key role in freshwater lotic ecosystems. A population decrease or breakdown e.g. due to pesticides may therefore have severe consequences for stream ecosystems. Streams may experience repeated pesticide pulses, which may be of short duration but have high pesticide concentrations. This may not lead to death of organisms but to reduced health, which is hard to detect with routine sampling methods. A stream mesocosm study was conducted in order to investigate the effects of short and repeated pulses of the neurotoxic insecticide imidacloprid on macroinvertebrate communities with special focus on behavioral and functional endpoints of the amphipod *Gammarus roeseli*. Prior to the study, laboratory toxicity tests were run with this species to determine the optimal sublethal pulse concentrations for the mesocosm study. In the mesocosm experiment, weekly 12 h pulses of 12 µg/L of the insecticide imidacloprid were set 3 times in 4 stream mesocosms in 2 series, one in spring and one in summer. Another 4 mesocosms served as controls. Prior to each pulse series, the mesocosms were equipped with sediment and water, and stocked with macroinvertebrates from an uncontaminated reference stream using straw bags as attraction devices. Within the investigation period of 11 weeks, gammarids were sampled regularly for the following endpoints: drift, activity, live counts, abundance and shredder activity of *G. roeseli*. In the toxicity tests, the EC50 (96 h) for *G. roeseli* varied considerably from 1.9 to 129.5 µg/L imidacloprid depending on the different experimental conditions. Besides effects of food and age, the origin of the test species taken from a field population in autumn or spring had strong effects on the sensitivity of *G. roeseli*. In the stream mesocosm study, abundance data and shredding activity did not indicate negative pulse effects, but strong effects were found for the endpoints drift and activity. Under more natural conditions including fish predation and higher flow velocity inactive or impaired gammarids may drift out of the system or be eaten by fish. A combination of different sublethal endpoints such as drift effects may provide a realistic view of what may actually happen in the field and help to predict detrimental effects for ecosystems.

**231 Extensive toxicity testing with the neonicotinoid imidacloprid shows a high chronic toxicity to mayfly larvae** **L. Roessink**, Alterra; L.B. Merga, Wageningen University; H. Zweers, Alterra, Wageningen UR; P. van den Brink, AlterraWageningen UR / (a) Aquatic Ecology and Water Quality Management Group; (b) Alterra. Although solely used in the terrestrial environment, the neonicotinoid insecticide imidacloprid also finds its way to surface waters, for instance via spray drift, leaching and run off. Most of the research to its effects on aquatic non-target invertebrates comprises acute tests and chronic data is scarce. A knowledge gap, since chronic exposure may be expected given the persistence of imidacloprid. The current study was designed to generate both acute and chronic toxicity data for a range of freshwater invertebrates. Both acute 96h and chronic 28d toxicity tests have been performed using glass jars placed in a water bath. Tested species comprised *Cloeon dipterum*, *Caenis horaria*, *Chaoborus obscuripes*, *Plecoptera minuttissima*, *Sialis* spp., *Asellus aquaticus*, *Gammarus pulex*, *Trichoptera* spp., *Notonecta* spp., and *Corixidae* spp. (the latter three only tested in acute tests). Both immobilisation and mortality parameters were scored and EC<sub>50</sub> and LC<sub>50</sub> parameters were calculated. These values were further used to construct Species Sensitivity Distributions (SSD). The acute tests showed that mayflies (*C. dipterum* and *C. horaria*) and caddisflies (*Trichoptera* spp.) are the most sensitive species tested. While *Notonecta* spp. and *Sialis* spp. did not show any treatment-related mortality in the acute experiments at all. Chronically, both

mayfly species (*C. dipterum* and *C. horaria*) were the showed a much higher sensitivity compared to the other species tested. Acute to chronic ratios were highest for *C. obscuripes* and the two mayfly species, and overall ranged from 4 to 85, suggesting that it is not recommended to use acute data to assess the effects of long-term exposure to imidacloprid.

### 232 Plant Species Sensitivity Distributions for ozone exposure **T.v. van Goethem**

van Goethem, Radboud University / Environmental Sciences; L.B. Azevedo, Radboud University Nijmegen / Department of Environmental Science; R. Van Zelm, Radboud University; F. Hayes, Environment Centre Wales / Centre for Ecology and Hydrology; M. Ashmore, University of York / Stockholm Environment Institute; M.A. Huijbregts, Department of Environmental Science. Current critical levels commonly used in environmental policy assessment for ozone exposure to natural vegetation are based on relationships between ozone concentrations and effects such as yield loss and biomass reduction. These levels are based on sensitive but ecological relevant species. These species, and corresponding critical levels, are used as indicators for species groups to determine risk for larger ecological units. For semi-natural plant communities, with the large range of species present, an approach based on a single indicator species ignores the wide range of sensitivity across all the component species. To date, an approach which gives the affected fraction of a species assemblage due to ozone exposure is lacking in risk assessment for semi-natural vegetation. Therefore, Species Sensitivity Distributions (SSD), representing a cumulative stressor-response distribution for species within a plant community, were derived for damage to natural vegetation caused by ozone exposure. SSDs were constructed for three species groups, i.e. trees, annual grassland and perennial grassland species, using species-specific exposure-response data. The SSDs were applied in two ways. First, critical levels were calculated for each species group and compared to current critical levels for ozone exposure. Second, spatially explicit estimates of the potentially affected fraction of plant species in Northwestern Europe were calculated based on ambient ozone concentrations in 2010. We found that the SSD-based critical levels were lower than the current critical levels for ozone exposure, with conventional critical levels for ozone relating to 8-20% affected plant species. The potentially affected fractions based on ambient ozone concentrations in 2010 indicate that in some regions potentially 13% of the perennial grassland species and 30% of annual grassland species have growth reductions of at least 10%. Furthermore, the mean values of the SSDs were significantly lower for annual than for perennial grassland species. This indicates that annual grassland species, as a species assemblage, are more sensitive to ozone than perennial grassland species. Our study shows that the SSD concept can be successfully applied to derive critical ozone levels as well as to estimate the affected fraction of a community along specific ozone gradients.

**233 Deriving species sensitivity distributions (SSDs) from species distribution models (SDMs)** A.M. Schipper; L. Postuma, RIVM / Lab. for Ecological Risk Assessment; D. De Zwart, RIVM / Centre for Sustainability, Environment and Health; M.A. Huijbregts, Department of Environmental Science. Species sensitivity distributions (SSDs) are commonly used to describe the inter-species variation in sensitivity to an environmental stressor. SSDs are typically established based on laboratory experiments where a few test species are exposed to various levels of a single stressor, usually a toxicant, under otherwise optimal conditions. These tests typically rely on a few easily cultivable species that may be absent in the field site of concern. More ecologically relevant SSDs may be obtained by establishing field-based SSDs (f-SSDs) from monitoring data, thus covering the actual species pool. However, unless the stressor of concern is the main, dominant stressor that influences species occurrence or abundance, such field-based response curves may be flawed by the confounding influences of other stressors. In the present study we aim at deriving f-SSDs from single-species distribution models (SDMs) that predict the occurrence of species under multiple environmental factors. We used a monitoring data set comprising 103 benthic macroinvertebrate taxa and 12 abiotic

variables collected at 349 sites in tidally influenced fresh water bodies in the west of The Netherlands. For each taxon in the database we constructed a distribution model relating the probability of occurrence to the 12 abiotic variables. To translate the predicted probabilities of occurrence into presence or absence, we identified taxon-specific thresholds of occurrence. Subsequently, we used the SDMs and thresholds to predict the presence-absence of each taxon as function of each single abiotic variable. This was done by varying the level of the abiotic variable of concern across its range while keeping the other abiotic variables at optimal levels, i.e., levels maximizing the predicted probability of occurrence of the taxon of concern. Finally, we aggregated the presence-absence for all taxa across the range of the abiotic variable, thus establishing an f-SSD showing the potentially not occurring fraction (PNOF) of taxa in relation to each abiotic variable of concern. Our preliminary results indicate the feasibility of establishing SSDs by applying multiple regression techniques on field data, thus yielding f-SSDs for multiple stressors based on actual species pools.

**234 Effects of herbicides and fertilizer on the plant community of field margins** J. Schmitz, Inst for Environmental Sciences / Institute for Environmental Sciences; C. Bruehl, University of Landau Institute for Environmental Sciences. The risk assessment of herbicides aims to protect non-target plants in off-crop habitats such as field margins from adverse effects of pesticides. Therefore, short-term phytotoxicity tests with single and annual plant species are performed in young development stages in greenhouse experiments. Although testing guidelines allow using non-crop species, the standard risk assessment uses crop plants for phytotoxicity testing even though non-crop species (annual and perennial species) are to be protected in field margins. Due to the short test duration of 21-28 days effects on reproduction cannot be detected with these test methods. Furthermore, the phytotoxicity tests are performed under standardized greenhouse conditions that differ markedly from field conditions (e.g. intra- and interspecific competition for resources). The present study was undertaken to investigate the effects of the misplacement of pesticides and fertilizer on the plant community of field margins. The study was a perennial field study, which started in 2010 and ended in 2012. The applications of the treatments and their application sequences mimicked the field management of winter wheat fields with their recommended agrochemical products and application rates. The applied fertiliser and pesticide concentrations were consistent with their inputs (drift+overspray) in the first meter of a field margin directly adjacent to a field under Good Agricultural Practices. To detect the effects of the applications, vegetation assessments were performed in May and June 2010 to 2012. Additionally, the seed production of selected species (*Ranunculus acris*, *Lathyrus pratensis*, *Vicia sepium*, and *Rumex acetosa*) was assessed in 2012. The plant density of the four species was significantly affected by the fertilizer and herbicide applications. The plant density of *R. acris* and *L. pratensis* was affected stronger in the fertilizer treatments than the herbicide treatment and the plant density of *R. acetosa* and *V. sepium* was similar affected through the fertilizer and herbicide treatment. However, the treatment combination of fertilizer and herbicide resulted in additive effects. In addition, the herbicide treatment reduced the seed production of *R. acris*, *L. pratensis* and *V. sepium*. The experiment showed that the effects in the field are complex, interaction effects between agrochemicals (e.g. herbicides and fertilizer) can occur and are certainly important for the sensitivity of species to agrochemicals.

**235 Does soil type influence the toxicity of silver to plants?** K. Langdon, CSIRO Land and Water; M. McLaughlin, CSIRO University of Adelaide; J. Kirby, CSIRO; G. Merrington, Environment Agency. Due to the extensive use of silver (Ag) in a wide range of consumer products, there is an increased interest in understanding the potential risks associated with this element in various environmental compartments. Although Ag in its ionic form ( $\text{Ag}^+$ ) has been shown to exhibit toxicity to aquatic organisms at low solution concentrations, it is likely that in the terrestrial environment interactions with a soil matrix will result in increased sorption and binding of Ag, making it less

bioavailable to soil-dwelling organisms. Research into on ecotoxicity of other metals has shown that soil properties can modify metal toxicity, with soil pH, organic carbon (OC), clay content and cation exchange capacity (CEC) shown to have the greatest influence in mitigating toxicity. The aim of this study was to examine the toxicity of Ag to plants in a range of soils with varying properties to determine if differing soil properties influence the toxicity of Ag in soils. A standard five day root elongation test was used, with barley as a test organism. Increasing concentrations of Ag significantly inhibited barley root elongation to varying degrees in the soils. EC50 values were found to vary over three orders of magnitude from approximately 80 to 1000 mg Ag/kg. Toxicity thresholds were found to be (positively) related to the soil OC content and CEC. These results indicate the need to consider soil properties when determining the predicted no effect concentrations for Ag in the terrestrial environment.

**236 Plant toxicity in Pb-salt spiked soils: unraveling effects of acidification, salt stress and ageing reactions** E.E. Smolders, Katholieke Universiteit Leuven; K. Cheyns, KU Leuven. This study was set-up to quantify the differences in lead (Pb) toxicity between  $\text{Pb}^{2+}$  spiked soils and field contaminated soils and to identify factors involved. Yield of wheat seedling was unaffected by total Pb (up to 14,000 mg Pb/kg) in field contaminated soils. In contrast, yield declined by factors 2 or more in corresponding soils freshly spiked with  $\text{Pb}^{2+}$ -salts at similar total soil Pb concentration. Toxicity of  $\text{Pb}^{2+}$ -salts in soil (21-8700 mg Pb/kg) to tomato and barley plants was stepwise reduced by leaching with pH correction and by five years ageing after spiking. Shoot-phosphorus (P) concentration strikingly explained the differences in toxicity among soils and treatments. Bioassays and soil analysis show that Pb toxicity in freshly spiked, unleached soils is primarily confounded by salinity stress and acidification. In addition, ageing processes do reduce Pb toxicity, likely due to reduced Pb induced P deficiency for plants. Soil leaching and pH correction after spiking are practical methods that increase the field relevance of test soils in laboratory assays.

**237 Single Effects of UV radiation to *Folsomia candida*** D.N. Nunes Cardoso, CESAM University of Aveiro. At the same time that organisms are exposed to chemical contaminants, they can also experience a large range of environmental fluctuations such as drought and flood conditions, temperature and pH or even UV radiation increments. In this study we have carried out the exposure of the collembolan *Folsomia candida* to different doses of UV radiation in order to attain for differences in exposure type effects. *F. candida* was highly sensitive to UV radiation, affecting its reproduction and survival. Two situations were tested when the organisms were exposed to UV radiation: direct (Plaster) and indirect (Lufa 2.2) exposure to the radiation. Additionally, we tested the influence of UV radiation to soil organisms in compacted soil. When radiation was applied directly, at the highest UV doses all exposed collembolans died. Curiously, the reproduction of collembolans was higher at the highest doses when they received indirect radiation. Also, with our results, we proved the negative importance of soil compaction on the soil fauna regarding the protection it can also provide to this kind of organisms, when evaluating UV radiation effects. Furthermore, UV radiation influenced the capacity of egg's hatching when their exposed to direct UV radiation. At high doses of UV, the number of eggs hatched was much lower than in control situations. With those bioassays, we proved that UV radiation influence the survival and reproduction of the organisms and the protection that soil give to organisms when they are exposed to UV radiation.

**238 Long-term sorption of Mn(II) by viable and dead *Shewanella putrefaciens*: speciation & bioavailability relevance** N. Chubar, Glasgow Caledonian University / School of Engineering and Built Environment; C. Avramut, Leiden University Medical Center / Department of Molecular Cell Biology. The interfacial processes at the microbial cell walls (due to variety of functional groups and metabolic activity of living cells) influence the cycling, speciation and



bioavailability of xenobiotics and nutrients. Additionally, these processes influence the composition of surface and groundwater, acid mine drainage and formation of soils and minerals. Taken together, these processes are of great importance for the development of bioremediation strategies. Although many researchers have evaluated metal sorption in viable and non-viable bacteria, forming conclusions about their similar adsorptive behaviour for short periods of contact time this is the first work where the difference between the adsorptive properties of viable and dead cells is clearly shown, due to the application of a longer contact time. Manganese is one of the most important redox chemical elements in the environment; it is a nutrient in smaller amounts and a contaminant at high concentrations, particularly at mining. The data reported here are the first insight into the ability of viable cells to remove metal ions over a one-month period. The long-term ability of the bacteria to remove  $Mn^{2+}$  over one month and an accelerated removal of this metal after 4 days is due to the formation of inorganic precipitates of Mn(II) which are a function of the contact time, metal loading, temperature and a total number of the microbial cells. This is the first study demonstrating the ability of the initially viable bacteria *S. putrefaciens* to synthesise manganese phosphate, and also showing how the other experimental condition can direct the living cells to form the other precipitate(s), such as manganese(II) carbonate. Dissolved organic substances released by viable *S. putrefaciens* did not complex  $Mn^{2+}$ , at least over 14 days. However, in the presence of Mn(II), an increased concentration of the released dissolved intracellular inorganic phosphate and the production of dissolved organic substances containing phosphate groups are possible reasons of the formation of the new inorganic Mn-containing phase, which is Mn(II) phosphate.

**239 The effect of soil pH on cadmium toxicokinetics in the springtail *Folsomia candida*** M.M. Ardestani, Ecological Science; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science. Heavy metals should be taken up by the organism to show toxic effects. Soil organisms are exposed to metals mainly through pore water. Therefore, availability of metals in the porewater is important factor which determines in what extent metals could reach their targets in the organisms' body. Bioavailability is a dynamic process, which is affected by physical-chemical soil properties including soil pH. Taking into account the effect of time might better explain the effect of soil properties on metal bioavailability in soil. We therefore performed a toxicokinetics study to assess the effect of soil pH on the uptake and elimination kinetics of cadmium in the springtail *Folsomia candida*. Animals were exposed to three cadmium concentrations (0, 5, and 20  $\mu g$  Cd  $g^{-1}$  dry soil) in LUFA 2.2 soil, using three pH levels (4.5, 5.5, and 6.5). Cadmium concentrations in the animals were measured by sampling the animals at day 0, 0.25, 1, 2, 4, 7, 10, 14 and 21 for the uptake phase and at the same sampling time points for the elimination phase after the animals were transferred to clean LUFA 2.2 soil. A first-order one-compartment model was fitted to the internal cadmium concentration data for each pH level and exposure concentration. At the beginning and the end of uptake and elimination phases, total and water or 0.01 M CaCl<sub>2</sub> extractable concentrations of cadmium were measured in the soil as well as concentrations in the pore water. Extractable and porewater concentrations decreased with increasing pH. The results showed a fast uptake of cadmium in the animals, with uptake rate constants ranging between 0.33 to 1.00  $g_{soil}^{-1} g_{animal}^{-1} d^{-1}$  based on total soil concentrations followed by a fast elimination with elimination rate constants between 0.15 to 0.45  $d^{-1}$ . Steady state was reached after approximately in three weeks of exposure. Cadmium uptake rate constants based on total soil concentrations were independent of soil pH and so were steady state concentrations in the earthworms. Uptake rate constants related to extractable and porewater concentrations, however, increased with increasing pH, which could be explained from the absence of proton competition at high pH. These findings support the biotic ligand model approach.

**240 Suitability of flow cytometric analysis of primary cultures of coelomocytes of *Eisenia fetida* for toxicity testing** A. Irizar Loibide, University of the Basque country UPVEHU / Department of Zoology

and Animal Cell Biology; C. Rivas, University of the Basque Country; F. Goni, J. Etxebarria, GAIKER Technological Centre. IK4 Research Alliance.; I. Marigomez, University of the Basque Country; M. Soto, University of Basque Country / Zoology and Animal Cell Dynamic. Soil pollution is a very common problem and the assessment of its effects on soil ecosystems has become a priority issue. Recently, primary cultures of earthworm immune cells, coelomocytes, have been used for *in vitro* toxicological research following the 3R principle, and the suitability of coelomocyte primary cultures for toxicity assessment was demonstrated. The autofluorescence of riboflavin molecules present in eleocytes, a subpopulation of coelomocytes, is a very useful characteristic for toxicity evaluation, since it has been seen that it decreases after exposure of earthworms to chemical stress (Plytycz et al., 2009). Several works have investigated the changes of autofluorescence levels after exposure of earthworms to contaminated soils with flow cytometry, since this technique allows the rapid and precise quantification of autofluorescent cells and can also give information about the changes in the fluorescence signal of the eleocytes. In the present work, coelomocyte primary cultures have been exposed in two different manners to a model metal, cadmium, in order to study its effects on coelomocytes and on their different subpopulations (the autofluorescent eleocytes and the non-autofluorescent amoebocytes) and to prove the suitability of the flow cytometric analysis of *in vitro* exposed coelomocyte primary cultures for toxicity assessment. The results show that primary cultures were sensitive to Cd exposure although the stabilization of cells in culture medium for 24h caused a decrease in their sensitivity. Among coelomocyte subpopulations, Cd caused toxicity in both studied cell populations, but viability of amoebocytes was affected at higher Cd concentrations than eleocytes, indicating different sensitivity levels. The comparison of those *in vitro* exposures and the parallel *in vivo* exposures showed an increased toxicity in the first ones. It can be concluded that primary cultures of coelomocytes exposed *in vitro* to contaminants are suitable for flow cytometric analysis and toxicity assessment since both coelomocytes subpopulations were sensitive to the presence of Cd, and their mortality was dose-dependent. However, the stabilization period before the exposure could cause a loss of sensitivity. The higher sensitivity of *in vitro* exposures made them suitable for toxicological studies.

**241 Activated Sludge, Respiration Inhibition Tests; Experiences with the Updated OECD 209 Test Guideline** C. Mead, Harlan Laboratories Ltd / Ecotoxicology dept; P. Roulstone, Harlan Laboratories Ltd. The OECD 209 Test Guideline describes a method to determine the effects of a substance on the respiration rate of activated sludge micro-organisms. The results obtained can be used to assess the effect that the test substance will have on a sewage treatment facility and / or an indicator of potential inhibitory effects in a biodegradation test. In July 2010 an updated Test Guideline was published which introduced replication of the test vessels and the possibility of determining inhibition of both carbon and ammonium oxidation rates. As a result of these changes to the Test Guideline it was increasingly difficult to conduct a 'full' study during a standard working day when using the suggested sequential preparation of test vessels. Here we describe some of the alternatives available for conducting 'full' studies and present a summary of the studies that have been conducted over the last 12 months to illustrate the applicability of these alternatives.

**242 Comparing concentrations of micropollutants and toxicological effect thresholds in wastewater-impacted rivers** R. Bloch, Helmholtz Centre for Environmental Research GmbH / UFZ; C. Hug, Helmholtz Centre for Environmental Research / Effect-Directed Analysis; P.C. Von der Ohe, UFZ Helmholtz Centre for Environmental Research; M. Krauss, Helmholtz Centre for Environmental Research / Effect-Directed Analysis; T. Schulze, W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis. Wastewater discharges result in ubiquitous contamination of surface waters by a large number of polar organic chemicals, which stem from households and industrial uses. These micropollutants are only incompletely

removed by conventional wastewater treatment plants (WTPs). Although occurring in rather low concentrations, micropollutants might cause adverse effects on aquatic organisms. A target screening for 120 compounds was done in 31 surface water samples from the catchments of the rivers Saale and Mulde in Germany. Compounds were chosen from different compound classes, such as pharmaceuticals, industrial chemicals, pesticides, biocides, and personal care products based on likely or known occurrence in the environment. A total of 82 target compounds were found in at least one sample. Among them, 34 compounds were detected at more than 50% of the sites. For the evaluation of the toxicological impact on the aquatic environment, predicted no-effect concentrations (PNECs) were derived for all compounds, using acute and chronic toxicity data. An exceedance of the PNEC was registered for 7% of positive detections. This illustrates that environmental concentrations of organic micropollutants, discharged by WTPs, can easily reach and exceed toxicological effect thresholds. Pharmaceuticals and pesticides are compound groups that are likely to cause effects on aquatic organisms and therefore monitoring programs have to be extended to address all relevant substances.

**243 Effluent Toxicity Testing in Ireland (1983-2013)** r. hernan, Shannon Aquatic Toxicity Laboratory; K. O'Rourke, Enterprise Ireland / Shannon Aquatic Toxicity Laboratory. **Effluent Toxicity Testing in Ireland (1983-2013)** Robert Hernan<sup>1</sup>, Kathleen O'Rourke<sup>1</sup>, Shannon Aquatic Toxicity Laboratory, Enterprise Ireland, Shannon Town Centre, Co. Clare, Ireland \nE-mail contact: robert.hernan@enterprise-ireland.com In Ireland the EPA, as part of their IPC program, regulate industrial effluent discharges. In the case of complex (non-simple) effluents, the EPA requires a combination of toxicity testing and the traditional substance by substance analysis. Approximately 25% of all licenses have a toxicity clause. Toxicity testing is carried out on a final effluent sample to either marine or freshwater fish/crustacean/algae/bacteria. Discharges to WWTP also require a respirometry test. The EPA has assigned a toxicity emission limit value of no more than 10 toxic units (TU) i.e.  $E(L)_{50} < 10\%$  vol./vol. to most effluent discharges. In 2011, 90% of the samples tested were within the 10 TU limit. Between 1991 and 2011, greater than 70% effluents were within the 10 TU limit however between 1985 and 1990, greater than 50% effluents were over the 10 TU limit. In 2011, *S. costatum* and *T. battagliai* were the most sensitive test species with fish the least sensitive. In general we have found that the fish tests, rainbow trout and turbot, are the least sensitive test species. Other species including *Lemna minor* and *Crangon crangon* have also exhibited low sensitivity to effluents. The marine alga, *Skeletonema costatum*, is the most sensitive. In 2011, 35% of effluent samples tested discharged to freshwaters, 34% to WWTPs and 31% to coastal waters. In recent years increasing numbers of discharges are going to tertiary WWTPs. Domestic wastewaters are licenced by the EPA through Wastewater Authorisation (WWDA'S) since 2008. Approximately 20% of large wastewater treatment plants (population equivalent >200) require toxicity testing. Initial testing shows these are mostly non-toxic. Keywords: toxicity testing, wastewater effluents, toxic units, species sensitivity

**244 In situ reprotoxic effects of urban and hospital effluent on the mudsnail *P. antipodarum*: role of biofilm and water column** M. Gust, Irstea; A. Berlioz-Barbier, CNRS; A. Bouchez, INRA; J. Labanowski, C. CREN, CNRS; J. GARRIC, Irstea. Municipal effluents are well-known contributors of pollution in aquatic ecosystems. Bellecombe (Sipibel, Haute-Savoie, France) Waste-Water Treatment Plant (WWTP) treats urban effluents, but also the effluent of the newly established hospital. Treated effluents are discharged directly in the Arve River. Previous reports (unpublished results) showed that chemicals (pharmaceuticals and endocrine disruptors) were released in the river by the effluents, and found directly in water and also in biofilms. *Potamopyrgus antipodarum* was shown to be a sensitive and adapted organism to assess the toxicity of effluents. It is able to feed naturally with biofilm, in submerged cages. Thus the contamination occurs both via water, and the ingested biofilm. The aims of this study

were: (i) to assess the reproductive effects of the exposure to the effluent discharge contaminated both by urban and hospitals pollutants, (ii), to assess the contamination in water, biofilm and snails, and (iii) to assess whether a link existed between the contamination of the different compartments and the observed biological effects (trophic transfert). Adults snails were placed at one upstream and two downstream sites, during 6 weeks in September-October 2012. Mortality was checked weekly, and reproduction in snails (number of embryos in the brood pouch) was assessed after 42 days. Energy status was quantified in snails as well as reproduction-related biomarkers. Chemcatchers were used to measure the chemical contamination of the water. Biofilm was collected at the end of the exposure to assess its diversity, and its load in various chemicals. Measurements of chemicals was also performed in snails at the end of the exposure. The reproduction in upstream snails was in the upper range of physiological values. Nonetheless, the effluent induced a significant increase of reproduction, which disappeared in the farther downstream site. As dietary causes seem to be excluded, a toxic cause is suggested. This will be further investigated when the contamination of biofilm (snail feeding), water and snails will be available. The possible trophic transfert of contaminants will be assessed.

**245 In situ reproductive bioassay with caged *Gammarus fossarum* (Crustacea): a tool to diagnose toxicity of wastewater effluents** C. Lopes, Université Lyon UMR CNRS; O. Geffard, Irstea; B. Xuereb, University of Le Havre, Laboratoire d'Ecotoxicologie-Milieux Aquatiques (UPRES EA3222); R. Coulaud, G. Jubeaux, H. Queau, A. Francois, A. Chaumot, Irstea. Monitoring the adverse effects of environmental contaminants, and especially wastewater effluents, on the reproduction of species from diverse taxa of biodiversity is an ongoing challenge in ecotoxicology. In this context, a chronic sub-lethal toxicity test was developed on *Gammarus fossarum*, a keystone species of European freshwater ecosystems, to detect adverse outcomes (such as endocrine disruption) of the exposure to chemical compounds. Moreover, protocols were developed for in situ bioassays with transplanted caged *G. fossarum* to diagnose toxicity in freshwater systems. Such field bioassays permit to control biotic factors known to influence endpoints measurements, but the impact of abiotic factors like water temperature and hardness can not be controlled. In previous laboratory ecophysiological studies, we shown that the moulting cycle of *G. fossarum* only depends on temperature. A temperature-dependent model was thus developed to describe the moulting development of *G. fossarum* according to water temperature. The aim of our presentation will be to show how we can use such a model to take into account the effects of temperature in the interpretation of responses measured during in situ assays. Based on ex situ and in situ case studies, we will firstly show the improvement of the the temperature-dependent model by transplanting gammarids throughout four seasons in two reference stations. Secondly, to illustrate the relevance of this model in interpreting the moulting development modulation of *G. fossarum* exposed to wastewater effluents, we will show the results from: 1- ex situ exposure performed to assess toxicity reduction by alternative tertiary treatments in pilot WWTP; 2- in situ exposure upstream and downstream from three WWTP effluent outputs discharged in Rhône-Alpes Rivers (France). Our study underlined the relevance of formalizing the impact of confounding factors on the responses of ecophysiological tests in order to have a good interpretation of in situ bioassays. Furthermore, the reproductive bioassay with caged *G. fossarum* appeared as a relevant tool to diagnose toxicity of wastewater effluents with potential adverse effect for reproductive processes within receiving ecosystems.

**246 Organic pollutants from wastewater treatment plants affect macroinvertebrate communities** K. Bunzel, Helmholtz Centre for Environmental Research UFZ / Department Bioenergy/System Ecotoxicology; M. Kattwinkel, Helmholtz Centre for Environmental Research UFZ / System Analysis, Integrated Assessment and Modelling; M. Liess, UFZ Center for Environmental Research / Department of System Ecotoxicology. More than 10 years after the adoption of the EU Water Framework Directive, the majority of German

rivers and streams are still at risk of failing to achieve the good ecological and chemical status by 2015. Pesticide contamination of streams is a major stressor for stream ecosystem health preventing the achievement of a good status. Emission from point sources such as municipal wastewater treatment plants (WWTPs) is an important pathway by which pesticides enter surface waters. However, to date, no studies have focused on the ecological effects of pesticide-contaminated WWTP effluent on macroinvertebrate communities. On the basis of governmental monitoring data of 247 sites in Hesse, Germany, we identified insecticidal long-term effects on the structure of the macroinvertebrate community up to 3 km downstream of WWTPs. The effects were quantified using the trait-based SPEAR<sup>pesticides</sup> index, which has been shown to be an effective tool for identifying community effects of pesticides (insecticidal toxicity). According to the ecological status class boundaries of SPEAR<sup>pesticides</sup> proposed by Beketov et al. (2009), a total of 75% of the sites with a WWTP within 3 km upstream had a moderate to bad ecological status, compared to only 31% of the sites with no WWTP within 3 km upstream. In addition, based on the German Saprobic Index, we revealed that WWTPs are still an important source of oxygen-depleting organic pollution, despite the extensive technological improvements in wastewater management over several centuries. According to the German WFD classification system, a total of 51% of the sites with a WWTP within 3 km upstream had a medium to bad ecological status. In contrast, 91% of the sites with no WWTP within 3 km upstream had a good or even high ecological status. For pesticide management, our findings emphasize the need to take municipal WWTPs into consideration in the management of river basins under the EU Water Framework Directive to achieve good ecological and chemical status for European streams and rivers.

#### **247 Active materials for the in-situ stabilization of contaminated soil**

S.E. Hale, Norwegian Geotechnical Institute; J. Jensen, Aarhus University; L. Jakob, Alfred Wegener Institute for Polar and Marine Research; P. Oleszczuk, Maria Curie-Skłodowska University; T. Hartnik, Climate and Pollution Agency; T. Henriksen, Lindum Ressurs og Gjenvinning; G. Okkenhaug, Norwegian Geotechnical Institute; P. Meynet, D. Werner, R. Davenport, Newcastle University; G. Breedveld, G. Cornelissen, Norwegian Geotechnical Institute. The project "Active materials for the in-situ stabilization of contaminated soil" was carried out in order to investigate how polycyclic aromatic hydrocarbon (PAH) and heavy metal contaminated soils could be remediated using materials capable of stabilizing these pollutants in-situ. Field tests were carried out in collaboration with a waste treatment company, in which activated carbon was added to stabilize PAHs and Fe(OH)<sub>3</sub> powder/limestone and Fe<sup>0</sup> granulate were used to stabilise metals. A great variety of end points were used to assess treatment effectiveness, including available pollutant concentrations, effects on microbes, invertebrates and plants. In addition the toxicity of the amendments themselves was tested. The field plots for the PAH and metal contaminated soils were constructed as lysimeter cells. For the PAH contaminated soil, three plots were constructed with buffer areas of about 5 m; one reference, one PAC amended and one GAC amended (containing 2 % AC). For the heavy metal contaminated soil, Fe(OH)<sub>3</sub> powder/limestone and Fe<sup>0</sup> granulate were mixed at 2 and 4 % in to a soil taken from near a shooting field at Steinsjøen. The available PAH concentrations measured with passive samplers were reduced 93 % and 76 %, 17 and 28 months after PAC amendment, compared to 84 % and 69 % for GAC. PAC had a negative effect on plant growth while the GAC increased the growth rate of plants. PAC was toxic to earthworms, demonstrated by a significant weight loss, while the results for GAC were less clear. Reproduction of *F. candida* was significantly increased when 2 and 5 % GAC and biochar were used. In avoidance tests, *E. fetida* showed no avoidance of the different amendments. After three years, total microbial cell counts and respiration rates were highest in the GAC amended soil. In the metal contaminated soil the porewater was analyzed for pH, EC, Pb, Cu, Zn, Fe, Ca, Mg, Sb(III)/Sb(V) and DOC. A good efficiency of Sb and Pb retention in the treated soil for both amendments was seen. These results show activated carbon was successful in reducing the leaching of organic contaminants from soils, and iron-based sorbents, were

successful in reducing antimony leaching from soils. This use of this method to stabilize contaminated soil is now being offered by the industry partner, Lindum Resources and Recycling, in this project.

#### **248 Bioturbation affects on natural attenuation and in-situ remediation with thin layer AC application**

D. Lin, Y. Cho, Stanford University / Department of Civil and Environmental Engineering; D. Werner, Newcastle University / Civil Eng. and Geosciences; R.G. Luthy, Stanford University / Department of Civil and Environmental Engineering. Capping hydrophobic organic carbon (HOCs) contaminated sediment with various clean materials, including sand and activated carbon and clay mixtures are effective in reducing sediment-to-water flux of HOCs and porewater concentrations in the bioactive layer. Natural deposition of clean sediments may also be effective in burying older contaminated sediment, although it is less clear how effective natural deposition is for capping contaminated sediment. This is especially the case in the presence of bioturbation, since natural sedimentation is usually slow (typically 1 cm/year) and bioturbation may continually re-mix clean and contaminated sediment. These processes were studied in microcosm experiments using DDT-contaminated sediment (2 mg/kg dw ?DDT) from Lake Maggiore, Italy. The microcosms consisted of an underlying control contaminated sediment with three different treatment methods with thin caps: 1) control, with no capping 2) natural deposition, comprising of a control sediment sublayer with a thin layer (0.5 mm) of background sediment cap (0.03 mg/kg dw ?DDT) and 3) in-situ treatment, comprising of a control sediment sublayer with a thin layer (0.3 cm) of virgin activated carbon cap. For each sediment treatment method, we conducted a set of microcosm experiments with *Lumbriculus variegatus* and microcosms without worms. The thin-layer sediment cap was only effective in reducing the sediment-to-water flux when bioturbation was not present, while the in-situ treatment AC cap was effective in reducing the ?DDT flux even in the presence of bioturbation by  $94 \pm 4\%$  compared to the control sediment with bioturbation. The 28-day bioaccumulation study showed that the thin-layer AC treatment method reduced bioaccumulation in the worms by  $66 \pm 33\%$  compared to the control. Potential ecotoxicological effects in lipid contents and survival rates were not observed in our study. Porewater concentration profile measurements at 0.5 cm resolution showed bioturbation creates a more uniform concentration profile by increasing the surface porewater concentration. These experimental results show thin activated carbon placement above sediment can be effective in reducing the short-term flux of contaminants from the sediment, even in the presence of bioturbation.

#### **249 Biochars as a Climate Change Mitigation Technology: The Implications for Bioavailability and Efficacy of Pesticides**

R.S. Kookana, S. Martin, CSIRO Land and Water; S. Nag, Central Inland Fisheries Research Institute. Biochar is an emerging technology worldwide for mitigation of climate change. However, little attention has been paid towards potential unintended consequences of such a practice, especially on the bioavailability and efficacy of pesticides. The objective of this study was to establish to what an extent the application of biochar to soil can influence the bioavailability of soil-applied pesticides to biota and adversely affect their efficacy. We evaluated the ability of several biochars in sorbing pesticides through batch sorption-desorption experiments. Sorption-desorption behaviour was also compared between soils freshly amended with biochar versus those with biochar aged under field conditions for two years. We also carried out a glasshouse study to evaluate a wheat straw biochar produced at 450° C for its ability to influence bioavailability and persistence of two commonly used herbicides with different mode of actions (atrazine and trifluralin) in two contrasting soils. Sorption was found to increase with increasing biochar contents in soil and even small amounts of biochar (0.1%) made a significant difference in not only sorption but also their desorption behaviour. In presence of 0.5 or 1% biochar in soils, the sorption was essentially non-reversible suggesting sequestration of the sorbed pesticide. The study showed that due to ageing (e.g. via organo-mineral interactions) in two years under field conditions, biochars can

lose their extraordinary sorption capability. The efficacies of both herbicides were reduced in the presence of biochar in soil, but the magnitude of effect was dependent on the herbicide and soil type. In the presence of 1% biochar in soils, the dose of atrazine required for 50% weed control needed to be increased by about 3.5 times in Calcarosol (from 1.17 kg ha<sup>-1</sup> to 4.16 kg ha<sup>-1</sup>) and 2.5 fold in the Ferrosol (from 1.45 kg ha<sup>-1</sup> to 3.48 kg ha<sup>-1</sup>). The corresponding increase was much smaller in the case of trifluralin. The effect was strongly dependent on the mode of action of the herbicide. Persistence increased in biochar amended soils, indicating the reduced bioavailability of herbicides. The study concluded that freshly applied biochars to soils can significantly reduce the bioavailability and efficacy of soil applied pesticides to the extent that up to 3-4 times the normal recommended rate may need to be applied for an effective control or weed/pest in a soil amended with 10 tons of biochar per ha.

**250 In situ activated carbon amendment reduces bioaccumulation in aquatic food chains** D. Kupryianchyk, M. Rakowska, Wageningen University; I. Roessink, Altera Wageningen; T. Grotenhuis, A.A. Koelmans, Wageningen University. In situ activated carbon (AC) amendment is a new direction in contaminated sediment management, yet its effectiveness and safety have never been tested on the level of entire food chains including fish. Here we tested the effects of three different AC treatments on hydrophobic organic chemical (HOC) concentrations in pore water, macrophytes, benthic invertebrates, zooplankton and fish (*Leuciscus idus melanotus*). AC treatments were mixing with powdered AC (PAC), mixing with granular AC (GAC), and addition/removal of GAC (sediment "stripping"). AC sediment treatments resulted in a significant decrease in HOC concentrations in pore water, macrophytes, benthic invertebrates, zooplankton, and fish. In 6 months, PAC treatment caused a reduction of accumulation of polychlorobiphenyls (PCB) in fish by a factor of 20 bringing pollutant levels below toxic thresholds. All AC treatments supported growth of fish, but growth was inhibited in the PAC treatment, which was explained from reduced availability of zooplankton.

**251 Bioavailability of Genotoxic Contaminants in PAH-Contaminated Soil Before and After Biological Treatment** J. Hu, The Dow Chemical Company / Department of Environmental Sciences & Engineering; M. Aitken, University of North Carolina / Environmental Sciences and Engineering; A. Adrion, University of North Carolina; D. Shea, North Carolina State University / Department of Biology; J. Nakamura, University of North Carolina at Chapel Hill / Department of Environmental Sciences & Engineering. Field-contaminated soil from a former manufactured-gas plant site was treated in a laboratory-scale, aerobic, slurry-phase bioreactor. The bioreactor was operated in a semi-continuous (draw and fill) manner, by replacing one-fifth of the reactor slurry with untreated soil every week. Desorbability and biodegradability of 14 polycyclic aromatic hydrocarbons (PAHs) and four oxygenated PAH metabolites (oxy-PAHs) in the soil were investigated throughout a treatment cycle. Of the four oxy-PAHs analyzed, the most prevalent was 9,10-anthracene dione (9,10-anthraquinone, or AQ). Desorbability was determined using a mixed-function HLB resin or Tenax beads in dialysis tubing suspended in the soil slurry. Toxicity and genotoxicity of the whole soil and the desorbable fractions were determined by DNA damage response analysis with the DT40 chicken B-lymphocyte isogenic cell line and its DNA-repair-deficient mutant *Rad54*<sup>cr</sup>, respectively. Biological treatment significantly removed PAHs and oxy-PAHs, and the desorbability of both PAHs and oxy-PAHs decreased throughout the one-week bioreactor treatment cycle. Collectively, oxy-PAHs were more desorbable and biodegradable than the PAHs. When comparing oxy-PAHs with their parent PAHs, we observed that 9,10-phenanthrenequinone and AQ were more desorbable and biodegradable than the parent compounds phenanthrene and anthracene, respectively; 9-fluorenone was less desorbable and biodegradable than the parent compound fluorene; and benz[a]anthracene-7,12-quinone was more desorbable but less biodegradable than the parent compound benz[a]anthracene. For both PAHs and oxy-PAHs, the percentage

removed in the bioreactor significantly exceeded the percentage desorbed from untreated soil, suggesting that the contaminants in less-accessible domains were susceptible to biodegradation. Toxicity of solvent extracts of the whole soil slightly decreased after biological treatment. However, genotoxicity of solvent extracts of the whole soil slightly increased after biological treatment. Both toxicity and genotoxicity of the desorbable constituents in the soil decreased after treatment, suggesting that any genotoxic constituents that may have formed during treatment were primarily associated with less-accessible domains in the soil. If the desorbable fraction of a contaminant approximates bioavailability to ecological or human receptors, this finding suggests that biological treatment may correspond to a decrease in overall risk.

**252 Evidence of limited exposure originating from desorption resistant PAHs in black carbon.** V. Gouliarmou, Aarhus University Science and Technology Faculty / Environmental Chemistry and Microbiology; E. Christiansen, Aarhus University / Department of Environmental Science; K.J. James, University of Saskatchewan / Toxicology Group and Soil Science; R. Peters, Soil Science Department / Toxicology Group and Department of Soil Science; S.D. Siciliano, University of Saskatchewan / Toxicology Group and Department of Soil Science; P. Mayer, Technical University of Denmark / Department of Environmental Engineering. Bioremediation of historically contaminated soils often ends up with a desorption resistant fraction of HOCs that is very difficult to remove even after intense treatment [1]. Additionally, during the last decades various black carbon sorbent amendments have been applied for soil remediation [2]. In this case, the aim is not to remove contamination but to eliminate or at least reduce the exposure pathway between the contaminants and the receptor. This approach takes advantage of strong sorption for the immobilization of the contaminants. However, the crucial question remains whether desorption resistant contaminants indeed give rise to no or at least very limited risk and exposure. In the current study we incubated wood soot in contaminant traps for more than one year in order to remove the readily desorbing PAH fraction from the soot. The exposure originating from the remaining desorption resistant PAHs was then studied in terms of (1) chemical activity, (2) *in vitro* oral bioaccessibility and (3) uptake in juvenile swine. The working hypothesis was that: 1) due to desorption resistance, the reduction in exposure is higher than the reduction in total PAH concentration and 2) limited exposure originates from the desorption resistant PAHs. All measurements confirmed limited exposure originating from the desorption resistant PAHs in black carbon (soot). The present study strongly supports the underlying assumption of bioavailability research, that desorption resistance gives rise to limited exposure. These findings further support that bioremediation and sorbent amendment can be used as efficient exposure reduction measures, even if they do not succeed to remove the desorption resistant contaminants.

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**253 Indirect effect of an insecticide on an ecosystem service: pollination by moths** M. Hahn, Institute for Environmental Sciences; C. Bruehl, University of Landau Institute for Environmental Sciences. Pollination is an essential ecosystem service in terrestrial habitats. In agro-ecosystems, field margins can provide habitat for reproduction and foraging for several species including many pollinators. However, insecticide inputs of adjacent fields via overspray and spray drift might affect insect pollinators in field margins and, hence, be detrimental to pollination services for crops and wild plants. We focused on the indirect effects of a field margin-relevant concentration of an insecticide (lambda-Cyhalothrin, formulation: Karate Zeon) on moths and the pollination services provided by them. As study organism we used the White Campion (*Silene latifolia*), a dioecious plant species specialized on nocturnal moth pollination. In our study, half of the test plants (6

plants, 36 flowers) were sprayed with the insecticide in an application rate consistent with the pesticide input due to overspray and spray drift in 1m wide field margins (30% of the field application rate). Such small field margins are common landscape features in German agro-ecosystems. The treated plants and untreated control plants (6 plants, 34 flowers) were exposed to natural pollination during one night in a semi-field design. Two weeks later, seed production of the flowers was compared between treated and untreated plants. Furthermore, it was assessed if the flowers were used for oviposition by a specialized moth pollinator (*Hadena bicruris*). Our results showed that flowers treated with the insecticide were less likely to be pollinated and used as oviposition sites compared to untreated flowers. It is known that Lepidoptera in juvenile as well as adult development stages are sensitive towards low rates of pesticides. The reduced pollination and oviposition observed in this study were possibly caused by repellence of moths due to the insecticide treatment. Since field margins constitute habitats for several Lepidoptera species in agricultural landscapes, inputs of insecticides might not only be detrimental to pests but also to non-target species and the ecosystem-services provided by them. Repellence is so far not included in a risk assessment approach; however, it might affect population size of pollinators in agro-ecosystems dramatically.

**254 Ecological models in chemical risk assessment – Recommendations of the SETAC workshop MODELINKU.** Hommen, Fraunhofer IME; A. Alix, Dow Agrosciences / Risk Management; D. Auteri, Auteri; P. Carpentier, ANSES / Direction du Vegetal et de l'Environnement; P. Dohmen, BASF SE / Landw. Versuchsstation, APD/RO; V. Ducrot, INRA / Ecotoxicology and Quality of Aquatic Ecosystems; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; V. Grimm, Helmholtz Centre for Environmental Research UFZ / Department of Ecological Modeling; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research; M. Reed, Chemicals Regulation Directorate, HSE; W. Schmitt, Bayer CropScience AG / Environmental Modelling; P. Thorbek, Syngenta / Environmental Safety; L. Wendt-Rasch, KEMI. Recently EFSA has published a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides. Within this framework ecological modelling is identified as a promising tool to link the results of ecotoxicological studies to such specific protection goals because it can facilitate extrapolation from standard test endpoints to higher levels of biological organization and can explore the influence of various kinds of ecological complexity on the degree of risk. However, currently there are no recommendations on which models are suitable or how to apply them to risk assessments. Therefore, the general objective of the SETAC Europe technical workshop MODELINK is to provide guidance for when and how to apply ecological models to regulatory risk assessment. Such guidance is essential because risk assessment questions, data and protection goals vary across chemicals, ecosystems and species and consequently the question of how to apply models to risk assessments is not trivial. MODELINK focusses on the risk assessment for plant protection products. Approximately 60 experts from diverse backgrounds and representing the tripartite structure of SETAC participate upon invitation. The workshop is built on two meetings over 3 – 4 days in October 2012 and April 2013 and a homework period in between to work within breakout groups on case study reports covering risk assessments without and with ecological models for birds and mammals, fish, soil invertebrates, terrestrial arthropods, aquatic invertebrates and macrophytes. These reports will be discussed and refined during the second meeting and serve as the basis to derive general recommendations on the use of effect models in pesticide risk assessment which will be presented.

**255 When Pests Pose Greater Risks to Ecosystem Services than Pesticides: A Case Study** D.P. Kreutzweiser, S. Capell, Canadian Forest Service / Natural Resources Canada; D. Nisbet, P. Sibley, University of Guelph / School of Environmental Sciences; P. Hazlett, Canadian Forest Service / Natural Resources Canada; T. Scarr, Ontario Ministry of Natural Resources / Forest Health and Silviculture. The

exotic invasive insect, emerald ash borer (EAB), is rapidly spreading through eastern North America and causing extensive mortality of ash trees. Many of these trees are in riparian (shoreline) areas that provide critical refuge habitats, movement corridors, and other ecological services including the support of a rich biodiversity and the protection of aquatic ecosystem health. We conducted microcosm experiments to test effects of systemic insecticides for control of EAB on non-target, leaf-decomposing organisms. We then designed field studies to compare a no-intervention option, to determine impacts of the insect pest on riparian forests and some of the ecosystem services they provide. Preliminary results indicate that EAB causes rapid and extensive mortality of all ash tree species in riparian areas. When ash trees compose about 30% or more of riparian forests, this mortality causes large and sudden canopy openings. Light penetration to forest floors is measurably increased, forest floor vegetation proliferates, incursions by invasive plants are about doubled, and nitrogen cycling in riparian soils is increased by about 4 times above baseline rates. Across our riparian plots, ash is always among the top 4 tree species contributing leaf litter to forest floors and adjacent water bodies, with an average contribution of 20% and ranging to 45%. Ash litter inputs have a distinct seasonal trend; always among the earliest inputs. Among litter from the 6 most common riparian trees, ash is preferred (decomposed) by aquatic invertebrates as first or second choice in selection microcosms. Further endpoints, including invertebrate communities on in-situ leaf packs with or without ash, are being assessed and will be reported. We show that risks posed by effective insecticides are quantifiable and manageable. Conversely, risks posed by the insect, if left unchecked, appear to threaten the ecological function of riparian forests in terms of canopy cover and shading, forest plant biodiversity, soil nitrogen cycling, leaf litter inputs, and the food web dynamics of adjacent water bodies, probably for several decades at least.

**256 Ecosystem Services in Pesticide Regulation: Soil Treatments used in Tomato Production in Italy** S. Deacon, ENVIRON UK Ltd; A. Alix, Dow Agrosciences / Risk Management; G. Quadri, ENVIRON Italy; E. Tescari, M. Miles, Dow Agrosciences; P. Burston, J. Nicolette, ENVIRON; M. Rockel, ENVIRON International Corporation. This project delivered a case study to inform pesticide regulation at Member State level where an ecosystem services approach can inform risk and integrated pest management (IPM) decisions under the European Plant Protection Products Regulation 1107/2009 and the Sustainable Use Directive 2009/128/EC. The *sustainable use* of pesticides calls for a holistic approach to be taken in decision making. An ecosystem services framework has been developed and is applied to a range of soil treatments for the control of nematodes in tomato cultivation and other salad crops in southern Italy. Farmers rely on soil sterilisation treatments as an important component of a pest control management strategy. The use of chemical (1,3-dichloropropene (1,3-D)), physical (solarisation) and biological (microbial) treatments limit the root damage to tomatoes caused by nematodes, greatly affecting the growth and yield of tomatoes and the income earned by farmers at harvest time. Surveys of tomato growers in the Puglia and Sicily regions of Italy were undertaken to gather information on the socio-economics and the management practices of tomato cultivation. A spatial model was developed to evaluate the use of a range of soil treatments in tomato farms in the two regions. Specifically, the results of the evaluation were used to determine: (a) changes in ecosystem services in the absence of nematicide use; (b) the influence of other pest management options in the absence of, or in combination with, nematicides; (c) the effect on socio-economics and the environment posed by the discontinued use of nematicides and the strategies that might be developed to minimise changes and enhance current ecosystem services for future generations.

**257 Climate change and species community responses between north and southern Europe** V.B. Oliveira, J. Scott-Fordsmand, Aarhus University / Department of Bioscience – Terrestrial Ecology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; M.J. Amorim, Universidade de Aveiro / Department of Biology and CESAM. Climate

change may have a severe impact on terrestrial ecosystems. Further, effects are expected to be more severe in the southern Europe than north. It is important to study regional variations occurring between soil species communities (e.g. soil types) and the associated services/functions as well as to anticipate the level of change that temperature may induce. Here, we studied species community changes and function using soil mesocosms - Soil Multispecies System (SMS) – assessing 1) the effect of a temperature range resembling northern European (10-14-19-23°C) conditions; 2) the effect of a temperature range resembling southern European (19-23-26-29°C) conditions and 3) the interaction between temperature and Cu for both 1 and 2. Further, 1 year field experiments were run on the respective countries and field conditions. SMSs contained six species representative of different functional groups and were sampled along three exposure periods (28, 61, 84 days). OM breakdown and feeding activities were also assessed. Soils were from two different regions in Europe (Portugal and Denmark). Higher temperature (19-29°C) caused a higher impact in species community than lower (10-23°C). Effect of Cu was observed at 10-23°C experiments whereas at 19-29°C the high temperature overruled the Cu toxicity. Differences between historical Cu contamination and freshly Cu spiked soil indicate large differences in bioavailability as verified by the measured total concentration (ca. 1000 and 100 mg/kg respectively). Major changes in communities occurred during the growth phase (28 days at SMS tests and late spring in field), after which, at stable phases, changes in populations dynamics will be more reflecting actual species interactions and matured ecosystem status being both less resilient to changes and better fitted to the exposure scenarios (Cu and temperature). Ecosystem services, e.g. biodiversity, agriculture and associated OM turnover, will be impacted. Effects are more pronounced at more extreme low (10°C) and high temperatures (29°C) and the momentum feeding activity at different time intervals will better describe such changes compared to OM breakdown cumulated along time. Effects on species community and ecosystems were less predictable at higher temperature conditions. This is partly related with differences in species optimum curves, more skewed at the higher end, hence smaller differences will impact very rapidly.

**258 Ecosystem services within sustainable soil management and area development using the Triple-D approach** S. Keuning, H. Wagelmans, Bioclear. The definition of Ecosystem Services was first formalised in the Millenium Ecosystem Assessment [1] although scientists have been working on the concept for decades. A wealth of information is available with the professionals that are working at the scientific part of ecosystem services but also with the professionals within area development, urban development, soil remediation and nature development and control. However, communication between all different expertises keeps hampering, even within organisations, which slows down or even puts area development to a stop. How can soil management, soil and area functions and spacial redevelopment be connected? How can we link air quality, biodiversity, water and soil? How can different and even opposing interests be weighed and considered? That needs a more-dimensional consideration framework. The ecosystem services approach combines two worlds that apparently are difficult to reconcile. Economy and ecology. Profit and sustainability. Wealth and environment. Exploiting and protecting our environment in one approach, that acknowledges and connects all these differences. This was the base for the development of the Triple-D approach that subsequently will be further described and presented. The strengths and opportunities of the Triple D-approach will be illustrated in three examples in which the approach was used to investigate the area in which the ambitions needed to be realized, with ecosystem services and the natural capital as starting point.

**259 Linking of exposure and effects in higher tiers of the assessment of the risk of pesticides to honey bees as proposed in the EFSA guidance document** J. Boesten, Alterra / ERA team; A. Rortais, EFSA / Emerging Risk Unit; F. Streissl, C. Szentes, EFSA. In the EFSA guidance proposal the assessment of the risk of pesticides to honey bees consists of two components, i.e. the effect assessment and

the exposure assessment, which have to be adequately linked. In the past five years considerable experience has been gained with adequate linking for the effect assessments of aquatic organisms which can be used for the risk assessment of honey bees. It is proposed to base the effect assessment primarily on the concentration in nectar and pollen entering the hive and to combine these concentrations with consumption rates of nectar and pollen by adult bees and larvae (later also contributions of guttation water and other water sources may be considered). Furthermore it is proposed to base the field-exposure assessment on a 90th percentile of the exposure concentration considering a well-defined statistical population of hives, i.e. all hives at edges of treated fields in the area of use of the substance. The 90th percentile concentration entering the hive may as a first approximation be combined with 90th percentile rates of consumption of nectar and pollen to achieve an overall 90th percentile pesticide intake rate. There are two types of exposure assessment in such a linked risk assessment procedure: (A) the exposure of the hive in the field resulting from the use of the pesticide in agriculture (resulting in the 90th percentile concentration described above), and (B) the exposure of the hive in the higher-tier effect studies. So (A) assesses the reality in agriculture whereas (B) assesses what happened in the regulatory studies. In the past, the concentrations in nectar and pollen in the hives in the regulatory studies were usually not measured; instead it was assumed that exposure was adequate e.g. because the hive was located at the edge of a treated oil seed rape field. However, it is questionable whether this is a defensible approach to achieve at least a 90th percentile exposure. Therefore we recommend to measure in higher-tier semi-field and field studies always the course of time of the concentrations in nectar and pollen entering the hive. For adequate linking it is needed that the exposure in the higher-tier studies is higher than the endpoint of the field-exposure assessment. Otherwise the outcome of such a higher-tier study may be that there are no unacceptable effects at a concentration that is lower than the endpoint of the exposure assessment which is a non-relevant result for the risk assessment.

**260 Higher tier refinements for birds and mammals under EFSA 2009: a regulator's perspective** J. O'Leary Quinn, Chemicals Regulation Directorate / Ecotoxicology. EFSA 2009 lays out the European approach to assessing the risk to birds and mammals for pesticide active substances. It has now been in use in practice for about three years allowing regulators to gain practical experience of higher tier refinements which have been successfully used in practice. The Chemical Regulations Directorate (CRD), the regulatory body for the United Kingdom, highlights refinement steps based on our direct experience and which have been successfully used, as well as some of the challenges that can arise. For the acute risk assessment for several active substances body burden modelling has been proposed however EFSA (2009) highlights that further research is needed first. Also where acute data are available for multiple species a geometric mean of the toxicity end point may be taken in certain circumstances. Field trials may also provide additional information to support the risk assessment but such studies need careful and detailed examination and this approach has had very limited use. For the reproductive risk, the most frequent refinements are the degradation time used for both single and multiple application as well as use of the use of relevant focal species to the crop together with an appropriate food intake rate for that species. For birds it may also be possible to identify certain periods of the year when a reproductive risk assessment is not required as the birds are not breeding, additionally the UK has some recent research on breeding phases for UK species which potentially could be used to with the phase specific approach. Another important approach for each higher tier assessment is to consider the uncertainties and level of conservatism of the risk assessment relative to the protection goals.

**261 Optimizing experimental setup and sampling techniques to assess pesticide effects on macroinvertebrates in aquatic mesocosm studies** T. Strauss, RWTH Aachen University / Research Institute Gaiac; M. Hammers-Wirtz, Research Institute gaiac / Research Institute Gaiac; S. Norman, Ridgeway Eco; U. Hommen, Fraunhofer IME.

Aquatic mesocosm studies are often used as a higher tier tool in the environmental risk assessment of plant protection products. To minimize the uncertainty in the extrapolation to the field situation it is necessary that vulnerable species are present in the test systems in sufficiently high numbers to allow the detection of effects caused by the test item. Here, vulnerability is related to the intrinsic (toxicological) sensitivity to the test item and the ecological sensitivity with respect to the species life cycle and recovery potential. An additional factor influencing species vulnerability, the exposure, is given by the experimental setup. For insecticides in particular, emerging insects such as mayflies and further arthropods such as isopods and amphipods are considered as ecologically relevant and representative vulnerable species to be included in such a study. In this poster, we will present results from an outdoor mesocosm study where the design was optimized for these groups of macroinvertebrates. Stainless steel tables with a sediment layer introduced into the mesocosms provided a shallow zone with planted macrophytes as a habitat for macroinvertebrates. Colonization of the mesocosms came from the introduced sediment taken from a natural pond, water samples from ponds or untreated mesocosms and, in the case of flying insects, via the air. In addition, approximately 250 individuals of *Asellus aquaticus* (Isopoda) and *Crangonyx pseudogracilis* (Amphipoda), respectively, were introduced in each mesocosm and supported by supplying additional food (*Populus* leaves). The macroinvertebrates were intensively sampled to increase the sampling efficiency and thus to reduce the sampling error. Emerging insects were sampled by two traps, one installed above the shallow macrophyte area and the other above the deeper water column area. The emergence rates of the two traps were compared with respect to abundance and community composition. The efficiencies of different types of epibenthic and floating non-destructive sampling devices were analysed for *Cloeon dipterum* (Ephemeroptera), *Asellus aquaticus* and *Crangonyx pseudogracilis*.

**262 An evaluation of five years experience in off-field NTA testing** F.M. Bakker, Mitox Consultants; S. Aldershof, Bioresearch Promotion. Spray drift of plant protection products occurs and effects on ecosystem services potential (ESP) inherent to non-target areas (NTA's) must be assessed. Bakker and Miles (2007) proposed a field study design for an ERA on arthropod communities present in the NTA (also see de Jong et al. 2010). These communities provide a suite of services, all of which require stable and complex food webs for sustainable delivery. The ESP of NTA's is thus in nature a biodiversity issue and measurements of changes in biodiversity on a regulatory relevant time scale are key to risk assessment. The time factor is important because it provides insight in the resilience of the ecosystem (the service provider) and thus in the acceptability of effects. Historically, the analysis uses PRC-techniques (van den Brink and Ter Braak, 1998, 1999) where changes in relative abundance can be conveniently plotted over time and relative to a control. With this paper we evaluate five years of experience in off-field testing with particular reference to the following questions: (1) Were faunistic studies in an off-field environment needed? The alternative are in-field studies, therefore we compare both the biodiversity and the sensitivity of the NTA community to an in-field situation. (2) Was the test design sufficiently precise to find dose-response relationships and derive relevant endpoints such as the NOEAER? Some selected but anonymized cases will be presented to illustrate this point. (3) Was the test design sufficiently robust to buffer against effect dilution (e.g. immigration) and yet still realistic for the habitat type under study? Time-response patterns following disruption were analysed. (4) Were habitats sufficiently diverse to capture spatial heterogeneities in exposure that may be expected to occur in true off-field exposure scenarios? Vegetation surveys, their analysis and their relation to the arthropod community will be presented to provide a first insight.

**263 Guidance Document of EFSA's PPR Panel on tiered effect assessment for plant protection products in edge-of-field surface waters** A. Aagaard, M. Arena, S.K. Bopp, J. Boesten, T. Brock, M. Klein, M. Liess, R. Luttik, D. Pickford, A. Tiktak, L. Wendt-Rasch, Members of the Working Group Aquatic Ecotoxicology of the PPR

Panel of the European Food Safety Authority (EFSA). This presentation aims to provide scientific background information on the aquatic effect assessment scheme developed by EFSA for authorisation of plant protection products (and their metabolites) in the EU. It has its focus on experimental approaches. Furthermore, the appropriate linking between exposure and effect assessment estimates is described, as well as the possible use of non-testing methods (in particular for metabolites). The Guidance Document (GD) describes first the specific protection goals (SPG) for aquatic organisms. The overall aim of the SPG is to protect aquatic plants and animals at the population level in surface water. However, the SPG selected for aquatic vertebrates aims at protection at the individual level. To protect populations of aquatic organisms, effect assessment schemes are developed that enable derivation of Regulatory Acceptable Concentrations (RACs). In the aquatic effect assessment scheme a distinction is made between acute and chronic effects and the following tiers are developed, viz: Tier 1 effect assessment based on toxicity data for standard test species Tier 2 effect assessment based on laboratory toxicity data for both standard and additional test species, i.e. the geometric mean (Tier 2A), the species sensitivity distribution (Tier 2B) and the refined exposure laboratory toxicity test (Tier 2C) approaches. Tier 3 effect assessment based on model ecosystem (micro-/mesocosm) studies.

**264 Apple orchards & chlorpyrifos: 1st year results of UK field monitoring study on species-diversity, abundance & reproduction of birds & mammals 2012** F. Sotti, Tier3 Solutions GmbH; B. Giessing; S. Norman, Dow Agrosciences; C. Wolf, Tier Solutions GmbH; G. Weyman, Makhteshim Agan (UK) Ltd. The aim of this long-term field monitoring study is to provide data on species-diversity, abundance & reproduction of birds & mammals in 10 large apple orchards in Herefordshire, UK. All 10 orchards were sprayed with chlorpyrifos in 2012, as normal. The work in 2012 was done from start of April to end of July, and will continue in '13 & '14. The methods were: bird-ringing (mist-netting), nest searching & monitoring (open nests & natural cavities), visual surveys, predator surveys & mammal trapping. In total, 1166 birds (931 adults, 223 juveniles & 12 nestlings) of 37 species were trapped; the most abundant were great tit (274), blue tit (236), robin (172) & blackbird (107). During visual surveys, 636 individuals of 33 species were observed; most frequent were wood pigeon (114), blue tit (95), blackbird (78) & great tit (69). Concerning breeding, a total number of 123 active nests of 20 species, were found & monitored. Among them, 45 open-nests of 12 species were located in the orchards & their immediate surroundings, mainly of blackbird (19) and wood pigeon (5). 962 tree-cavities were found in 4 orchards (with older trees). Active nests were in 68 cavities, mainly of blue tit (30) & great tit (18). Predators were recorded & motion-sensitive cameras were used to include nocturnal species. 448 observations of potential predators were noted, mainly carrion crows, common buzzards, domestic dogs & badgers. For mammals, 809 individuals of 7 species were trapped. The most common were bank vole (334), wood mouse (272) & yellow-necked mouse (177). Despite only 20% of traps being in the off-crop habitat within a few metres of the orchards, the majority of captures (89.5%) occurred in these traps (7 species). In contrast, *within* the orchards only wood mice & one individual bank vole were trapped. Overall, the results showed a diverse community of birds. Notably, the most abundant species trapped (great tit & blue tit) were small insectivores, the group highlighted in EU pesticide assessment schemes as being at greatest risk. For mammals, in this landscape, the off-crop appeared to be much more attractive than inside the orchard. This is logical due to cover from predators by long grass & food availability. The results are valuable for plans in 2013, including use of nest boxes.

**265 Development of characterization factor to assess biodiversity damage due to salinity increase in a coastal wetland** M. Amores Barrero, Universitat Rovira i Virgili / Chemical engineering department; F. Verones, Institute of Environmental Engineering (IfU); C.E. Raptis, Institute of Environmental Engineering; R. Juraske, S. Pfister, F. Stoessel, ETH Zurich; A. Assumpcio, IRTA; F. Castells, Universitat Rovira i Virgili / Chemical engineering department; S. Hellweg, ETH Zurich / Institute of Environmental Engineering. Coastal wetlands are



among the most productive, valuable, and yet most threatened ecosystems in the world. They provide a critical interface between terrestrial and marine environments, where fresh water and salt water are often mixed. Coastal wetlands in arid and semi-arid zones experience periods of increasing salinity as a consequence of high evaporative conditions, variability of inflows, impacts of human pressure and their proximity to the sea. In this paper, a new method has been conducted close to our case study, so in order to develop a methodology for salinity impacts authors selected a coastal Spanish wetland called “Albufera de Adra” as a case study. It is located in a semi-arid region (South-East of Spain), where agricultural activities require substantial irrigation and areas with native vegetation and fauna are restricted to some small patches and wetlands. This wetland is situated closer to the sea and it is predominantly fed by groundwater. This work derived the first Characterization Factor (CF) for salinity impacts in a coastal wetland defined as the change in the Potentially Affected Fraction (PAF) of species due to a change in salinity and extraction of groundwater for crop irrigation. A Life Cycle Impact Assessment method was developed to evaluate the environmental impacts associated with salinity on biodiversity in this Spanish coastal wetland. This CF for the salinity impact is defined as the change in the PAF of species due to a change in salinity which is caused by the changed of groundwater for irrigation in the vicinity of the Albufera de Adra. The developed CF consists of a fate (FF) and an effect factor (EF) and it indicates a “potential loss of 0.32 m<sup>3</sup> ecosystem” for a water consumption rate of 1 m<sup>3</sup>yr<sup>-1</sup>. The FF was calculated from seasonal water and salt balances of the wetland Albufera de Adra and the EF was obtained from the fitted curve of PAF of native wetland species due to salinity. In order to test the applicability of the CF, an assessment of water consumption of greenhouse crops in the area (mainly 86% of groundwater) was conducted as a case study. Results converted into ecosystem quality damage using the ReCiPe method were compared to other categories. **Keywords:** life cycle assessment, salinity, ecotoxicity, coastal wetland. **Session:** Life cycle analysis (LCA) and sustainability **Presentation preference:** platform presentation

**266 Developing an assessment methodology for the impact of consumptive water use on the biodiversity of wetlands of international importance** E. Veronesi, Institute of Environmental Engineering (IfU); S. Pfister, ETH Zurich; D. Saner; C. Rondinini, Università di Roma La Sapienza / Department of Animal and Human Biology; D. Baisero, Università di Roma La Sapienza; S. Hellweg, ETH Zurich / Institute of Environmental Engineering. Wetlands harbor large varieties of species, but they also belong to the world’s most threatened ecosystems. Half of their global area was lost during the last century. So far no approach exists in life cycle impact assessment which acknowledges the vulnerability and importance of wetlands and provides impact factors for this kind of ecosystems on a global scale. We use 1184 inland wetlands which are currently designated as sites of international importance under the Ramsar Convention for developing regionalized Fate Factors (FF) and Effect Factors (EF) for consumptive water use. We distinguish between surface water-fed and groundwater-fed wetlands and between surface water and groundwater consumption. FFs quantify the change of wetland area caused per m<sup>3</sup> water consumed and EFs quantify the “global species”-equivalents which are potentially lost for each change in wetland area. Since we are assessing the impact per wetland area, our assessment methodology is compatible with impact assessment methodologies of land use impacts. EFs are calculated for waterbirds, non-residential birds and water-dependent mammals. Characterization factors (CFs) which are derived with the FFs and EFs of the individual wetlands are distributed over each wetland’s individual catchment. In areas where catchments overlap, the CFs are summed up, since obviously several wetlands will be damaged from consumptive water use at this location. Thus global, spatially differentiated maps of impacts from both consumptive surface water and groundwater use are derived for waterbirds, non-residential birds and water-dependent mammals. A case study of rose production in Kenya is calculated in order to assess the impacts of both consumptive surface and groundwater use in the region around Lake Naivasha, which is one of Kenya’s designated wetlands of international importance. The case

study highlights the importance of incorporating these impacts into life cycle assessment, since impacts of consumptive water use proved to be in the same order of magnitude like agricultural land occupation and several orders of magnitude more important than freshwater eutrophication or terrestrial acidification.

#### **267 Towards including regionalized soil-water consumption in LCA**

M. Núñez, UR050, Laboratoire de Biotechnologie de l’Environnement; S. Pfister, ETH Zurich; P. Roux, Irstea; A. Anton, IRTA. This research aimed to address in life cycle assessment (LCA) the impact pathway of soil-water consumption, also denoted as green water consumption in the literature, which is of great importance for dry lands. The developed approach consists on quantifying, at the inventory stage, the net change in soil-water consumption under a specific human land use compared to the natural reference situation. The potential natural vegetation (PNV) was selected as the reference situation. To allow the approach to be applied, we estimated water consumption of PNV as a function of location for global dry lands of the world. Two methods were combined to estimate soil-water consumption of PNV: an empirical approach based on annual precipitation and potential evapotranspiration data, and another approach based on remote sensing models for actual evapotranspiration of natural areas. The combination of both approaches enables to obtain more robust results. Water consumption of the regionalized natural reference situation was derived on three spatial scales of aggregation: 10 arcmin grid cells, ecoregions (501 units) and biomes (14 units). This facilitates inclusion of soil-water consumption in LCA for different detail of the geographic information available. The method is intended to be used in contexts of rain-fed agriculture and rainwater harvesting, which includes direct soil moisture uptake by plants and rainwater harvested and reused in production systems. The approach provides a basis for impact assessment of soil-water consumption. Specific characterisation factors and impact assessment model have not been proposed so far. The life cycle inventory framework and the associated mapped results are already usable for several situations of land use and land use-changes. Impacts of soil-water consumption should be subsequently assessed by characterisation factors (CFs) for water consumption on the impact assessment (LCIA) stage. The approach is of high relevance for water-use-dependent land uses such as agriculture and forestry.

#### **268 Linkage with land use and freshwater availability in the context of water footprint**

M. MOTOSHITA, National Institute of Advanced Industrial Sci and; K. TAHARA, National Institute of Advanced Industrial Science and Technology; A. INABA, Kogakuin University. Water footprint is a tool for assessing freshwater availability loss caused by artificial activities. Water consumption as a result of direct withdrawal from freshwater resources is basically discussed and focused in many present cases. However, freshwater availability seems to be affected by not only withdrawal of water but also artificial land use. Land cover change will result in the infiltration of rain water to soil and groundwater. The change of infiltration amount of rain water will vary directly the amount of groundwater and indirectly the amount of surface water through changing freshwater outflow from ground to surface freshwater resources. While the linkage between water and land use have been potentially pointed out in some studies [1][2], quantitative evidence has not been provided yet. The focus of this study is to assess the effect of land use on water availability and clarify the significance of land use in the context of water footprint. Water deprivation intensities for each artificial land use type could be calculated as -0.0065 [m<sup>3</sup>/m<sup>2</sup>] for planted forest, 0.156 [m<sup>3</sup>/m<sup>2</sup>] for paddy field, 0.0161 [m<sup>3</sup>/m<sup>2</sup>] for arable land, 0.452 [m<sup>3</sup>/m<sup>2</sup>] for urban area, on average in Japan. Average freshwater resource availability in Japan is calculated as 1.23 [m<sup>3</sup>/m<sup>2</sup>] by dividing total amount of available freshwater resource with total land area. Thus, in case of land use as urban area, the loss of freshwater availability caused by land use is accounted as almost 37% of freshwater availability per land area on average. While water consumption by withdrawal is still significant in many industries, water deprivation by land use is more dominant in some industries. If the availability loss of water by land use were taken into account in water footprint, the value

of water footprint will be 14 times (at the maximum) more than that calculated by conventional concept (only considering withdrawal-based consumption). Water deprivation by land use affects on the freshwater availability and the effect on water footprint may be accounted up to 14 times. In addition, large regional difference could be found in the intensity of water deprivation by land use. The interaction between the amount of availability loss by land use and regional impact factor may affect on the results of water footprinting.

**269 Diving into water scarcity indicators: model components, choices and uncertainties** A. Boulay, CIRAIÉ École Polytechnique de Montréal / Chemical engineering department; S. Pfister, ETH Zurich; C. Bulle, CIRAIÉ Polytechnique Montreal / Chemical Engineering; M. Margni, Ecole Polytechnique de Montreal / Department of Mathematical and Industrial Engineering. This paper presents preliminary results of the third deliverable of the WULCA project (Water Use in LCA) of the UNEP/SETAC Life Cycle Initiative performing a quantitative comparison of the available water impact assessment methods in order to enhance the understanding of differences, similarities, modeling choices, data used and uncertainty related to the choice of model. This paper aims to 1) compare selected methods addressing water scarcity as a midpoint indicator by identifying and quantifying the key elements and modeling choices driving their results, and 2) assess individual regional model uncertainty and propose recommendations towards a consensual indicator. Results from water scarcity methods are first normalized using a reference flow of a world average water  $m^3$  and then compared using the Spearman rank correlation coefficient and the Gini mean difference coefficient. The former expresses the consistency of model response for different flows and the latter expresses the mean difference between two model results for the same flows. Similar models have high rank correlation and low mean difference. These tests are also performed for specific modeling choices to assess their relative influence: source of data, source of water (surface water vs. groundwater), consideration of temporal variation, use of consumption or withdrawal based scarcity index, choice of regional resolution scale and of aggregation methods. Results show that the type and scale of regional resolution, the source of data used and the consideration of consumed water to assess scarcity have a significant effect on the results. However to enlighten these choices, questions need to be answered as to which data source is the most representative, how should scarcity be defined when using consumption-to-availability ratios and what is the most relevant spatial resolution. Choices to include source of water or seasonal variation could be limited only to more sensitive regions in the world as they are time and data consuming to apply and will not add to the discrimination power of the indicators in most regions of the world.

**270 Assessing cosmetic products life cycle water use impacts focused on water pollution** S. Vionnet, Quantis; I.M. Francke, Natura Cosméticos; S. Humbert, Home. Water use impact assessment, or water footprint, methodologies are quickly evolving on one hand. On the other hand, companies need to measure water footprints of their products in order to better manage their impacts on water resources. Water use impact assessment methodologies within the frame of life cycle assessment will answer this need, although there is no consensus on the method to use yet among the various options. In this work we explored methodologies addressing the degradative use of water. This is particularly a challenge for cosmetic products that incorporate complex ingredients. Key parameters of those ingredients, like human toxicity or degradability, are usually difficult to find resulting in difficulty to apply the methodologies. We assessed five methodologies that address degradative use of water: Grey water footprint (GWF), Critical Dilution Volume (CDV), Water Impact Index (WIIX), USEtox ecotoxicity and Ridoutt and Pfister 2012 methodology. The endpoints methods used for calculating this latter were eutrophication (ReCiPe), acidification (IMPACT2002+) and ecotoxicity (USEtox). Two products of a cosmetic company called Natura were assessed: a facial cream and a body oil made out of 32 and 10 different ingredients respectively. The assessment encompassed the entire life cycle of both products, from

cradle to grave. The functional unit were defined regarding the use of one entire packaged product. Each methodology and associated results were then assessed in term of science, communication and deployment criterias in order for Natura to select the more sound methodology to deploy its water strategy. The results showed that each methodology had different life cycle stages contributing at various extent to the impacts. The parameters considered by each method (toxicity, degradability, local water stress index, amount of water consumed, etc) are first responsible for the differences between methodologies results. The data gaps and uncertainty was the second source of variability in results. Overall the Ridoutt and Pfister 2012 methodology allows for the best communication (with units in  $m^3$ -eq), is based on the best science available (including USEtox methodology) and is the most comprehensive in term of pollution issues. Its applicability shortcomings are possible to overcome in the future, although it will require new development from the scientific community.

**271 Spatially resolved approach for linking emission to human exposure: a case study for Europe** S. Sala, Joint Research Centre European Commission / Sustainability Assessment Unit - Institute of Environment and Sustainability; B. Ciuffo, Joint Research Centre / Sustainability Assessment Unit - Institute of Environment and Sustainability; D. Marinov, JRC EC / Institute for Environment and Sustainability; M. Trombetti, Joint Research Centre / Institute of Environment and Sustainability. Spatial differentiation is a topic of increasing interest within ecological risk assessment (ERA) and Life Cycle Impact Assessment (LCIA). Consequently, multimedia models are increasingly adopted for supporting the analysis of the elements affecting the spatial variability of ecotoxicity and human toxicity impacts. A key issue to be addressed in the impact assessment methods, models and corresponding impact factors is the level of spatial detail required and uncertainties related to the use of generic impact factors when the exact location of the activities is unknown. For human exposure via inhalation, we run the spatially resolved multimedia model MAPPE Europe ( $1^\circ \times 1km$ ), in order to assess the relative influence of scale and input parameters in calculation of Intake Factors (iFs) under different emission scenarios, such as: (1) a multiple uniform emission coming from highly populated and industrialised areas. The amount of emission in each European country has been scaled up by the population density. In these runs, two substances have been evaluated: 1,1,2,2 tetrachloroethane - representative of highly volatile chemicals; Nitrobenzene - representative of multimedia chemicals; acephate - representative of hydrophilic chemicals; (2) a point source emission coming only from a single country (Spain, Luxemburg and Slovenia); (3) a multiple non uniform emission, accounting for real industrial emission of 1,2 dichloroethane (representative of volatile chemicals), as reported in the *European Pollutant Release and Transfer Register* (E-PRTR), from the countries where these emissions are reported and then from Belgium only. The results were, then, compared to those of a nested box model (USEtox) in order to address the uncertainty related to the adoption of a non-spatially resolved model. The iFs for the different tested substances vary of 1 order of magnitude amongst countries in Europe and the values are mainly affected by the physical chemical properties of the substances and by the population density. The location of the emission affect the results, as for example, considering the case of a single emission per country, emitting the same quantity from Luxemburg and Slovenia implies a factor of 2 of differences. Comparing the results with USEtox, as average, the iFs at continental scale are one order of magnitude higher whereas at urban scale two orders of magnitude higher than in USEtox.

**272 Predicting honeybee exposure to pesticides from vapour drift using a combined pesticide emission and atmospheric transport model** T.S. Geoghegan, K.J. Hageman, University of Otago / Chemistry; M. Scheringer, ETH Zuerich / Institute for Chemical and Bioengineering. Modelling pesticide emissions to air is important for understanding pesticide vapour drift and its potential effects on non-target environments. Several environmental fate models have been developed for pesticides but few use plant or species-specific descriptors

to describe volatilisation from crops. As a chemical's environmental fate is a function of its physical-chemical properties and the nature of the environmental media in which it resides, both factors should be considered when estimating pesticide volatilisation. This work proposes a new way to estimate pesticide emissions from a sprayed field using soil-air and plant-air partition coefficients. It then links those emissions to potential effects in the near-field non-target environment using risk assessment methods and honeybees as an indicator species. This risk assessment approach is unique in that the emphasis is on the effects caused by the process of vapour drift rather than by particular pesticides. The objectives of this work were to (1) develop a model for estimating pesticide emissions from agricultural fields that considers the interactions of pesticides with soil and plants (including plant size and surface characteristics), (2) combine emissions data with a chemical transport model to determine downwind concentrations of pesticides in air and plant phases and (3) use modelled results to calculate risk quotients for honeybees exposed to volatilised pesticides in a non-target area, thereby assessing the importance of vapour drift as an environmental exposure mechanism. Volatilisation emissions were calculated using mass balance and flux equations, and had a good correlation ( $R^2=0.8$ ) with literature values. Downwind air concentrations were calculated from persistence and characteristic travel distance data determined using the Small World model. Pesticide concentrations in plants in the non-target area were estimated from deposition flux to plant surfaces. Honeybee exposure was calculated for respiratory, contact and oral routes which were combined to give total daily exposure. Preliminary results indicate that, for some pesticides, vapour drift alone could contribute enough pesticide to a non-target area to cause an adverse affect on foraging honeybees; while for other pesticides it is an important contributing factor to environmental contamination. Therefore vapour drift should be considered in traditional risk assessments along with other environmental exposure mechanisms.

**273 Evaluating the environmental fate of short-chain chlorinated paraffins (SCCPs) in the Nordic environment using a dynamic multimedia model** L.S. Krogseth, Norwegian Institute for Air Research; K. Breivik, Norwegian Inst for Air Research; J.A. Arnot, ARC Arnot Research Consulting / Department of Physical Environmental Science; M. Schlabach, Norwegian Inst. for Air Research. Short chain chlorinated paraffins (SCCPs), also called polychlorinated *n*-alkanes, are mixtures of compounds of molecular formula  $C_nH_{2n-2}Cl_x$  containing 10-13 carbon atoms and usually 30-70 % degree of chlorination. They have a range of industrial applications, but there are concerns regarding their potential for environmental persistence, bioaccumulation, adverse effects and long-range transport. However, the environmental fate and exposures of SCCPs to humans and ecological receptors is poorly understood. The technical mixtures consist of thousands of isomers, which make analysis and modeling of these compounds very challenging. The goal of this study was to evaluate the overall understanding of the link between emissions of SCCPs, environmental levels and human exposure in the Nordic environment, and to identify the more critical knowledge gaps. Additionally, we wanted to assess the extent of expected variation of environmental fate within the complex mixture of SCCPs, and to evaluate the merits and limitations of using an average property estimate for the whole group. The 46 theoretically possible formula groups of SCCPs were represented by one isomer each, and physical-chemical properties were estimated. Realistic emission scenarios for sum SCCPs in the Nordic countries were estimated using a high-throughput screening method and data on chemical usage of SCCPs in the Nordic countries. The physical-chemical property and emission estimates were used to parameterize an integrated, non-steady state environmental fate and bioaccumulation multimedia model (CoZMoMAN), and the model results were compared to measured levels in the Nordic environment, as well as to predictions based on the "average" SCCP properties used in the EU Risk assessment report of SCCPs. Preliminary results show that the EU "average" SCCP does not capture the large range in possible environmental behaviors. In general the model underestimated

concentrations for all compartments compared to measured levels, implying that the emission estimate was underestimated. Results will be presented and discussed with emphasis on the more critical research needs with respect to the overall fate and exposure of SCCPs.

**274 Highly dynamic multimedia fate models in risk assessment of contaminated sites: why is "one fits all" unsuitable?** M. Morselli, University of Insubria / Department of Science and High Technology; G. Porto, A. Biasiolo, Copernico S.r.l.; A. Di Guardo, University of Insubria / Department of Science and High Technology. Subsurface contamination is one of the environmental problems of most concern for human health and environment. The most acknowledged references offering guidelines for the managements of sites affected by this type of contamination are the American Society for Testing and Materials (ASTM) Risk Based Corrective Action (RBCA) standards for evaluating petroleum release sites and chemical release sites. In these documents, the use of modelling tools is encouraged. However, even the most used modelling tools, such as RBCA Toolkit for Chemical Releases, consider a static environment, characterized by environmental properties which are constant in time. This often leads to extremely conservative predictions. In order to understand the potential improvement deriving from a more accurate description of the environment, a new dynamic multimedia fate model (SoilREM) was developed. SoilREM is based on the site-specific dynamic model SoilPlus, in which two atmospheric layers interact with a litter/soil multi-layered system. The height and wind speed of the atmospheric layers, representing the planetary boundary layer (PBL) and the atmospheric residual layer, are computed on an hourly basis starting from standard meteorological observations, in order to reflect the high variability which is usually observed in this part of troposphere. The development of SoilREM implied the nesting of 3 SoilPlus units in order to simulate different spatial scales, the smallest of which can represent a specific outdoor point as well as a building located within the contaminated site. The simulations with SoilREM were performed for a semi-urban site located near Milan (Northern Italy) for two selected chemicals (benzene and phenanthrene); results showed that air chemical concentrations directly respond to the short-term variations occurring in the lower troposphere (i.e., PBL height and wind speed oscillations). A comparison with the results produced by RBCA Toolkit for Chemical Releases confirmed the excessively conservative nature of the latter approach. For this reason, a dynamic model such as SoilREM could be useful for the assessment of the occurrence and magnitude of exposure peaks and could provide vital information for performing adequate monitoring campaigns in contaminated sites.

**275 Development of a realistic environmental scenario for a dynamic bioaccumulation model of organic contaminants in plant biomass** E. Terzaghi, University of Insubria Como / Dept. of Science and High Technology; M. Morselli, University of Insubria / Department of Science and High Technology; B. Cerabolini, University of Insubria; A. Di Guardo, University of Insubria / Department of Science and High Technology. The approach currently employed for environmental risk assessment need to be improved in the next few decades since it lacks of ecological realism. This goal can be achieved through the development of a number of more realistic scenarios which consider temporal and spatial variability of environmental parameters and their adoption in dynamic multimedia fate model capable to predict time and space variable concentrations. Models which consider variability in exposure concentrations and in environmental and compartment parameters are scarce; additionally, even fewer models include a dynamic vegetation compartment. The aim of the present work was to develop a fully dynamic scenario that consider the variability of exposure concentrations, meteorological and compartment parameters and to adopt this scenario in a dynamic bioaccumulation model (SoilPlusVeg) to predict the temporal uptake and release (on hourly basis) of some Polycyclic Aromatic Hydrocarbons in a mixed broadleaf wood located in Northern Italy. SoilPlusVeg is a multimedia fate model based on the fugacity approach which incorporates a double layered dynamic atmosphere compartment which varies in height on hourly basis, a multi-

layered soil (bare or covered by up to three litter horizons) and a vegetation compartment. The vegetation compartment can be composed of a mono-specific or multi-specific forest canopy which includes roots, stem and leaves. While a preliminary version was presented before, now the model is improved considering a dynamic LAI (Leaf Area Index) and SLA (Specific Leaf Area) to obtain a leaf biomass that change with time. Air and leaf concentrations of 16 PAHs and two ecological parameters (LAI and SLA) measured weekly in a small broadleaf wood located in Northern Italy were used to develop the realistic scenario. Hourly or daily values of meteorological parameters were instead provided by the Regional Environmental Protection Agency (REPA). Simulations were performed to evaluate the role of air concentration variations, meteorological and compartment parameters variability in influencing the change of foliar biomass concentration with time. Comparison of measured vs predicted leaf concentrations produced results within the same order of magnitude. A preliminary sensitivity analysis showed the relevance of a number of parameters which need to be calibrated in order to obtain a better fit.

#### **276 Using passive samplers to assess sources, transport and fate of legacy and emerging organic pollutants in a U.S. North East coast estuary**

**R. Lohmann**, University of Rhode Island / Graduate School of Oceanography. The emergence of passive samplers in recent years has made the sampling of hydrophobic organic contaminants in water and porewater easy and reliable. A group of volunteer citizen-scientists was recruited to deploy passive samplers in the Narragansett Bay (NB) watershed, greatly expanding the study area. Freely dissolved concentrations of emerging contaminants (triclosans, alkylphenols, and PBDEs) as well as legacy contaminants (OCPs) were determined. Triclosan, previously undetected in the Bay was detected in the NB watershed suggesting releases in coastal waters. Tetra, penta, and hexa PBDEs were consistently detected in the NB watershed at concentrations ranging from 100's to 1000's of fg L<sup>-1</sup>, with higher maximum concentrations than previously reported within the NB. Lastly, OCPs such as hexachlorobenzene, *trans*-chlordane, *cis*-chlordane, *t*-nonachlor, *p,p'*-DDE and methoxychlor were widely detected within the watershed. Methyl triclosan and the PBDEs showed higher concentrations in the Providence River and the head of the bay where human influence is greatest and flushing is lowest. Alkylphenols and OCPs in contrast, did not show the same pattern of distribution suggesting that there is substantial release from historically contaminated sediments. Passive samplers are suited for deployment by citizen-scientists and can help raise awareness of pollutants and identify spatial/temporal trends and potential point sources.

#### **277 Endocrine disrupting chemicals and antibacterial agents in the coastal environment**

**Z. Xie**, Helmholtz Zentrum Geesthacht.

#### **278 Contaminants of emerging concern in the Norwegian marine environment**

**K. Thomas**, NIVA.

#### **279 Panel discussion: Future issues - contaminants in estuarine and coastal environments**

**J. Readman**.

#### **280 Intestinal uptake of copper nanoparticles in rainbow trout using an in vitro intestinal perfusion, involves nystatin and vanadate-dependent pathways**

**R.D. Handy**, Ecotoxicology Research and Innovation Centre; H. Soliman, University of Plymouth. The production of manufactured nanoparticles is increasing, making their release into the environment inevitable. There is an emerging literature on the toxic effects of nanoparticles in a variety of aquatic organisms, although the uptake mechanisms for nanoparticles are not entirely understood. In this study, whole gut sacs from rainbow trout were used to identify the regions of gut involved in Cu NP absorption. Exposure of whole gut sacs to 1 mg l<sup>-1</sup> Cu as CuSO<sub>4</sub> or Cu NP in the luminal solution caused Cu accumulation mostly in the mucosa (62% or more) rather than the underlying muscularis. Tissue total Cu concentrations indicated that Cu was predominantly absorbed in the mid and hind intestine, regardless of the nano form. Perfused intestines were used to investigate details of the

uptake mechanisms and showed good viability criteria including low LDH leak (-1) and steady perfusate flow. The accumulation of Cu from CuSO<sub>4</sub> was higher than that from Cu NP in both mid and hind intestine. Serosal applications of the P-type ATPase inhibitor vanadate (100 μmol l<sup>-1</sup>) caused 3.1 and 5.35 fold decreases in net Cu uptake from copper sulphate and copper nanoparticles, respectively. Also, additions of 120 μmol l<sup>-1</sup> nystatin caused a 1.9 and 3.75 fold decline in net Cu flux to serosal compartment from CuSO<sub>4</sub> and Cu NP, respectively. The present study shows that copper as CuSO<sub>4</sub> or Cu NP are mostly absorbed across the mid and hind intestine, with the uptake rate of Cu from Cu NPs being especially sensitive to inhibitors. The uptake mechanisms involve a vanadate sensitive pathway, as well as a nystatin-sensitive endocytosis across the mucosal membrane.

#### **281 Uptake kinetics of silver nanoparticles and silver nitrate by *Pseudokirchneriella subcapitata* related to Ag speciation in solution**

**E. Ribeiro**, University of Aveiro CESAM / department of Biology & CESAM; J. Gallego, University of Gothenburg / Department of Chemistry and Molecular Biology; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science; E.V. Soares, Chemical Engineering; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; S. Loureiro, Universidade de Aveiro / Biology. Freshwater algae plays an important role in lakes and ponds as primary producers, representing a great fraction of the diet of zooplankton feeding on suspended particulate matter. A change in the productivity and/or population structure of algae has the potential to affect the entire food chain of a lake, and therefore it is important to consider such organism as a key species for toxicological assessments. This study assessed the uptake kinetics of AgNP (3-8nm, AMEPOX) and silver nitrate (AgNO<sub>3</sub> from Sigma Aldrich, 99% purity CAS 7761-88-8) in the green algae *Pseudokirchneriella subcapitata*, in order to relate internal concentrations in the algae with Ag speciation in the colloidal suspension to which they were exposed. To describe the bioconcentration of silver in *P. subcapitata* over time, a first order kinetics model was used to calculate uptake and elimination rate constants (k<sub>1</sub> and k<sub>2</sub>). Toxicokinetics were calculated relating Ag uptake to each of the different colloidal fractions that were analysed. To take into account the decrease of silver concentrations in the different fractions during the uptake phase, a decay rate constant was estimated based on first order kinetics. This decay rate constant was included in the first order uptake kinetics model. Bioconcentration Factors (BCF's) were calculated as for each exposure, being K<sub>1</sub> (uptake constant) and K<sub>2</sub> (elimination constant) Considering the non-filtered fraction, both uptake and elimination were fitted to the kinetics model. Assuming that K<sub>1</sub> is more related to exposure and bioavailability, we therefore will center our conclusions based on these values. Even though the BCF values look similar for both concentrations of AgNO<sub>3</sub>, we obtained different values of K<sub>1</sub> meaning that at 15 μg/L of AgNO<sub>3</sub>, algae were able to uptake silver faster than at 30 μg/L. For silver nanoparticles, the picture looks quite dissimilar from AgNO<sub>3</sub> since K<sub>1</sub> for both concentrations are almost identical to each other. This suggests that silver nanoparticle uptake by algae is not a concentration-dependent parameter, whereas it can be considered an Ag-speciation dependent parameter.

#### **282 Bioaccumulation and Toxicity of Metal NanoParticles to Sediment-Dwelling Organisms**

**H. Selck**, Roskilde University; G.T. Banta, Roskilde University / Environmental, Social and Spatial Change; V.E. Forbes, University of Nebraska-Lincoln / School of Biological Sciences. Nanotechnology is expanding rapidly, thus increasing the release of engineered nano-particles (NPs) to the aquatic environment. Sediment is likely to be the environmental compartment that is most exposed to metal-bearing NPs, and organisms that feed on sediment are likely to be particularly at risk from metal-NP exposure. Due to their increased surface area and reactivity, NPs may be more bioavailable and toxic than their macroscale counterparts. However, very little is known about the environmental impact of NPs and whether they behave similarly in the environment compared to their macroscale counterparts, and these factors make it difficult to perform environmental risk

assessments and to set quality standards for NPs. Some of the questions we are facing are whether *nano-specific properties lead to unexpected and new biological effects? If so, is the potential for such effects diminished when NPs are introduced to a complex environment such as the sediment compartment?* Acknowledging that we are not yet able to characterize NPs once they have been introduced to the sediment compartment, we used a comparative approach to assess toxicity and bioavailability of metal NPs. This presentation will show that by using realistic exposure scenarios, applying different well-characterized forms and sizes of metals to the sediment compartment, and examining subsequent bioavailability and toxicity, we found evidence that bioavailability was affected by the metal form introduced even though metal inevitably will change speciation once it enters the sediment compartment. The effect and importance of metal form added to sediment for metal bioavailability and subsequent toxicity is not straightforward, however, and depends both on biological factors, such as digestive complexity, and physical-chemical factors affecting the fate and speciation of metals in the sediment compartment. To better understand these mechanisms we need to focus future research efforts on developing methods to characterize nanomaterials in complex environments, including sediment, soil and diet, that are robust, reliable and yet relatively easy to implement in a standard laboratory.

### 283 ZnO nanoparticle fate and effects on *Folsomia candida* in soil

P. Waalewijn-Kool, VU University; M. Diez Ortiz, Centre for Ecology and Hydrology; C. van Gestel, VU University. In this contribution we will provide an overview of the short-term and long-term fate and effects of coated and uncoated ZnO-NP on the springtail *Folsomia candida*. Also, the effect of soil pH on the toxicity of ZnO-NP will be shown for a field soil amended to three different pH levels. In a 28-day toxicity test, springtail reproduction was reduced in a dose-dependent manner and EC<sub>50</sub> values of 1964, 1591 and 298 mg Zn/kg were estimated for uncoated ZnO-NP, non-nano ZnO and ZnCl<sub>2</sub>, respectively. Coated ZnO-NP (EC<sub>50</sub> = 873 mg Zn/kg) was more toxic than uncoated ZnO-NP. Based on porewater Zn concentration EC50 values for all Zn forms were in the same range (7.94-16.9 mg Zn/l), suggesting that the zinc ions released from the ZnO rather than the particles themselves are responsible for the observed toxic effects. ZnO-NP dissolution and related toxicity continued changing for one year equilibration of spiked soil. Our toxicity tests showed that an equilibration period of three months or longer is able to reduce toxicity for *F. candida*, except for coated ZnO-NP which showed reduced toxicity only after 12 months equilibration. ZnO addition caused Zn porewater concentrations to steadily increase with time for at least one year. Already after three months, porewater concentrations peaked at intermediate concentrations for ZnO-NP (67.1 mg Zn/l after 12 months) and non-nano ZnO (66.5 mg Zn/l), while for coated ZnO-NP such a clear peak was only seen after 12 months (36.6 mg Zn/l). Dose-related increases in soil pH may explain decreased soluble Zn levels due to fixation of Zn released from ZnO at higher soil concentrations. For the pH amended soils, the effect on reproduction decreased with increasing soil pH for all three Zn forms, with 28-d EC<sub>50</sub> values of 553, 1481 and 3233 mg Zn/kg for uncoated ZnO-NP and 3301, 732 and 1174 mg Zn/kg for ZnCl<sub>2</sub>, at pH 4.5, 5.9 and 7.2, respectively. EC<sub>50</sub> values based on porewater Zn concentrations increased with increasing pH for uncoated ZnO-NP from 4.77 to 18.5 mg Zn/l, while for ZnCl<sub>2</sub> no consistent pH-related trend in EC<sub>50</sub> values was found (21.0-63.3 mg Zn/l). In comparison with ZnCl<sub>2</sub>, uncoated ZnO-NP toxicity is lower based on total Zn concentrations and higher based on porewater Zn concentrations. This indicates that toxicity of uncoated ZnO-NP can not be solely attributed to the dissolved Zn fraction. The fact that different Zn forms do affect soil pH differently may contribute to differences in toxicity.

### 284 Bioavailability and toxicity of sediment-bound nanoparticles

T. Ramskov, Roskilde University / Department of Environmental, Social and Spatial Change; H. Selck, Roskilde University; G. Banta, Roskilde University / Department of Environmental, Social and Spatial Change; D. Berhanu, Natural History Museum; S.K. Misra, E. Valsami-Jones,

Natural History Museum, University of Birmingham; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences. Due to the widespread applications of metal bearing nanoparticles (NPs), such as copper oxide (CuO), in our daily life, both human and environmental exposure is likely to increase. Once released into the aquatic environment, CuO NPs are likely to agglomerate and/or aggregate, whereby it is expected that they will partition to sediments. Deposit-feeding organisms may be at particular risk of exposure to CuO NPs. Thus, in a number of studies we have used an environmentally realistic exposure scenario by examining the fate and effects of metal oxide NPs added to the sediment compartment. The overall aim of the studies performed has been to explore the relative importance of metal form (aqueous vs. NP) and shape (rods, spheres, spindles) added to sediment for subsequent bioaccumulation and effects in the deposit-feeding snail, *Potamopyrgus antipodarum*. Our results show that both particle form and shape have an important influence on the toxicity and bioaccumulation of Cu in *P. antipodarum*, and that correlations between these parameters are not straightforward. Besides summarizing highlights from the performed studies, we will also focus on how sediment exposure can increase our understanding of the behavior of nanoparticles in the environment, and thus how increasing ecological relevance in ecotoxicological studies can add value to environmental risk assessment of NPs.

### 285 Environmental conditions alter the ecotoxicity of silver nanoparticles to *Daphnia magna*

F. Seitz, Inst for Environmental Sciences / Institute for Environmental Sciences; R.R. Rosenfeldt, University of KoblenzLandau Institute for Environmental Sciences / Institute for Environmental Sciences; K. Storm, University of Koblenz-Landau / Institute for Environmental Sciences; R. Schulz, University of KoblenzLandau / Institute for Environmental Sciences; M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment. Products containing silver nanoparticles (nAg), e.g. textiles, are increasingly used. Hence, nAg may finally enter aquatic environments, where they potentially pose a risk to wildlife. For this reason, several studies investigated the aquatic toxicity of different nAg products, frequently focusing on implications for the waterflea *Daphnia magna*. However, there is only limited information about ecotoxicological effects of nAg when considering varying environmental conditions, such as pH or dissolved organic carbon (DOC) levels. Therefore, the present study assessed the 48-h acute toxicity of nAg products with varying particle size (citrate coated; 20, 60, 100 nm) and silver nitrate, under different pH (6 and 8) and DOC (0 and 8 mg TOC/L) levels for *D. magna*. Independent of pH and DOC level, the results clearly indicated a size dependent toxicity of nAg. This was displayed by up to 10 fold increased 48-h EC<sub>50</sub> when comparing 20- and 100-nm nAg. Moreover, the toxicity of nAg increased with decreasing pH (~3 times) and DOC level (up to ~1.3 times). Silver nitrate, in contrast, displayed – regardless of pH and DOC level – a 48-h EC<sub>50</sub> of 2.07±0.83 µg/L. The findings of the present study hint at the pathway of nAg toxicity, which has been controversially discussed in literature previously. As the total amount of silver ions in the media strongly depends on the present pH, particle coating (citrate or DOC) as well as on the actual particle size, most likely ions play the major role in nAg toxicity to *D. magna*. However, this hypothesis needs to be further assessed by chemical analysis. In conclusion, the present study underpins the importance of environmental parameters for the fate and ecotoxicity of nanoparticles, which should be further investigated in future.

### 286 Body Residues and Responses of the Midge *Chironomus riparius* to Sediment Associated Fullerene C60

G.C. Waissi-Leinonen, University of Eastern Finland; K. Pakarinen; S. Bold, University of Eastern Finland; E.J. Petersen, National Institute of Standards Technology; J. Akkanen, University of Eastern Finland / Department of Biology; M.T. Leppanen, Finnish Environment Institute / Department of Biology; J.V. Kukkonen, University of Jyväskylä / Biological and Environmental Science. The research of nanoparticles

toxicity to organisms has faced dilemma of appropriate testing methods and concepts to deal with them. Common ecotoxicity test do not encounter well to nanoparticles due to their unique chemical characters and thus may cause misleading conclusions. In this study a benthic invertebrate *Chironomus riparius* larvae were exposed to fullerene (nC<sub>60</sub>) masses in sediment using an environmentally realistic method. By simulating a sensitive exposure route for *C. riparius* feeding habits larvae body residues were analyzed using spectrophotometric measurements and gut content investigated with transmission electron microscopy. Exposure conditions were created to test short-term effects after exposure for 10 d consisting instars from first to fourth. In addition to 10d standard exposure, body residues of fourth instar larvae were investigated also after 12 and 24 h exposure times. Results showed clear evidence that extraction method was able to use and perform when studying body residues of *C. riparius* and indicated that body residues were lower after 15 days than 10 days. This might stem from change in behavior of larval feeding during the development time or possibly after 10 d time larvae have reached the steady state. It was also shown that after short exposures (12 and 24 h) larvae were rapidly packed by fullerenes. Fullerene exposure might have an effect on development time which could be taken as a possible response of stressed larvae.

**287 Nanomicex – Ecotoxicity of pristine and modified pigment nanoparticles** B.K. Gaiser, School of Life Sciences; J.H. Kinross, D.M. Brown, A. Kermanizadeh, V. Stone, Heriot-Watt University; T.F. Fernandes, HeriotWatt University / School of Life Sciences. A variety of nanoparticles are used as pigments in paints, inks and. Exposure to these particles can be experienced in an occupational setting or by the end user. Nanoparticulate pigments can also reach the environment through waste, spills, abrasion and wash-off. Nanomicex is a recently launched FP7 project combining partners from industry and academia, in which seven different nano-sized pigments (Ag, TiO<sub>2</sub>, ZnO, CoAl<sub>2</sub>O<sub>3</sub>, Cd/Se QDs, Al<sub>2</sub>O<sub>3</sub> and Fe<sub>3</sub>O<sub>4</sub>) manufactured by the industrial partners are investigated for their toxic effects in the environment and on human cell lines. The aim of the project is hazard assessment of nanoparticles and the modification of any deemed hazards following those tests in order to reduce adverse effects in the environment and human cells, while preserving the properties which make the particles valuable as paints and dyes. In addition, the project seeks to establish structure-activity relationships and to investigate realistic exposure scenarios. A variety of endpoints including mortality/lethality and sub-lethal effects and mechanisms of action are investigated in *Daphnia magna*, *Lumbriculus variegatus* and *Pseudokirchneriella subcapitata* as environmental model organisms, and in human A549, C3A and J774 cells (alveolar epithelial cells, hepatocytes and macrophages). The pilot studies carried out to date indicate that the TiO<sub>2</sub> NPs studied were of low toxicity to the models and endpoints studied. Further tests will be conducted to verify these results. In contrast, ZnO NPs were found to exert a level of toxicity and therefore further studies will be conducted after NP surface modifications. The collaboration with the industrial partners, the combined information on human and environmental hazards, as well as the facilities to modify particles to keep them suitable for their intended application while minimising the hazard to environmental and public health, make Nanomicex a very promising project for investigating realistic steps towards safe, environmentally compatible use of nanomaterials.

**288 Interactions of CdSe/ZnS quantum dots with the ciliated protozoa *Tetrahymena thermophila*** M. Mortimer, Aquatic Biogeochemistry and Ecotoxicology, Institute F.-A. Forel, Faculty of Sciences; A. Kahru, National Institute of Chemical Physics and Biophysics / Laboratory of Environmental Toxicology; V. Slaveykova, University of Geneva / Aquatic Biogeochemistry and Ecotoxicology, Institute F.-A. Forel, Faculty of Sciences. Understanding the environmental impact of engineered nanomaterials has become an active area of research over the past years due to their possible entrance to ecosystems, including the food chain. Research in this area is still insufficient for a complete environmental risk assessment. In the current

study carboxyl functionalized CdSe/ZnS core shell quantum dots (QDs) were studied for their cellular uptake, localization and clearance. To consider both the cellular and organism level effects, the current study employed a unicellular eukaryote, freshwater protozoan *Tetrahymena thermophila* as a model organism. The QDs were not found to be cytotoxic to *T. thermophila* at concentrations up to 80 nM, which could be explained by the low solubility of the studied QDs: the dissolved fraction of Cd in the exposure medium was negligible and the concentration of dissolved Zn remained below toxic concentrations to *T. thermophila*. As evidenced by the optical microscopy imaging the investigated QDs, with an average hydrodynamic diameter of 12 nm, were readily phagocytosed by the protozoan upon the exposure to QDs in HEPES buffer. Flow cytometry analysis showed that the food vacuoles of *T. thermophila* became filled with QDs in time and dose dependent manner. The maximum filling of the food vacuoles with QDs was detected after 2-h exposure, while after 24-h exposure the tendency towards the clearance of the food vacuoles was detected. The process was shown to be dose dependent – the cells exposed to lower concentrations of QDs were able to excrete the content of the food vacuoles more efficiently. After replacing the QD-containing medium with the QD-free buffer about 50% of the protozoan cells had cleared their food vacuoles of QDs after 20 h. Confocal microscopy studies of QD-exposed *T. thermophila* revealed that phagocytosis was not the only pathway of entry for QD in the cell. In addition to larger aggregates of QDs in the food vacuoles also smaller-sized fluorescent particles were detected in the cells, suggesting QD internalization through cell membrane by alternative mechanisms (e.g. endocytosis). The intracellular QDs localized outside the food vacuoles were not subject to cellular clearance. The current study highlights that inherently non-toxic NPs may pose possible risks by accumulating in the cells and becoming bioavailable for the organisms at higher trophic levels.

**289 Multiwalled carbon nanotubes affect long term recolonization and structure of benthic communities** I. Velzeboer, Wageningen University; K. Peeters, VITO; A.A. Koelmans, Wageningen University / Environment. Whereas for traditional pollutants knowledge evolved from studying effects in the laboratory to studying effects in situ, assessment of in situ effects for ENPs still is in its infancy. Most ecotoxicity studies focus on single species laboratory tests. This kind of tests is less realistic with respect to multiple exposure routes, dynamic particle mixing, 'fouling' and aging of ENPs, or ecological processes like community effects or recolonization. Consequently, assessment of in situ effects is urgently needed because laboratory studies can be expected to be poor predictors of effects under field conditions, especially for ENPs. Aim of this study was to assess long term community effects of macroinvertebrates exposed to sediment contaminated with multiwalled carbon nanotubes (MWCNTs). This study focussed on the influence of MWCNT contaminated sediment on the recolonization by benthic macroinvertebrates in a long term controlled and replicated field experiment (15 months). MWCNT treatment effects were assessed and quantitatively compared to community composition after 3 months and to similar treatment effects of another carbonaceous material (activated carbon; AC) which was applied simultaneously in parallel systems in the framework of another study. A remarkable finding of this recolonization study is that MWCNT contaminated sediment showed significant effects on the benthic community structure after 15 months. After 3 months, effects were not (yet) significant. A dose of 0.0002 – 0.2 % MWCNTs explained 9.9% of the community composition (biodiversity and species abundance,  $p=0.012$ ). The field-relevant lowest dose tested also caused significant effects ( $p=0.020$ ). For the first time this shows that despite aging and burial, MWCNTs affect the structure of natural communities. Exposure to AC showed a comparable impact at a 50x higher dose, which suggests that the MWCNTs were about 50 times more potent than AC.

**290 Effects of nanoparticles of TiO<sub>2</sub> on food depletion and life-history responses of *Daphnia magna*.** C. Barata, CSIC / Environmental Chemistry; B. Campos, IDAEACSIC / Environmental Toxicology; C. Rivetti, CSIC; P. Rosenkranz, INIA; J. Navas, INIA -

Madrid. The extent to which different forms of nanoparticles of titanium dioxide (nano-TiO<sub>2</sub>) aggregated with microalgae, decreased food levels and hence impaired growth, reproduction and fitness of *Daphnia magna* individuals were studied. Treatments included three different types of nano-TiO<sub>2</sub> differing in their coating or crystalline structure but of similar primary size (20 nm) plus a micron-sized bulk material, two exposure levels (1, 10 mg/l) and two food ration levels of the microalgae *Chlorella vulgaris* that included a non limiting (1.5 µg C/ml) and a limiting one (0.3 µg C/L). Effects were assessed using standardized chronic tests and assays that maximized food depletion in the water column under semi-static and re-suspension conditions. Results indicated that the high ion levels in culture medium lead to the aggregation of nanoparticles followed by particle destabilization. Nanoparticle aggregates interacted with the algae cells, forming clusters. Large TiO<sub>2</sub>-algae agglomerates settled readily depleting dramatically the concentration of edible food for *D. magna*. At limiting food ratios food depletion by nanoparticle aggregation had dramatic effects on reproduction and fitness of exposed *D. magna* at 1 mg/L irrespectively of the particle form. At high food rations effects were only observed for one of the nano-TiO<sub>2</sub>, P25, at high exposure levels (10 mg/l) under both semi-static and particle re-suspension conditions, which suggest that P25 effects were mediated by clogging the gut and hence diminishing food acquisition. These results indicate that nano-TiO<sub>2</sub> may affect the transfer of energy throughout the planktonic aquatic food webs increasing the settlement of edible particles from the water column.

**291 Integrating bioaccessibility and community analyses into ecological risk assessment of metal contaminated sites: A shooting range case study** S.R. Bowman, The Ohio State University / Evolution Ecology and Organismal Biology; J.L. Bryant, The Ohio State University / Department of Entomology; R.P. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology. Although lead (Pb) occurs naturally, anthropogenic activities have caused Pb to be elevated beyond normal background concentrations in some areas. Since metal bioavailability varies with soil modifying characteristics and species-specific uptake and elimination, it is sometimes necessary to use site-specific measures such as bioaccessibility estimates and analysis of biotic communities when evaluating these areas. Our study site is a private shooting range located in central Ohio, USA. Study areas include the main shotfall zone of a trap and skeet range (2899 ± 1228 mg/kg soil Pb), an area where Pb pellets were excavated (Fall 2009; 628 ± 44 mg/kg soil Pb), and a reference area (1356 ± 86 mg/kg soil Pb) that does not directly receive spent shot. Our first objective was to determine how ground beetle (Carabidae) community structure (i.e., diversity and abundance) changes along a Pb gradient. Ground beetles were collected from 2008 to 2012 using pitfall traps in shotfall, reference, and Pb extracted areas. Forty-five species were collected during the first two years. In 2009, overall abundance was greater in the reference area than shotfall. Overall species richness did not differ between sites; however, an indicator species analysis suggested that six species were indicators of the reference site. With additional years of sampling (and a more robust dataset), we expect that we will be able to understand temporal and spatial trends in ground beetle communities at our study site. Our second objective was to adapt *in vitro* bioaccessibility assays to small mammal physiology so that we can better estimate exposure to Pb from ingestion of soil and prey. Preliminary results suggest that soil bioaccessibility is 5-10% of the total Pb in the field soils. For earthworms (*Eisenia fetida*) exposed to field soils for 30 days, bioaccessible Pb ranged from < 1% - 4% of the total earthworm Pb. Our next step is to field collect invertebrates, shrews (*Blarina brevicauda*) and voles (*Microtus pennsylvanicus*). We will analyze gut content and incidental soil ingestion of shrews and voles from the shooting range and control sites. We will use diet composition, incidental soil ingestion and bioaccessibility estimates to adjust exposure estimates at our field site. We anticipate that results from our study will provide evidence for the usefulness of IVG analyses and invertebrate community analyses in site-specific ecological risk assessment of metals.

**292 Application of the OECD 307 study to assess the persistence of Gas to Liquid (GTL) Fuel** G. Whale, Shell Health; C. Bayliss, Harlan Laboratories Ltd.; S. Forbes, Shell Global Solutions (UK); J. Dawick, Shell Health; C. Mead, Harlan Laboratories Ltd.. The main study currently used to assess the biodegradation of chemicals in soil is the OECD Guideline 307 for testing chemicals: Aerobic - Anaerobic Transformation in Soil (April 2002). This test was originally designed to provide degradation rate data for crop protection products but this is now being undertaken to provide data for other 'chemicals' under the EU Reach regulations. Many do not believe the current guidelines are suitable to assess the fate of complex substances. For example, the current approach recommended by CONCAWE (Conservation of Clean Air and Water in Europe) for complex hydrocarbon substances is to model persistence of the constituent hydrocarbon blocks. However as part of the registration of a new substance the European Chemicals Agency (ECHA) have stipulated to the registrants that an OECD 307 study should be undertaken to determine potential persistent hydrocarbon components of a Gas to Liquid (GTL) Fuel. This has proved challenging and in the course of our investigations a number of lessons have been learnt. In this presentation we provide an overview of some of the limitations and issues encountered with the conduct of the OECD 307 test, steps which could be taken to improve the test and finally the relevance of such tests to assess the fate of complex hydrocarbon substances in soil.

**293 Closing the bottleneck: evaluation of higher tier options for refinement of endpoints obtained by Collembola and Hypoaspis reproduction tests** P. Kabouw; S. Mastitsky, BASF / Crop Protection - Ecotoxicology; S. Royer, BASF SE, Global Ecotoxicology, Crop protection; F. Staab, M. Bergtold, BASF SE. The registration process of plant protection products includes tests on soil organisms such as Collembola and *Hypoaspis*. When refining endpoints obtained by Collembola OECD 232 and *Hypoaspis* OECD 226 reproduction tests risk assessors are limited by the test systems they can deploy. To overcome this bottleneck we evaluate previously published test systems for refinement of Collembola/*Hypoaspis* endpoints. Test systems we evaluate are aged residue tests, multi-generation tests, and RAMAS ® population models. All methodologies were evaluated based on the following criteria; simplicity in lower tiers, a higher level of realism in higher tiers, and the potential of these methods to address recovery. Weaknesses and strengths of the three methodologies will be demonstrated using case studies. Aged residue tests with Collembola/*Hypoaspis* evaluate the potential for external recovery. This approach is similar to aged residues tests conducted with NTAs. The outcome of the test system is dependent on how soils are aged and on the properties of the test compound. Soils can be aged by considering different environmental factors. Multigeneration tests are similar to aged residues test. However, in contrast to aged residue tests that consider external recovery, multigeneration tests consider internal recovery through reproduction. In this test system potential effects that are transferred over generations can be assessed. Both aged residues and multigeneration tests experimentally examine long term toxicity and take recovery into consideration. In both test systems ageing of treated soil leads to decreased toxicity only when the toxic effect is caused by readily degradable formulation additives or active substances. The RAMAS ® model projects population development based on generated data by OECD 226/232 tests. Such a modeling exercise can, therefore, be conducted without performing additional experimental work. At the same time multiple generations are considered increasing realism and taking recovery from reproduction into consideration. The projection assumes constant and homogeneous exposure of Collembola/*Hypoaspis* to the potential stressor. We conclude that the above mentioned refinement options are a suitable approach to bring more realism in risk assessment for soil organisms.

**294 Collembolan two-generation study within a tiered soil risk assessment** G. Ernst, Bayer CropScience / Ecotoxicology; U. Frommholz, U. Menke, P. Neumann, Bayer CropScience. An increasing conservatism in the tier 1 risk assessment (RA) of plant protection



products (PPP) for soil organisms is expected due to changing requirements in exposure modeling (e.g. reduced crop interception rate, PEC calculation for 0-1 cm soil layer). However, the exposure of PPP in soil is often limited in time because of various degradation and dissipation processes of the product in soil. In some cases this leads to a fast reduction in toxicity. Thus, the risk, e.g. for *Collembola*, might be overestimated in a highly conservative tier 1 RA with substances degrading fast. Therefore, a *Collembola* two-generation study is proposed as an intermediate tiered laboratory test system in order to assess the inherent recovery potential for *Collembola* from the treated area/population itself. Juvenile individuals hatched in a *Collembola* reproduction test after 28 d (OECD 232; 1<sup>st</sup> bioassay) will be transferred into a 2<sup>nd</sup> consecutive bioassay containing soil with aged test substance that was treated together with the soil from the 1<sup>st</sup> bioassay. This procedure aims to assess if populations which were initially impacted in the 1<sup>st</sup> bioassay (e.g. at or above the LOEC) are not impacted in their reproduction performance in the 2<sup>nd</sup> bioassay and thus able to build up a new population. A dose response test was performed with Lindane EC150 (emulsifiable concentrate; 150 g/L) following the proposed design. The 1<sup>st</sup> bioassay leads to a NOEC of 0.1 mg/kg and after the 2<sup>nd</sup> bioassay a NOEC of 0.25 mg/kg was determined indicating only a moderate reduction in toxicity to *Collembola*. Furthermore, a dose-response Terrestrial Model Ecosystem (TME) is available as well with Lindane EC150 (Scholz-Starke et al., submitted) considering application rates between 24 and 2400 g Lindane/ha. The *Collembola* community was shown to be not even initially impacted at a rate of 240 g Lindane/ha. Comparing the NOEC from the two-generation study with the application rate being safe for the *Collembola* community in the TME the conservatism of the proposed intermediate tiered RA approach is assessed. A risk (TER 0.68) would still be indicated at an exposure of 240 g Lindane/ha (NOER in TME) even with an a priori TER trigger value of 1. The consideration of the potential for inherent recovery in an intermediate tiered risk assessment approach is a valuable tool to fill the gap between an overly conservative tier 1 and higher tier *Collembola* risk assessment for substances dissipating fast.

**295 The chemical quality of urban soils in Glasgow, UK, with reference to anthropogenic impacts and current toxicologically-based soil guideline values.** F. Fordyce, Geochemical Baselines and Medical Geology; S. Nice, B. Lister, B. O'Dochartaigh, British Geological Survey. Until recently systematic data on the chemical quality of urban soils was lacking in many countries as traditional soil survey programmes focussed on rural environments. The advent of environmental protection legislation in the UK in the 1990s has driven the need for urban soil quality information to ensure healthy and sustainable environments. Since 1992, the British Geological Survey (BGS) has completed soil surveys in 27 UK cities under the Geochemical Baseline Survey of the Environment (G-BASE) programme. This included a survey of Glasgow and surrounding rural areas to link to a wider geoenvironmental assessment: the BGS Clyde and Glasgow Urban Super Project (CUSP). The survey provides an overview of land quality in Glasgow and is based upon the collection of 2 and 4 per km<sup>2</sup> in rural and urban areas respectively. The samples are analysed for total concentrations of c.50 chemical elements by X-ray fluorescence spectrometry. Glasgow is Scotland's largest city and was a major industrial centre until the mid 20<sup>th</sup> century. Much of this industry has now declined and the city is undergoing regeneration. However, the legacy of the industrial past remains. Results for 1381 urban and 241 rural soils reveal that concentrations of many metals are elevated in urban soils relative to the rural background regardless of the geological parent material. Elements that are commonly associated with anthropogenic pollution such as Pb, Sb and Sn show greatest enrichment in urban versus rural soils (2.6 – 3.3 times, based on median values). Median topsoil Cr (108 mg kg<sup>-1</sup>) and Ni (47 mg kg<sup>-1</sup>) concentrations in Glasgow are higher than in many other UK cities due to the presence of volcanic bedrock and history of metal processing in the city. In terms of toxicologically-based soil quality assessments, with the exception of Cr, only a small proportion of soils exceed the current UK human Contaminated Land Exposure Assessment (CLEA) residential Soil

Guideline Values despite the city's industrial heritage – Cr (22%), Pb (5%), As, Cd, Ni (2%) and Se (0%). However, a much greater proportion exceed the proposed UK Ecotoxicity Soil Screening Values – Cr (100%), Ni (94%), Zn (86%), Pb (30%), Cu (17%) and Cd (4%). The G-BASE data allow these thresholds to be evaluated in terms of typical soil element abundances to aid ecotoxicological assessments and inform policy.

**296 Results from a Workshop on Ecological Soil Levels—Next Steps in the** R.S. Wentsel, Exponent / Ecological Sciences; A. Fairbrother, Exponent Inc / EcoSciences. This paper presents the results from a workshop to develop a process for establishing ecological soil clean-up values (EcoSCVs) in the U.S. The goal of the workshop was to leverage advances from research conducted in support of REACH and the Australian risk assessment approaches by providing regulators with methods and processes to incorporate bioavailability, normalize toxicity thresholds, address food-web issues, and incorporate background concentrations. These recent major terrestrial research projects have significantly advanced our understanding of the behaviour and toxicity of metals in soils. Large data sets were developed that are useful for risk assessment of metals in soil environments, and were used by workshop participants as case studies in the development of the ecological standards for soils. Manuscripts from the workshop discussed bioavailability adjustments based on pH, cation exchange capacity, and organic carbon; application of leaching and aging factors; and consideration of the source and form of metals in the soil. Incorporation of soil microbial processes and a path forward for wildlife toxicity reference dose and food-chain modeling were also discussed in prepared manuscripts. In addition, one of the workgroups described the processes needed to gain regulatory acceptance as a directive or guidance by North American state and federal governments.

**297 Simulation of age-dependent survival of *Daphnia magna* following time-varying exposure of pentachlorophenol and dimethoate** Y. Wang, C.D. Brown, University of York / Environment Department. Toxicokinetic-toxicodynamic models (TKTD models) have been recommended as a promising tool to simulate the time-course of toxic effects at organism level and even beyond test conditions over time. Wider use is partially restricted at present because there has not been extensive application of the approach to the key risk assessment species. This study consisted of TK and TD experiments for pentachlorophenol and dimethoate. Each experiment included three independent tests which were started with different age groups of *Daphnia magna* (i.e. less than 24-hour old neonates, 7-day old juveniles, and 14-day old adults). The dry weight of *Daphnia* was used as a measure of the growth of the organism. A TKTD model was established to simulate effects of time-varying exposure with correction for growth dilution. This is the first detailed study of using a TKTD modelling approach to simulate the effect of time-varying exposure of pesticides on the age-dependent survival of *Daphnia magna*. This study demonstrated that: i) growth greatly influenced uptake and elimination of the two compounds, causing a large reduction in BCF for greater than 1 order of magnitude between neonates and adults; ii) sensitivity of *Daphnia* to the two compounds varied by up to a factor of 2 between different life stages with LC<sub>50</sub>s increasing and decreasing with age for pentachlorophenol and dimethoate, respectively; iii) the TKTD model gave a good simulation of the survival of *Daphnia* both for the TD experiments and an independent validation test with long-term time-varying exposure. TKTD models do not currently account for changes in sensitivity between life stages, so this needs to be incorporated to develop fully-functional models for use in chronic risk assessment. **Key words: time-varying exposure, TKTD models, model evaluation**

**298 A method to predict and understand fish survival under dynamic chemical stress using standard ecotoxicity data** R. Ashauer, University of York / Environment; P. Thorbek, Syngenta / Environmental Safety; J. Warinton, Syngenta; J.R. Wheeler, Syngenta Ltd; S. Maund, Syngenta Crop Protection AG. We present a method to predict survival of fish under exposure to fluctuating concentrations and

repeated pulses of a chemical stressor. The method is based on toxicokinetic-toxicodynamic (TKTD) modelling using the General Unified Threshold model of Survival (GUTS) and calibrated using the raw data from standard fish acute toxicity tests. The model was validated by predicting fry survival in a fish early life stage test. Application of the model was demonstrated by using surface water exposure patterns (FOCUS-SW) as model input and predicting the survival of fish over 485 days. Exposure patterns were also multiplied with a factor of five and ten to achieve higher exposure concentrations for fish survival predictions. Further we quantified how far the exposure profiles were below the onset of mortality by finding the corresponding exposure multiplication factor for each scenario. We calculated organism recovery times as additional characteristic of toxicity as well as number of peaks, interval length between peaks and mean duration as additional characteristics of the exposure pattern. We also calculated which of the exposure patterns had the smallest and largest inherent potential toxicity, respectively. Sensitivity of the model to parameter changes depends on the exposure pattern and also differs between GUTS-IT (individual tolerance) and GUTS-SD (stochastic death). Possible uses of the additional information gained from modelling to inform risk assessment are discussed.

### **299 Role of ecological modelling in soil risk assessment: *Folsomia candida* model supports the definition of eco(toxico)logically relevant depths**

**F. Scherr**, Bayer CropScience AG; K. Hammel, Bayer CropScience AG / Environmental Safety; G. Ernst, Bayer CropScience / Ecotoxicology; V. Roeben, RWTH Aachen University Institute for Environmental Research / Environmental Safety; G. Goerlitz, Bayer CropScience AG / Environmental Safety. After application of plant protection product (PPP) to a field crop, soil organisms are exposed to a pronounced concentration gradient. Conversely in laboratory studies soil organisms are exposed to a homogeneous distribution of the test substance in the medium. In a typical risk assessment (RA) the effect concentrations observed in the laboratory test are then compared with a predicted environmental concentration (PEC) averaged over the so called eco(toxico)logically relevant soil depth ERD. In a recent opinion the EFSA PPR panel has proposed scenarios and models to calculate PEC in soil as a function of time and soil depth. While EFSA experts also suggest to base risk assessment on species dependent ERDs, e.g. 1 cm for collembolans, little is actually known on the vertical distribution of soil organisms and how these interact with a realistic concentration profile of a PPP. Consequently an imbalance exists and the refinement brought into the RA on the exposure side is not yet matched by refinements on the effect side, e.g. the derivation of the ERDs for soil inhabitants. In this study we employ an individual based, ecological model of *F. candida* to illustrate how biological variations of soil inhabitants and environmental parameters as well as the distribution of the pesticide in the soil profile can influence the effects on a population of soil organisms. Furthermore we propose how the ecological model may be used in adequate determination of ERDs for soil risk assessment. Results from a range of compounds on the reproduction performance of *F. candida* s under different environmental conditions were gathered. The results were evaluated to derive the appropriate ERDs by comparing the effects of realistic concentration profiles with homogeneous concentration profiles. The model performance was validated by simulating the findings of the TME study. Results show the importance of environmental variations for the vertical distribution of *F. candida*. Calculations with a range of compounds clearly show that realistic concentration profiles are relevant in order to define the appropriate ERD, which deviates from the 1 - 5 cm currently proposed. The results from the present study show that ecological modelling has the potential to support the definitions of risk assessment schemes as proposed by EFSA. The presented ecological model helps to derive an appropriate ERD for tier 1 in the area of soil risk assessment.

### **300 Soil organisms exposed to plant protection products – analysis of exposure and effects over different soil layers**

**A. Toschki**; U. Hommen, M. Klein, Fraunhofer IME; W. Koenig, S. Pieper, Federal

Environment Agency; C. Possberg, Institute for Environmental Research RWTH; J. Roembke, ECT Oecotoxikology; M. Ross-Nickoll, A. Schaeffer, Institute for Environmental Research RWTH; A. Scheffczyk, ECT Oecotoxikology; B. Schmidt, B. Scholz-Starke, Institute for Environmental Research RWTH; M. Hammers-Wirtz, gaia, Research Institute for Ecosystem Analysis and Assessment. It has been questioned in the last years whether the risk assessment of Plant Protection Products (PPP) in soil addresses properly the relationship between pesticide exposure and effects on soil organisms, especially with respect to the time distribution of the PPP over time in relation to the distribution of different species in the soil layer. To understand this relationship, a study with outdoor Terrestrial Model Ecosystems (TME) using intact soil cores was conducted, analysing the distribution of two pesticides and their effects on soil organisms (collembolans, oribatid mites, enchytraeids, earthworms) at the same time. Two insecticides were tested in 68 TME (Ø 467 mm, height 400 mm), in two concentrations each; untreated TME served as controls. Different soil layers (0-2.5 cm, 2.5-5 cm, 5-10 cm, 10-20 cm) were analysed separately for PPP concentrations and species abundance and community composition over 12 months. The results of chemical analyses for the different soil layers show how the pesticides move through the soil column. The abundance of soil organisms in the different soil layers over time will be given for the different treatments and concentrations. Univariate statistical analyses for single species as well as multivariate analyses were used to assess effects of the investigated PPP on the soil organism community. The results of this experiment allow a direct linkage of exposure and effects on soil organisms under realistic environmental conditions. The relevance of the results for refinement of exposure calculation and risk assessment for soil organisms will be discussed.

### **301 Development of archetype exposure scenarios for use in risk assessment in Asia**

**O. Price**, Unilever / Colworth Science Park; A. Franco, Unilever; O. Jolliet, University of Michigan / School of Public Health; P. van den Brink, AlterraWageningen UR / (a) Aquatic Ecology and Water Quality Management Group; (b) Alterra; C.M. Holmes, Waterborne Environmental Inc. Environmental risk assessment frameworks used in Europe for regulatory purposes are protective of ecosystems but lack environmental realism. Such assessments fail to address the ecological consequences of environmental contaminants on ecosystem structure and function via the simplistic use of default parameters and assessment factors. They fail to provide a mechanistic framework to assess ecological consequences at high hierarchical levels of ecological organisation (e.g. communities), which makes the usefulness of molecular approaches (e.g. as early warning systems) difficult to incorporate within risk-based decision making frameworks. There is a clear need to develop mechanistic exposure and ecological scenarios to enable risk assessors to ascertain spatial and temporal ecological consequences of single and multiple stressors (including chemical mixtures) on ecosystem health. Our research programme aims at developing archetype scenarios for use in risk assessment, including the development of models to: provide spatially resolved emission estimates, predict the fate and elimination of chemicals in wastewater treatment plants, predict chemicals transport and distribution using multiscale multimedia fate models, and link them with water quality models. The ultimate aims are to integrate these exposure models within a single platform to (1) assess spatially and temporally resolved environmental concentrations, (2) compare the fate of these stressors in multiple stressed environments, (3) develop archetype scenarios for use in prospective risk assessment and (4) integrate ecological effects models. This presentation gives an overview of the project, an update on each model and initial results on their integration within a single platform, currently applied to aid the identification of relevant exposure scenarios in Asia. We present the initial results of the integration of these models into one platform to assess source to receptor impacts for two test chemicals that are widely used in home and personal care products across Asia. We present the initial development of a suite of exposure scenarios that try to capture the variability of scenarios across Asia for use in a prospective risk assessment framework. The exposure

models developed to date still need further refinement and there is a need to update them to better address chemicals with a wide range of physicochemical properties (e.g. ionisables) to better assess site-specific bioavailability.

**302 Acute risk of organic compounds in European river basins** E. Malaj, Helmholtz Centre for Environmental Research/UFZ / Department of Effect-Directed Analysis; P.C. Von der Ohe, UFZ Helmholtz Centre for Environmental Research; M. Grote, EDF RD / National Hydraulics and Environment Laboratory; R. Schaefer, University Koblenz-Landau. Compliance with environmental quality standards for 33 priority substances is required from Water Framework Directive to achieve at least a good chemical status of European water bodies, while all other compounds which could affect freshwater ecosystems should be identified as river basin specific pollutants. Our aim was to estimate the ecological risk for freshwater ecosystems from organic compounds on the European level, as well as to identify the chemicals of highest concern. We evaluated a total of 5208 sites distributed over 29 countries and 38 major European river basins, for a total of 239 compounds. Potential risk was assessed for the green algae *Pseudokirchneriella subcapitata*, the invertebrate *Daphnia magna*, and the fish *Pimephales promelas* using experimental and predicted acute toxicity data. Phthalates, PAHs and BFRs were the chemical groups most frequently detected, while pesticides were the most toxic group for the standard test organisms. Furthermore, 56% of the most toxic compounds (e.g. dichlorovos or acenaphthene) were not listed as priority pollutants. We found that the ecological risk depends on the number of toxic compounds present in the monitored sites. Although potentially underestimated, organic compounds, especially pesticides, pose a threat to the ecological status for a high proportion of monitored sites in Europe, where species at 1/6 of these sites are likely to be acutely affected. To our knowledge, this analysis represents the most thorough study on the spatial distribution of the ecological risks from toxicants on the continental scale.

**303 Multidisciplinary research for the design of “green” plasticizers** V.V. Yargeau, McGill University / Chemical Engineering; M. Maric, R.L. Leask, R. Bernard, McGill University. Phthalates are high production volume chemicals, used to make poly vinyl chloride flexible and easy to process. The phthalates in many consumer products can leach out into the environment. Consequently, human exposure to phthalates is widespread. Animal studies have shown that phthalates act as endocrine disruptors at environmentally relevant concentrations; *in utero* exposures result in abnormal development of the male reproductive system. Epidemiological studies have reported an association between phthalate exposure during pregnancy and an elevated incidence of hypospadias in the offspring. Plasticizers are needed for the production of an immense range of consumer products. The challenge is to develop “green” plasticizers to replace phthalates in poly vinyl chloride. Green plasticizers should not be toxic and, ideally, should be biodegradable so that they do not accumulate in the environment. We have established a multidiscipline-based methodology for the design, synthesis and testing of alternative plasticizers. The chemical engineers on the multidisciplinary team have designed and synthesized potential plasticizers. They have tested many of these candidates for their mechanical and rheological properties and have elucidated the biodegradation pathway of many of these compounds. This has allowed us to identify lead compounds within series of compounds from different classes of plasticizers, such as dibenzoates, succinates and poly(caprolactone) diols. In parallel, the toxicologists have used cell-based assays to determine if our candidate plasticizers affect cells and future testing is currently on-going using animal studies. Members of the team are also investigating the ethical and legal implications of testing novel green chemicals prior to their release into the marketplace. This concerted research effort provides essential information with respect to the identification of safer new “green” replacement plasticizers and to knowledge translation strategies to make this information accessible to regulators, policy makers and the public.

**304 Comparative assessment of the toxicity of “Green” household products** J.E. Weinstein, The Citadel / Department of Biology; J.A. Miller; A.D. Gray, Department of Biology. Although it is generally assumed that green household products contain individual ingredients that are less toxic and/or more degradable than conventional formulations, little research on this topic has been conducted. This study examined the toxicity of several green household products—before and after degradation treatments—using either larvae of the estuarine daggerblade grass shrimp, *Palaemonetes pugio*, or adults of the freshwater cladoceran, *Daphnia magna*. Seven green product formulations (Seventh Generation Dishwashing Gel, Green Works All-Purpose Cleaner, Green Works Dish Detergent, Earth Options Insect Killer, Tom’s Mouthwash, Martha Stewart Bathroom Cleaner, and Seventh Generation Laundry Detergent) were compared to that of categorically equivalent products using 48-hour toxicity tests. Of the seven categories of household products tested, in only two cases were the green products less toxic than either of the conventional formulations. In three cases, there was no difference in the toxicity between the green products and the conventional formulations. And, in one case, the green product was more toxic than both tested conventional products. Following a biodegradation treatment involving activated sludge, none of the green products became less toxic. In five cases, the biodegradation treatment did not significantly alter product toxicity. And, in one case, the green product became more toxic after the biodegradation treatment. By contrast, the biodegradation treatment decreased toxicity of the conventional product formulations in eight cases, increased toxicity in five cases, and had no significant effect on toxicity in one case. Following a photodegradation treatment for a subset of these green products, in only one case did the green product become less toxic. Photodegradation did not significantly alter toxicity of the other three tested green products. This research demonstrates that green household product formulations are not necessarily less toxic and/or more degradable than their conventional counterparts. These results also suggest that the toxicity and degradability of end product formulations need to be considered in the overall framework for green product evaluation.

**305 Designing non-PBT chemicals by QSARINS** P. Gramatica, University of Insubria / QSAR Res. Unit Environ. Chem. Ecotox./Dep. Structural & Functional Biology; N. Chirico, University of Insubria / QSAR Research Unit/Department of Theoretical and Applied Sciences; E. Papa, QSAR Res. Unit Environ. Chem/Dep. Structural Functional Biology. The chemicals that are jointly Persistent, Bioaccumulative and Toxic (PBTs) are substances of very high concern (SVHC) and subject to an authorization step in the European REACH regulation, which includes plans for safer substitutions of recognized hazardous compounds. The limited availability of experimental data necessary for the hazard/risk assessment of chemicals and the expected high costs have increased the interest for alternative predictive *in silico* methods, such as Quantitative Structure–Activity (Property) Relationships (QSA(P)R). In the Green Chemistry approach the design of a safe molecule is the earliest phase of the long process of placement on the market new, safe substances. Molecular modeling approaches, such as QSAR, can be successfully applied in “safe Chemical Design”. A structurally-based approach was proposed by our research group for a holistic screening of potential PBTs in the environment. The PBT Index, obtained by combining Persistence, bioconcentration and toxicity data into a Principal Component Analysis, was defined to rank compounds according to their cumulative PBT behaviour. A simple, robust and externally predictive QSPR model, based on four molecular descriptors, was developed for the PBT Index. This model is a hazard screening tool for the early identification and prioritization of not yet known PBTs, only on the basis of their molecular structure, and is now implemented in the new software QSARINS. QSARINS was recently developed by our research group for the development and validation of MLR-OLS QSAR models. The PBT index model, implemented in QSARINS, can be applied to any chemical, without experimental data and not yet recognized as PBTs or even not yet synthesized, to verify the potential PBT behaviour, just starting from the chemical structure. The software

verifies, by the Insubria graph, if a new hypothesized compound is in or out the structural applicability domain of the PBT Index model, in order to be confident only on the interpolated predictions. Finally some case studies will be presented regarding how to screen the PBT potential behaviour of existing and new chemicals. This structure-based approach responds to two levels of action in relation to the management, according to REACH regulation, of chemicals of highest concern: a) the need for tools for identification and prioritization, and b) the design and production of safer alternatives, according to the green chemistry philosophy of "benign by design".

### **306 The Chemistry Scoring Index (CSI): A Hazard-Based Scoring and Ranking Tool for Chemicals and Products Used in the Oil and Gas Industry** T.A. Verslycke, T. Bowers, K. Reid, Gradient; S. Thakali, URS; J. Sanders, D. Tuck, Halliburton Energy Services, Inc..

A large portfolio of chemicals and products is needed to meet the wide range of performance requirements of the oil and gas industry. Included within this portfolio are chemicals that exhibit a variety of potential hazards to human health, safety, and the environment. These hazards have historically been managed by the industry through various measures that effectively limit exposure to these chemicals. Nevertheless, the oil and gas industry is under increased scrutiny from regulators, environmental groups, the public, and other stakeholders for its use of chemicals. In response, industry is increasingly incorporating "greener" products and practices, but is struggling to define and quantify what exactly constitutes "green" in the absence of a universally-accepted definition. As one of the world's largest providers of services to the oil and gas industry, Halliburton Energy Services, Inc. recently developed a tool for scoring and ranking hazards of products in its portfolio. The Chemistry Scoring Index (CSI) is ultimately intended to be a globally implementable tool that comprehensively scores and ranks hazards to human health, safety, and the environment for products used in oil and gas operations. CSI scores are assigned to products designed for the same use (e.g., surfactants, catalysts) on the basis of product composition, intrinsic hazard properties, and data availability for each product component. As such, products with a lower CSI score within a product use group are considered to have a lower intrinsic hazard compared to other products within the same use group. The CSI provides a powerful tool to evaluate relative product hazards; to review and assess product portfolios; and to aid in the formulation of safer products.

### **307 Environmentally compatible flame retardants: The ENFIRO approach** P. Leonards, VU University Institute for Environmental Studies / Chemistry & Biology; S. Brandsma, IVM institute for environmental studies / Faculteit der aard- en levenswetenschappen; H. Hendriks, Utrecht University; N. Jonkers, IVAM; J. Parsons, University of Amsterdam / IBED; S.L. Waaijers, University of Amsterdam/IBED Institute; R. Westerink, Utrecht University; C. de Wit, Stockholm University; J. de Boer, VU University, Institute for Environmental Studies. Some brominated flame retardants (BFRs) have unintended negative effects on the environment and human health. Some of them show a strong bioaccumulation in aquatic and terrestrial food chains, some are very persistent, and some show serious toxicological effects such as endocrine disruption. During the last decade, an increasing number of reports have presented evidence of these negative effects caused by some BFRs. A number of BFRs (in particular polybrominated diphenyl ethers (PBDE's), hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A)) can be found in increasing concentrations in the human food chain, human tissues and breast milk. Less toxic alternatives appear to be available already but comprehensive information on their possible toxicological effects are lacking. The European Commission-funded project ENFIRO investigates the substitution options for some BFRs and compares the hazard, exposure, fire performance and application of the alternatives versus the BFRs. In addition, a risk assessment and comparative life cycle assessment was carried out. The current paper shows the main outcomes of ENFIRO and shows the ENFIRO approach "how to substitute chemicals which are environmentally compatible". Information on the hazards, exposure,

leaching behavior, emission to air, and life cycle assessment of the alternative halogen free flame retardants (HFFRs) compared to the BFRs are discussed. ENFIRO showed that viable alternative flame retardants are available. Some HFFRs show less risk for the environment and human health, and show similar fire performance and technical application capabilities as BFRs. The lower risk is mainly due to the lower hazards of the HFFRs, and probably not due to a lower exposure. In conclusion, for the substitution of chemicals a complete substitution cycle is needed: technical/application performances, hazard, exposure, and impact assessments. Such an assessment can only be performed with a group of experts from different disciplines (material experts, toxicologist, chemist, life-cycle experts etc).

### **308 What is the industry doing to develop safe flame retardants based on phosphorus, nitrogen and inorganic chemistries?** A. Beard, Clariant GmbH / BU Additives - Flame Retardants; U. Wietschorke, WTC Consulting; K. Priestersbach, .

The environmental behaviour of flame retardants has been studied since the 1990ies. Mainly brominated flame retardants were found in many environmental compartments up to human milk, and some individual substance were found to have toxic properties. Therefore, alternatives have been demanded by authorities, NGOs and equipment manufacturers. The producers of alternatives have formed an industry association for phosphorus, inorganic and nitrogen flame retardants (pinfa) in order to better inform flame retardant users and the public about technical options, the properties of their products and develop flame retardants with an improved health and environmental profile. pinfa has run a pilot project with GreenScreen, an assessment tool for users of chemicals to determine the environmental and health profile of halogen free flame retardants. In addition, the pinfa participated in the EU-funded collaborative research project ENFIRO ([www.enfiro.eu](http://www.enfiro.eu) , EU research project FP7: 226563) which aimed at evaluating alternatives to the major established brominated flame retardants. In order to make the evaluation fully comprehensive, it was decided compare also material and fire performance as well as attempt a life cycle assessment of a reference product containing halogen free versus brominated flame retardants. About a dozen halogen free flame retardants were studied representing a large variety of applications, from engineering plastics, printed circuit boards, encapsulants to textile and intumescent coatings. A large group of the studied flame retardants were found to have a good environmental and health profile. For several years now, the US-EPA has been carrying out a series of projects related to the environmental assessment of alternative flame retardants, the "design for environment" projects on flame retardants for printed wiring boards and alternatives to decabromo diphenylethers and hexabromocyclododecane (HBCD) which are all expected to be finalized in 2013. pinfa members have actively engaged in the process and industry's view on these hazard focussed assessments will be presented.

### **309 SimpleTreat evolution: applicability to ionisable chemicals** A. Franco, T. Gouin, Unilever / Safety and Environmental Assurance

Centre; O. Price, Unilever / Colworth Science Park; J. Struijs; D. van de Meent, RIVM / Institute of Wetland and Water Research. SimpleTreat is the sewage treatment plant (STP) model implemented in the EU framework for the environmental risk assessment of chemicals under different regulations (e.g. REACH regulation, Biocidal Products Directive). Although the previous version (SimpleTreat 3.1) was adapted to describe ionisation, the fate of organic ions was limited to the unbound aqueous phase, which seriously restricted the applicability domain. A recent model update aimed at enlarging (and defining) the applicability domain with respect to ionisable organics is presented here. SimpleTreat 3.2 includes improved estimates of the sludge-water partition coefficient normalized to organic carbon ( $K_{oc}$ ) of monovalent acids and bases from the octanol-water partition coefficient ( $K_{ow}$ ), the dissociation constant ( $pK$ ) and the pH. An evaluation study was carried out with ten test chemicals, specifically selected to challenge the applicability domain and to cover a wide range of functionalities and physical-chemical properties. The comparison of modelling results with monitoring data for activated sludge STPs collected from the literature

lend confidence to the use of SimpleTreat 3.2 with respect to monovalent ionics and neutral organics. The accuracy of the new sludge  $K_{oc}$  regressions is acceptable for monovalent acid, but is lower for bases, for which measured sludge  $K_{oc}$  is highly recommended, until better estimation methods become available. It was also shown that the approach remains unsatisfactory for ionic surfactants and organic ligands, for which measured  $K_{oc}$  is necessary. This may limit the applicability of SimpleTreat using a basic input dataset, as available from regulatory registration data.

**310 Ionogenic organic compound sorption models: Advancing qualitative concepts for quantitative descriptions** A. Mackay, University of Connecticut / Environmental Engineering Program; D. Vasudevan, Bowdoin College / Department of Chemistry. Suitable models are not yet available to predict the sorption of ionogenic organic compounds to soils because they do not account for contributions of cation exchange, surface complexation and cation bridging sorption mechanisms. Two elements must be addressed to advance qualitative understanding of these mechanisms to quantitative models: (i) an ability to quantify the abundance or availability of appropriate receptor sites, and (ii) knowledge of the influence of ionogenic organic compound structure on such interactions. A promising approach that addresses both of these challenges is the use of mechanism-specific probe compounds with structure based sorption models. Sorption of mechanism-specific probes (e.g. for cation exchange and for surface complexation) provides a implicit measure of the site availability and baseline sorption. Structure-based sorption models for cation exchange and surface complexation, respectively, would enable probe sorption measures to be extrapolated to other compounds that can participate in those sorption mechanisms. Existing sorption studies highlight consistent trends with structure in the sorption of organic compounds by cation exchange or surface complexation; however, more extensive observations with consistent experimental conditions (pH, ionic strength, etc) are needed to develop mathematical tools to describe structure trends in ionogenic organic compound sorption.

**311 Improving risk assessment modeling of the soil sorption affinity of cationic organic compounds** S.T. droge, Utrecht University / IRAS; K. Goss, Eawag / AUC. Using a consistent experimental data set for 70 bases, we examined how the soil properties and molecular structure of organic cations affects the sorption affinity to natural soil. Current risk assessment models are not suitable to predict the sorption affinity of organic cations because they are not sufficiently focused on the ion-exchange sorption processes that occur in soil. Under fixed medium conditions (pH, ionic composition), we used a HPLC method to test whether specific sorption coefficients to natural organic matter (NOM, micronized pahoekoe peat) and clay (illite) can be summed to predict sorption coefficients to soil (eurosoils). From the two Eurosoils, we derived a sorption model based on specific sorption coefficients to NOM and clay. The observed differences in ion-exchange affinities for organic cations are not explained by calculated differences in octanol-water distribution coefficients ( $K_{ow}$ ), as recently proposed in risk assessment procedures. We show that in "NOM rich / clay poor" soil the sorption is dominated by NOM, whereas in the "NOM poor / clay rich" soil, sorption to clay needs to be accounted for besides NOM, otherwise sorption is strongly underpredicted up to 3 orders of magnitude.

**312 Do we need to include protein interactions in risk assessment models? The case of perfluorinated alkyl acids.** C.A. Ng, ETH Zurich / Institute for Chemical and Bioengineering; K. Hungerbuehler, ETH Zurich / Institute for Chemical and Bioengineering. Environmental risk assessment as applied in the context of REACH was largely developed with neutral organic chemicals in mind, and is well-suited to assess risk from traditional legacy chemicals (e.g. PCBs). However, new classes of chemicals with very different properties have since gained a wider share of the market. Some, once regarded solely within specialized contexts (e.g. pharmaceuticals), are now recognized as important environmental contaminants. Ionic and ionogenic chemicals are particularly ill-suited to the 'neutral-centric' risk assessment

paradigm. Predictive hazard measures that depend on the  $K_{ow}$  to describe bioaccumulation potential or on the narcotic LC<sub>50</sub> as a proxy for toxic effect can miss substantial activity by these chemicals.<sup>50</sup> Perfluorinated alkyl acids (PFAAs) are some of the best studied yet still quite poorly understood examples. Although PFOS is now included in the Stockholm Convention, the mechanisms behind the bioaccumulation of perfluorinated chemicals still require elucidation. This represents a worrisome lag in our understanding given that intensive industrial production of a wide variety of fluorine chemistries continues to increase. Here, we explore what is known about the properties of PFAAs and how they relate to observed bioaccumulation patterns in fish. We focus in particular on the acid dissociation constant, pK<sub>a</sub>, and the (measured) protein-water distribution coefficients for bovine serum albumin ( $K_{BSA-W}$ ). We then propose a new albumin-binding model in rainbow trout, *Oncorhynchus mykiss*, and compare its performance in predicting BCF for C6-C12 PFAAs to a model based on equilibrium partitioning of only the neutral fraction. Our model evaluations indicate that the neutral fraction alone cannot account for PFAA bioavailability; some facilitation exists for the ions as well. More comprehensive studies are needed to measure  $K_{ow}$  for PFAAs along a wide range of chain lengths. More also needs to be known about the nature and distribution of PFAA-binding proteins in fish, particularly if we wish to investigate observed tissue distributions, which show marked preferences for some tissues (e.g. liver, kidneys) over others (adipose, muscle) and cannot be fully explained using our current knowledge. Our specific binding model holds promise for the prediction of PFAA bioconcentration and as a way to investigate gaps in our knowledge about these important chemicals.

**313 Modelling the accumulation and internal tissue distribution of PCBs and their hydroxylated metabolites in polar bears (Ursus maritimus)** J. Armitage, University of Toronto Scarborough / Department of Physical and Environmental Sciences; J.A. Arnot, ARC Arnot Research Consulting / Department of Physical Environmental Science; F. Wania, University of Toronto at Scarborough / Department of Physical & Environmental Sciences. It is well established that neutral hydrophobic organic contaminants such as polychlorinated biphenyls (PCBs) exhibit the highest wet weight concentrations in adipose tissue followed by other tissues with relatively high total lipid content. Recent biomonitoring studies have also shown that hydroxylated PCB metabolites (i.e., OH-PCBs) exhibit a different internal tissue distribution with higher wet weight concentrations in blood and liver compared to adipose. While interactions with plasma proteins may be an important factor explaining these observations, we suggest that another important factor to consider is that OH-PCBs are ionogenic (i.e., dissociate *in vivo* and hence are present in a neutral and charged form). The physical-chemical properties of hexachlorobenzene (HCB, neutral organic) and pentachlorophenol (PCP, weak acid, pK<sub>a</sub> ~ 4.7) provide a useful analogy when considering PCBs and OH-PCBs. Here we apply a recently developed Physiologically-Based Pharmacokinetic model (PBPK) for ionogenic organic chemicals (IOCs) to simultaneously model the accumulation and internal tissue distribution of a set of PCBs and their OH- metabolites. The model distinguishes between neutral (storage) lipids, zwitterionic (neutral) phospholipids, plasma proteins and other non-lipid organic matter (NLOM). As there is a relationship between degree of chlorination and estimated pK<sub>a</sub> (as # of chlorines ?, pK<sub>a</sub> ?, degree of ionization at pH 7.4 ?), it is important to recognize that all OH-PCBs do not distribute internally in the same manner. Besides whole blood, OH-PCBs exhibiting a high degree of ionization (i.e., predominantly charged *in vivo*) tend to favour tissues with elevated phospholipid content such as the liver whereas OH-PCBs exhibiting a low degree of ionization (i.e., predominantly neutral) behave like the parent compound (and hence exhibit the highest wet weight concentrations in adipose tissue). Assumptions regarding biotransformation (Phase I and II processing) are also influential. While the current model application is focused on PCBs and OH-PCBs, the findings have implications for the distribution of the hydroxylated metabolites of other POPs such as OH-PBDEs (i.e., biotransformation products of polybrominated diphenyl ethers).

### 314 Recovery of populations and communities after pesticide contamination

**M. Kattwinkel**, Helmholtz Centre for Environmental Research UFZ / System Analysis, Integrated Assessment and Modelling; **M. Liess**, UFZ Center for Environmental Research / Department of System Ecotoxicology; **J. Roembke**, ECT Oekotoxikologie GmbH. Assessing population recovery from toxicant effects plays a crucial role for the protection of biodiversity and ecosystems. However, little is known on the recovery potential of different taxa and the information is scattered in the scientific literature. To enable an informed assessment and to aid the revision of the Guidance Documents we address the following questions: (1) What scientific information is available on population and community recovery after contamination? (2) What are the main drivers of recovery that can be used for field level estimations based on laboratory or semi-field experiments? (3) What is missing in the current procedures for an effective risk assessment? We evaluated the recovery potential of populations and communities after pesticide contamination by an expanded literature review. Information dealing mainly with experimental studies were retrieved for the following taxa groups: mammals, birds, soil invertebrates, non-target arthropods (NTAs), bees, soil microorganisms, reptiles, terrestrial plants, fish, aquatic invertebrates, amphibians, aquatic microorganisms, algae, and aquatic plants. Although the investigated literature was very diverse and the possibility for direct comparisons was limited, some general conclusions are drawn: Based on the results for aquatic invertebrates, most taxa recover within five generation times. The absolute time of internal recovery depends strongly on the reproduction capacity. Migration from uncontaminated areas is a main driver for external recovery. In many studies including such re-colonization sources recovery occurred within one generation. If recovery from external sources is assumed, it has to be ensured that the magnitude of re-colonization is a realistic estimation in landscapes heavily influenced by agriculture. Environmental stress generally acts in addition or synergistically to pesticide stress and hence recovery has to be evaluated within the ecological context. This is especially true for endangered species that are under particular stress. Indirect effects based on competition and predation can play an important role on effect magnitude and recovery time.

### 315 Long-term incubation of *Nereis virens* in metal-spiked sediment: behavioural, biochemical, cellular and genotoxic responses.

**G. Watson**, Institute of Marine Sciences; **J. Pini**, University of Portsmouth. *Nereis virens* is an ecologically and commercially important temperate polychaete of intertidal soft sediment and an ideal species to investigate long term effects of metals. Using a spiked sediment approach juveniles are being incubated for 9 months in environmentally relevant concentrations of sediment (5 kg per box) spiked with copper, zinc and copper and zinc together at low (copper: 70 mg kg<sup>-1</sup>, zinc: 200 mg kg<sup>-1</sup>; medium: copper 120 mg kg<sup>-1</sup>, zinc: 270 mg kg<sup>-1</sup> and high: copper 575 mg kg<sup>-1</sup>, zinc: 1160 mg kg<sup>-1</sup>) concentrations. These concentrations are based on an extensive sampling regime of sediment, pore water and worms from seven UK sites with different levels of copper and zinc contamination. Worms are being fed during the experiment, but maintained under ambient conditions (temperature and photoperiod) in a flow-through seawater system. Throughout the experimental period feeding rates and out-of-burrow activity levels will be recorded using video with boxes sacrificially sampled at 3, 6 and 9 months. Using BCR sequential extraction, labile (bioavailable) amounts of metals in the sediment will be assessed in addition to pore water and tissue concentrations. The effects of these metals will be measured using a suite of biochemical, cellular, genotoxic and molecular endpoints. Scope for growth and metabolic changes (e.g. lipid, protein and carbohydrates levels) will assess the trade-offs between growth and responses to metal toxicity. Metallothionein concentrations, acetylcholinesterase activity, lysosomal membrane stability, micronucleus and comet assays and expression levels of key genes will all be used to build a 'global' picture of the effects of these metals on this species. It is expected that this study will show for the first time the relationship between biomarkers at different levels of

organisation and enable their relative sensitivity to be ascertained from a long term study. Ultimately this work will show how these individual responses directly link to the ecology and population of benthic polychaetes.

### 316 Statistical Analysis of Fish Early Lifestage Experiments

**J.W. Green**, DuPont / Applied Statistics Group. OECD TG 210 for fish early lifestage experiments has been updated recently. As part of that update, many GLP studies of freshwater and marine species have been re-analyzed under proposed revised guidelines and extensive computer modeling of both NOEC and ECx approaches have been done based on those studies. Responses to be analyzed under TG210 are size (length and weight), egg hatching success, larval mortality and abnormalities, first and last day of hatch, and first and last day of swim-up. It is the purpose of this presentation to present the results of these analyses and computer modeling studies, including specific hypothesis tests and regression models. It will be shown that regression provides good estimates of EC20 and EC30 in 75-90% of studies for size, hatch, and larval mortality, dependent on study conditions, but fails in 10-25% of studies for each of those responses. EC10 estimates are less often reliable, especially for hatching and mortality. NOECs are shown to be protective in most studies for all responses except egg hatch and larval mortality, where the power to detect a 20% effect is less than 80% for the most variable species or data. Guidance will be given on statistical tests for each response and models for ECx estimation, as well as criteria by which to evaluate the quality of ECx estimates and NOEC determinations.

### 317 Can we use kinetic data to estimate chronic effect levels for exposure situations different from laboratory EDC testing?

**M. Teigeler**, Fraunhofer IME / Ecotoxicology Dept.; **C. Schaefer**, FraunhoferInstitut / Department of Ecotoxicology. In our study, two fish life cycle tests were performed using an anti-estrogen, considering both flow-through and static peak exposure design. Effect threshold concentrations on population relevant endpoints of both test designs were compared. In a further step, we tried to derive a linkage between exposure and resulting effects using a kinetic approach. The calculation was based on data on lipophilicity and time weighted average concentrations taken from the static Fish Full Life Cycle (FFLC) test. The aim was to validate the relation of the exposure linked effect levels via kinetics. Two life cycle tests were performed using zebrafish (*Danio rerio*). As a test substance the selective estrogen receptor modulator Fulvestrant was chosen. In a static test three life stages of zebrafish (fertilized eggs, juveniles, spawning adults) were exposed simultaneously to a single substance peak. A second test was performed under continuous flow through design. For estimation of effect levels related to decreasing test substance concentrations we used a kinetic approach for which different assumptions were made. The fertilisation success was found to be the most sensitive population relevant endpoint in the static FFLC. Fertility was recorded daily, thus, data was available on effect duration and the timepoint of highest effect intensity. A daily uptake as well as the daily depuration was calculated considering the exponentially decreasing concentrations. The geometric mean of the substance concentrations in fish of each day in relation to the previous day were determined. The depuration constant k<sub>2</sub> was calculated using data on lipophilicity of the substance. The uptake constant k<sub>1</sub> was determined by iterative fitting of the concentration curve against the observed effect levels taken from the static FFLC and from a pre-test. Using the kinetic approach, a LOEC was estimated to be around 16 µg/L, the effect threshold was determined to be approx. 11 µg/L. Based on these findings, the concentration range for the flow through study could be outlined (40, 13, 4.0 and 1.3 µg/L). The kinetic approach can be considered as a useful tool to assess effect levels after short term exposure based e.g. time weighted average concentrations. A broad range of applications is conceivable. Beside the assessment of short term exposure with plant protection products, also the assessment of effects on fish passing short term peaks of substance e.g. from sewage treatment plant effluents, is possible.

**318 Investigating population relevance of the effects observed in standard rat reproduction studies** S. Mastitsky, BASF / Crop Protection - Ecotoxicology; M. Ebeling, Environmental Safety; N. Kreling, BASF SE / Crop Protection - Ecotoxicology; P. Thorbek, Syngenta / Environmental Safety; W. Schmitt, Bayer CropScience AG / Environmental Modelling. Wild mammal reproductive risk assessment is typically based on results from rat reproduction studies conducted for toxicological risk assessment. Since such toxicological studies are primarily conducted with the purpose of ensuring a high level of individual protection for humans, the NOAEL from these studies is sometimes based on effects on endpoints of questionable relevance for wild mammals, where the protection goal is the sustainability of populations whereas the fate of single individuals plays a minor role. In regulatory practice, it is particularly challenging to assess a threshold that should be considered as ecotoxicologically relevant for wild mammal reproductive risk assessment when it comes to effects on body weight of dams and/or offspring. In order to contribute to the development of scientifically sound decision criteria for such cases, we report an evaluation of regulatory guideline rat reproduction studies on a number of active substances of various classes and modes of action. Statistical analysis was conducted to identify systematic relationships between the body weight effects and reproductive performance parameters deemed of relevance for population sustainability. In addition to a better understanding of the population relevance of sublethal effects in general, the results of our study could also be helpful when developing and applying population models, such as those investigated during the MODELINK workshop.

**319 Using individual based modeling to quantify the importance of sub-lethal effects on population level - a case study for *Nitocra spinipes*** T. Bui, Institute for Environmental Research Biology / Chair of Environmental Biology and Chemistry; E. Lundstrom, Stockholm University / Dept. of Applied Environmental Science; M. Breitholtz, Department of applied environmental science; A. Schaeffer, RWTH Aachen University / Chair of Environmental Biology and Chemodynamics; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research. In order to protect populations of organisms, identifying important endpoints for ecotoxicological risk assessment is necessary. Therefore, the relevance of sub-lethal effects on population level should be assessed. Current ecotoxicological tests usually measure on individual level and consider each endpoint separately. However, statistically significant effects observed on individuals or a group of individuals are not necessarily relevant on population level. This aspect is essential because the goal of ecotoxicological risk assessment is to protect populations and communities. Chemicals can also have combined effects, resulting in much difficulty to detect effects at population level in the laboratory or in the field. In order to assess these problems and study the relevance of sub-lethal endpoints, individual based population modeling was considered to extrapolate effects detected on individuals to population level. We used an individual based model for *Nitocra spinipes*, a harpacticoid copepod, to investigate several adverse effects in combination with food dependent life cycle parameters and intra-specific competition on food. Model simulations showed the inhibition of the nauplius development to have a stronger adverse effect on the survival of a population than an increased overall mortality, requiring higher food levels for the population to maintain the same chance of survival. A detailed analysis of the population dynamics and population structure revealed the reason for this to be (1) higher amplitudes within the population fluctuations and (2) a shift towards and higher percentage of nauplii in the population. Hence, this study demonstrated the importance and relevance of sub-lethal endpoints for the survival of a population based on modeling results. The potential of those endpoints on population level should therefore be considered in risk assessment.

**320 Regionalised Global Assessment of Health Impacts of Non-Toxic Air Pollutants based on TM5-FASST** P. Preiss, econcept AG / Institute for Energy Economics and the Rational Use of Energy (IER); R. Van Dingenen, F. Dentener, Joint Research Centre - Ispra / Institute

for Environment and Sustainability, Climate Change Unite. This paper presents new globally differentiated intake fractions (iF) and characterization factors (CF) based on TM5-FASST source-receptor-matrices (SRM). The assessment of impact to human health includes effects of a) emission of primary particulate matter (PPM) and b) emission of the precursor pollutants NMVOC, NO<sub>x</sub>, NH<sub>3</sub> and SO<sub>2</sub> regarding the reactants ozone (i.e. so called ground level or photochemical ozone) and secondary inorganic aerosols (SIA). Human health damages need to be assessed in a spatially resolved context in order to increase the accuracy of impact assessment results [1]. Research on LCIA for transboundary pollutants have been reviewed for example by [2], suggesting that spatially differentiated fate modelling can be crucial. Based on results of chemical transport models (CTM) such as described in [3], [4], [5] impacts to human health have been expressed per unit of emission of the above mentioned pollutants already in the past. The present work aims to fill the gap of consistent global modelling by developing an approach to derive globally applicable and spatially explicit values. The advantages and disadvantages of global modelling based on a global CTM are explained by providing a comparison between model results with a different degree of spatial resolution and sophistication. A novel approach using the TM5-FASST model has been applied to derive globally applicable and spatially explicit impacts of the emission of the transboundary pollutants (PPM, SO<sub>2</sub>, NO<sub>x</sub>, NH<sub>3</sub>, NMVOC). In this context, the TM5-FASST model allowed to assess the whole globe as source and receptor area. This is a clear advantage of global models compared to only regional (continental) models. Moreover, sensitivity assessment regarding assumptions of the effects of different qualities of particulate matter and region specific sensitivity of receptors are possible. For the application within LCA it is necessary to adjust the results to archetypal conditions. It is desirable to rerun the global CTM models in order to better reflect the demands of LCIA. These are for example, a higher resolution of receptor areas, smaller marginal change of emissions, industry and sector specific model runs, etc..

**321 Fate process modelling in LCI: improving inventory quality or double counting?** T.J. Dijkman, Technical University of Denmark / DTU Management Engineering; M. Birkved, M.Z. Hauschild, Technical University of Denmark. In order to reliably determine environmental impacts, LCIA models require good inventories of flows to and from the technosphere as well as accurate characterization factors. Calculations of characterization factors require, amongst others, an understanding of the fate of chemicals that are emitted to the environment. However, fate processes not only occur in the ecosphere. Before emission, i.e. in the technosphere, chemicals can undergo a variety of fate processes. These processes determine a chemical's pathway before it crosses the border between technosphere and ecosphere, and should therefore be considered in the LCI. The aim of this presentation is to demonstrate the relevance of fate modelling in LCI and the necessity of choosing appropriate technosphere boundaries, using pesticide emissions as an example. Pesticide emissions from agricultural land were modelled using 2 approaches, after which freshwater ecotoxicity and human toxicity impacts were calculated using USEtox characterization factors. The first approach uses PestLCI, a LCI model to calculate pesticide emissions to air, surface water and groundwater applicable under European circumstances. PestLCI defines the technosphere as the field up to 1 meter of depth and the air column above it, up to 100 meters above the soil. Only when a pesticide crosses these system boundaries it is considered an emission. Within this technosphere a number of processes is modelled. Some of these processes (for example volatilization and runoff) lead to emissions to the environment. Other processes (such as degradation) result in removal of the pesticide from the system. Current LCI practice, the second approach, usually ignores these processes. It is assumed that all pesticide is emitted to soil. The pesticide hence enters the ecosphere upon application. Comparing the toxicity impacts found using the PestLCI approach shows a clear difference with the currently prevalent approach, with the current approach being more conservative. The observed variation can be explained by the difference in system boundaries on the one hand and



the difference in emission routes and their characterization factors on the other hand. Concluding, fate modelling is a useful tool to improve the inventory quality, thus reducing uncertainty. Moreover, in the case of PestLCI, emissions can be spatially differentiated. Combined with spatially resolved characterization factors, LCIA can reach a higher degree of accuracy.

### **322 Spatial differentiation for toxic emissions in LCA: How well the (nested) USEtox model mimic a truly spatially differentiated model?**

**A. Kounina**, Quantis EPFL; M. Margni, Ecole Polytechnique de Montreal / Department of Mathematical and Industrial Engineering; S. Shaked, University of Michigan / Applied Physics and School of Public Health; C. Bulle, CIRAIG Polytechnique Montreal / Chemical Engineering; O. Jolliet, University of Michigan / School of Public Health. Multimedia and multi-pathways exposure models have been recognized being suited characterization models to generate characterization factors for ecotoxicity and human toxicity impact categories in LCA. Given that human and eco-toxicity impacts are highly influenced by the emission location, recent multimedia and multi-pathway model developed spatially differentiated capabilities to reduce model uncertainty and improve accuracy and therefore confidence in LCA results. This work aims at selecting an appropriate model and spatial resolution to evaluate freshwater ecotoxicity and human toxicity impact through freshwater pathways. We evaluated *inter-continental variation* by comparing a developed nested USEtox model based on the sub-continental versus a fully connected IMPACT World model. Variation of fate and intake fraction represents up to 2 orders of magnitude among the 17 zones assessed with IMPACT World and USEtox model. Difference in continent specific landscape and population parameters is a key step to improve model relevance. Differences between the two models are explained by choices in model algorithms, e.g., modeling of freshwater outflow and volatilization algorithm. The model architecture of the surrounding of the continental box generally does not have a significant influence on results with the exception of volatile and persistent pollutant both in air and water. *Intra-continental variation* was analyzed on a finer resolution with two consistent versions of the same model: IMPACT Europe single zone model adopting the typical multimedia approach without spatial distinction, and IMPACT Europe spatial model accounting for spatial differentiation through 135 land zones (watersheds). Results showed that a-spatial models might overestimate the chemical fate and characterization factors for freshwater ecotoxicity up to a factor 5 when compared to a spatially differentiated model for unknown emission location. When the emission location is known, a spatially differentiated model can improve the model accuracy about 2-3 orders of magnitude in the case of IMPACT Europe model, because of its ability to accurately predict the freshwater residence time to the sea depending on the emission location. We then demonstrated that a developed archetype model based on water residence time to the sea can mimic aquatic fate and intake fractions results with up to a factor 5 of difference with results of the spatial model, whilst minimizing model sophistication.

**323 Development of characterization factors for metals in 7 EU water archetypes** **Y. Dong**, Section of Quantitative Sustainability Assessment, DTU Management Engineering; M.Z. Hauschild, Technical University of Denmark / Department of Management Engineering. Toxicity potential of most metals in the freshwater are estimated in current life cycle impact assessment (LCIA) models without taking the spatial differentiated speciation behavior of the metals into consideration. Using a novel approach developed by Gandhi and Diamond (Gandhi et al. 2010), new characterization factors (CF) representing freshwater ecotoxicity potentials are calculated for metals (e.g. Cr, Be and Ba) in 7 EU water types, taking into account the influence of speciation behavior on metal bioavailability and metal fate in seven different EU water types. USEtox is used to model the fate of the metals, WHAM 7.0 is used to model the metal speciation, Kd values and bioavailability, while the Free Ion Activity Model (FIAM) is used to model the ecotoxicity effect. The resulting archetype-specific CFs show up to ~4 orders of magnitude difference for Cr and Be. This indicates

that the toxicity potential of these two metals is strongly dependent on differences in water chemistry. In comparison, Ba shows a constant bioavailability ratio and toxicity effect across the modeled water chemistries. Thus CFs are strongly correlated with fate, which results in a more narrow range of CFs. The differences in water chemistry not only changes the absolute values of the CFs for the individual metals, but also their ranking in terms of freshwater ecotoxicity potential, illustrating the relevance of taking water chemistry into account when modeling metal ecotoxicity potential in LCIA. In order to support LCIA in the frequent situation where no information is available of the specific water type into which the metal emission occurs, site generic average factors are also calculated and different approaches to averaging across archetypes are investigated and discussed. Reference: Gandhi, N., M. L. Diamond, D. van de Meent, M. A. J. Huijbregts, Wjgm Peijnenburg, and J. Guinee. 2010. New Method for Calculating Comparative Toxicity Potential of Cationic Metals in Freshwater: Application to Copper, Nickel, and Zinc. *Environmental Science & Technology* 44 (13):5195-5201. Key words: metal, characterization factor, life cycle impact assessment (LCIA), ecotoxicity

**324 Considering temporal variability for the characterization of metals aquatic ecotoxicity impacts in LCA** **F. Lebaillly**, CIRAIG, Ecole Polytechnique de Montreal; A. Levasseur, CIRAIG École Polytechnique de Montréal / Chemical Engineering; R. Samson, Ecole Polytechnique de Montreal; L. Deschenes, Ecole Polytechnique de Montreal / Genie Chimique. LCA is a recognized tool to assess potential environmental impacts of products or services, and researchers are undergoing efforts to reduce the uncertainty of outcomes. Temporal variability is a major source of uncertainty associated to both inventory and impact characterization phases. The ecotoxicity impact category is particularly sensitive to temporal aspects because characterization deals with contaminants behaviour in the environment that depends on dynamic processes. Moreover, impacts are integrated over an infinite time horizon so that long lived pollutants such as metals have very high characterization factors (CFs) compared to others. Time-related issues regarding metals toxicity are often raised and CFs are sometimes qualified as “interim” factors. In this paper, dynamic CFs are developed in order to assess aquatic ecotoxicity impacts of metals and point out relevant temporal information. The method following a dynamic LCA approach provides the distribution of impacts over time and is based on inventory temporal disaggregation and time-dependent characterization. CFs have been generated introducing time in fate calculation so that metal mass in freshwater is known for each time step. All data are provided by the USEtox model. Both bioavailability and metal forms of emission can be integrated in the calculations. Two types of characterization factors are obtained. While instantaneous CFs provide the impact of metals at the time “t” following a pulse emission, cumulative CFs provide the total time-integrated impact since the pulse emission occurred. Results demonstrate that CFs strongly depends on the metal itself and on the emission compartment as these two variables determine maximal mass and also persistence time in freshwater compartment. Cumulative CFs converge to the traditional steady state values of USEtox for each metal but time horizon seems to be an important issue as metals can require thousand of years to produce their total impact. The use of traditional values might be questioned as they correspond to extended time perspectives that are not necessarily relevant in the context of LCA and differ from one impact category to another. Relative impact of metals compared to organic substances should also decrease as it was expected. Dynamic characterization, coupled to speciation and temporal inventory, improves interpretation and has an important influence on decision-holder conclusions.

**325 How policy makers use TMF** **N. Eckbo**; B. Nordboe, Norwegian Climate and Pollution Agency. The aim of the presentation is to show how policy makers use *trophic magnification factors* (TMFs) in risk assessment. In addition, the talk will address uncertainties when using TMF in monitoring programs, and our needs in future development of the TMF. For policy makers, TMFs is a useful tool for assessing the biomagnification potential of different environmental

pollutants. The Climate and Pollution Agency (Klif) is responsible for monitoring contaminants in the Norwegian environment. The primary goal is to get an overview of the state of the environment, in relation to Norwegian environmental goals and international obligations. Recently, biomagnification factors were included in some of the monitoring programs as a part of the risk assessment of contaminants, e.g. the monitoring of Norwegian lakes and the Norwegian coast line. Three new monitoring programs will also include TMFs. The monitoring results are used in national and international environmental management negotiations. By comparing the TMFs of well-known chemicals with TMFs of new contaminants with unknown chemical properties, the TMF may be a tool to identify B-properties of emerging contaminants. Our monitoring also results in time trends of TMFs, hence providing knowledge on how TMFs may change over time.

**326 Eutrophication may lead to lower mercury concentrations in aquatic food webs of East African lakes** A. Poste, Norwegian Institute for Water Research; D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division; S. Guildford, R. Hecky, University of Minnesota-Duluth. Much of what we know about mercury in aquatic food webs is based on extensive study of temperate systems with low to intermediate primary productivity, and the behaviour of this contaminant in eutrophic tropical systems lacks the same level of understanding. The primary objectives of this study were twofold: 1) to characterize accumulation and biomagnification of Hg at 8 East African study sites; and 2) to explore Hg dynamics in hypereutrophic lakes where phytoplankton productivity is high year-round. The study sites ranged in size from crater lakes to East Africa's great lakes (e.g. Lakes Albert and Victoria) and ranged in productivity from mesotrophic to hypereutrophic. Comprehensive water, plankton and fish samples were collected between 2007-2009 and were analyzed for both THg as well as stable carbon and nitrogen isotopic ratios. We found that THg concentrations in fish were generally low, and THg trophic magnification factors (TMFs) ranged from 1.88 (in hypereutrophic Lake George) to 5.6 (in mesotrophic Lake Nkuruba). We found that TMFs were significantly higher in hypereutrophic lakes than in meso- and eutrophic lakes, and found that  $\log(\text{THg})$  vs. trophic level regression slopes were negatively related to chlorophyll *a* concentrations. Furthermore, our results indicated that phytoplankton accumulate relatively less THg per unit biomass in higher productivity systems. These observations suggest that growth and/or biomass dilution may act to reduce Hg concentrations at the food web base, while growth dilution at consumer trophic levels may act to reduce Hg biomagnification rates. THg concentrations in fish appeared to be determined, at least in part, by processes at the base of the food web, with year-round high phytoplankton biomass and growth rates reducing the potential for high THg in fish in these productive tropical lakes.

**327 Controls on the Trophic Magnification Factor of Organic Chemicals in Aquatic Foodwebs** M. MacLeod, ITM Stockholm University / Department of applied environmental science; J.A. Arnot, ARC Arnot Research Consulting / Department of Physical Environmental Science; K. Borga, Norwegian Institute for Water Research; M.S. McLachlan, Stockholm University. Measured trophic magnification factor (TMF) greater than 1 has been proposed as the top-tier of a framework for assessing the bioaccumulation potential of chemicals. TMF greater than 1 will be observed when the average biomagnification factor (BMF) between trophic levels of a food web is greater than 1. Therefore, the TMF can be viewed as a field-measured BMF where the measurements have been conducted – and thereby averaged – across several trophic levels. We modeled BMFs using the Arnot & Gobas fish bioaccumulation model with the goal of identifying the processes that control BMF, and thus TMF, for different chemicals. Our modeling approach considered a broad range of combinations of  $\log K_{ow}$  from -2 to 12, and a range of rate constants for whole-body biotransformation by fish of  $0.00001 - 10000 \text{ day}^{-1}$ . Modeled BMFs and the dominant processes that remove chemicals from fish over the entire range of chemical properties were determined. The model identifies chemicals with biotransformation rate constants less than  $0.1 \text{ day}^{-1}$  and

( $4.5 < \log K_{ow} < 7.5$ ) as having  $\text{BMF} > 1$ , and thus having the potential to have  $\text{TMF} > 1$ . The nature of the process that controls BMF has implications for the variability in TMFs that should be expected in the field. Near the lower  $\log K_{ow}$  boundary gill elimination is the controlling process. At the upper boundary of biotransformation rate constant the potential variability between species and between systems is poorly characterized. Finally, at the upper boundary of  $\log K_{ow}$  there is clearly large variability between species and between aquatic systems in the growth rate of fish and other biota. Therefore, our modeling results indicate that we should expect high variability in TMF values determined in different systems for chemicals with  $\log K_{ow}$  above 7, with lower values in systems where growth rates are higher. We discuss the implications of these findings for the use of TMF in regulatory assessments of bioaccumulation potential conducted at national and international levels.

**328 Trophic dilution of cyclic volatile methylsiloxanes (cVMS) in the pelagic marine food web of Tokyo Bay, Japan** D.E. Powell, Dow Corning Corporation / Health & Environmental Sciences; N. Suganuma, Silicone Industry Association of Japan (SIAJ); S. Ushioka, Environmental Control Center (ECC); K. Kobayashi, T. Nakamura, K. Ninomiya, M. Itai, Silicone Industry Association of Japan (SIAJ); K.B. Woodburn, Dow Corning Corporation / Health & Environmental Sciences. Trophic transfer of three cyclic volatile methylsiloxanes (cVMS) and two polychlorinated biphenyl (PCB) congeners was evaluated in the pelagic marine food web of Tokyo Bay, Japan. The cVMS materials were octamethylcyclotetrasiloxane (D4; CAS No. 556-67-2), decamethylcyclopentasiloxane (D5; CAS No. 541-02-6) and dodecamethylcyclohexasiloxane (D6; CAS No. 540-97-6). The PCB congeners were 2,3,4,5,2',4',5'-heptachlorobiphenyl (PCB-180; CAS 35065-29-3) and 2,4,5,2',4',5'-hexachlorobiphenyl (PCB-153; CAS 35065-27-1), which are "legacy" chemicals known to bioaccumulate in aquatic organisms and biomagnify in aquatic food webs. The PCB congeners were used as a benchmark chemical (PCB-180) to calibrate the food web and as a reference chemical (PCB-153) to validate the results. The interrelationship between BSAF and TMF was applied to the field data to minimize bias and uncertainty associated with the nitrogen-15 enrichment factor (used to estimate relative trophic level position), omnivorous feeding, and migration, and to correct for variable exposure across concentration gradients. The field data was used to derive other measures of bioaccumulation (e.g., BCF, BAF, BMF) and associated properties (e.g. concentrations in water, food web partition coefficients, water-sediment fugacity ratios) directly from the natural food web. When not benchmarked against PCB-180 or corrected for variable exposure across concentration gradients, the mean TMF for PCB-153 was 2.2 (95%CI=1.5 to 3.1) with a coefficient of correlation ( $r^2$ ) of 61%. When benchmarked against PCB-180 (TMF=4.0) and corrected for variable exposure, the mean TMF for PCB-153 was 3.8 (95%CI=2.6 to 6.1) with an  $r^2$  of 86%. The increase in  $r^2$  from 61% to 86% indicated that correction for variable exposure was appropriate. When benchmarked against PCB-180 (TMF=4.0) and corrected for variable exposure, mean TMF values for the cVMS materials ranged from 0.8 to 0.9 (95%CI=0.5 to 1.3) with  $r^2$  values ranging from 1.9% to 8.5%. In contrast to PCB-153, correction for variable exposure had minimal impact on TMF for cVMS. Probabilistic uncertainty analyses demonstrated with greater than 80% certainty that TMF values for cVMS were less than 1.0 for the pelagic food web. These results for Tokyo Bay are comparable to results observed for other pelagic and demersal food webs in marine and freshwaters, and further demonstrate that cVMS materials do not biomagnify in aquatic environments.

**329 Revisiting TMFs for cVMS and legacy pollutants in Norwegian lakes** K. Borga, E. Fjeld, Norwegian Institute for Water Research; A.H. Kierkegaard, M.S. McLachlan, Stockholm University. A recent study of the food web biomagnification of cyclic volatile methyl siloxanes in the pelagic food web of Lake Mjøsa, Norway, found surprisingly high levels of decamethylcyclopentasiloxane (D5 CAS no. 541-02-6) with trophic magnification factors (TMF) significantly higher than 1. There are presently few studies of food web magnification of

cVMS. The two other available studies of cVMS TMF have demonstrated a lack of food web biomagnification in benthic associated food webs, with TMFs below 1. To address this difference between studies, and to increase our understanding of the cVMS food web biomagnification, their potential sources and differences between lakes, the pelagic food web of Lake Mjøsa was revisited (resampled) July-September 2012, in addition to a comparable lake (Lake Randsfjorden) and a reference site (Lake Femunden), far from any known sources of contaminants. Results from the present study support both the high concentration in Lake Mjøsa and food web biomagnification for D5 in pelagic food webs of Norwegian Lakes. The levels in the control lake were low and only measured in sediments, brown trout and one arctic charr, and almost all samples were below the limit of quantification except for a few of the brown trout that were just above. The factors affecting TMFs of D5 in the different lakes will be discussed along with comparison to benchmark chemicals such as PCBs, and potential sources of D5 to these ecosystems.

**330 What is the cause of biomagnification of PCBs and organochlorine pesticides in the aquatic environment?** M.T. Jonker, Utrecht University; S.A. van der Heijden, Institute for Risk Assessment Sciences / Institute for Risk Assessment Sciences. For decades, researchers have observed concentrations of hydrophobic organic chemicals (HOCs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides, to increase throughout food chains. The increase in concentrations of these chemicals with trophic level is referred to as “biomagnification” or “trophic magnification”. The phenomenon is not completely understood, but the general perception is that biomagnification is predominantly caused by slow elimination kinetics of hydrophobic chemicals that cannot keep up with the ongoing intake via food ingestion. In this presentation, this view will be challenged by showing that lipid-normalized bioaccumulation of a series of PCBs and organochlorine pesticides varies among different (non-mammalian) species by as much as almost one log unit and tends to increase with trophic level. Adjusting biomagnification factors (BMFs) from the literature for these different lipid affinities seems to level out the factors to unity (i.e., no or little increase in concentrations with trophic level). It is not the intention of this presentation to invalidate the existing paradigm of slow elimination kinetics, but we will discuss an alternative cause of biomagnification, based on partition experiments with different organism constituents (lipids, proteins). This hypothetical cause basically questions the existence of a true causative relationship between HOC concentrations and trophic position.

**331 Food, the fish gap and multi-trophic aquaculture** K. Black, SAMS.

**332 Alternative feeds for Atlantic salmon (*Salmo salar*) using novel plant-based meals and oils to replace fishmeal & oil** G. Bell, Institute of Aquaculture University of Stirling Scotland.

**333 Sea lice and their relevance to the sustainability of salmon aquaculture** C. Todd, University of St Andrews / Biology.

**334 Ensuring environmental sustainability of sea lice treatments** J. McHenry, NOVARTIS Animal Vaccines.

**335 Salmon culture as a source of POPs and metals to the Scottish marine environment?** C. Robinson, Scottish Government.

**336 Discussion** T.F. Fernandes, HeriotWatt University / School of Life Sciences.

**337 Conclusions** T.F. Fernandes, HeriotWatt University / School of Life Sciences.

**338 Comparing the sensitivity of *Lemna* spp. to herbicides and plant growth inhibitors with that of non-standard macrophytes** A. Arenas, Wageningen University; M. Daam, DPPF/CEER; G. Arts,

Alterra Wageningen University and Research Centre / Environmental Risk Assessment. There has been an increasing concern in the last few years regarding the representativeness of the standard macrophyte test species *Lemna gibba* and *Lemna minor* for the sensitivity of other aquatic macrophytes. Differences in life history, growth strategy, exposure route or sensitivity to chemicals with specific modes of action (MoA) could be some of the reasons for this. These differences are currently being discussed in the light of the revision of the Aquatic Guidance Document in Europe. This document recognizes that additional testing might be required on other macrophyte species for modes of action or exposure routes for which *Lemna* spp. is not sensitive. However, it is still unclear for which MoA additional macrophyte testing is needed and which are the most sensitive plant species or growth forms for these MoA. Our research aimed at addressing these issues in the context of the update of the Aquatic Guidance Document. Aquatic macrophyte EC50 values were selected for synthetic herbicides and plant growth inhibitors from the (US?)EPA ECOTOX database. Due to low data availability, the relative tolerance (Trel) approach was used to enable the comparison of macrophyte toxicity data grouped by MoA. Subsequently, Species Sensitivity Distributions were constructed to evaluate differences in sensitivity between *Lemna* spp. and other aquatic macrophytes. Algae toxicity data were also included in the final sensitivity analysis. Our results showed that the percentage (%) of species with a Trel value < 1 (i.e. greater sensitivity than *Lemna* spp.) was 66.78, 49.2, 45.5, 35.4 and 22 for synthetic auxins, ALS inhibitor (Metsulfuron methyl), microtubule inhibitors, PS II inhibitors and cell synthesis inhibitors, respectively. However, when a safety factor of 10 was applied to the toxicity value of *Lemna*, the % of unprotected species (i.e. Trel < 0.1) was 10.3 for synthetic auxins and below 5 for the other MoA. For the majority of the compounds, algal toxicity values were higher than those of *Lemna* spp. This indicates that aquatic macrophytes with higher sensitivity to herbicides than *Lemna* are not protected by algae testing. Implications for the risk assessment of herbicides and indications for future research are discussed.

**339 Aquatic macrophytes – does their sensitivity depend on their systematic position (monocotyledons / dicotyledons)** H. Christl, Tier Solutions GmbH. Many selective herbicides act specifically on certain terrestrial macrophytes, often either on monocotyledons (i.e. Liliopsida) or on dicotyledons (i.e. Magnoliopsida and Rosopsida). The current revised data requirements suggest to test further aquatic macrophytes also based on their systematic position. However there is indication in the literature that in contrast to terrestrial macrophytes the systematic position of aquatic macrophytes appears not to define their relative sensitivity. To verify or falsify these contradicting hypotheses, available macrophyte data collated by the AMDAP SSD group and evaluated in Giddings (2010) have been further analysed based on the taxonomic position of the assessed data. One problem of all analyses is that available parameters vary data sets (i.e. one study may have only assessed shoot length, another study provided several endpoints based further variables. The original SSD analysis always used the lowest endpoint of a given study / species. While this take reflects the prevailing regulatory approach (always use the lowest endpoint), the lower tail of these distributions is more affected by the type and number of parameters assessed (see above). A central toxicity point per species is considered to be more robust for a comparison of the relative sensitivity of different taxonomic groups. The outcomes of analyses either based on the lowest toxicity estimate or on the central points are presented and compared, and in addition individual SSD distributions for monocots and dicots are presented for several active substances, including the most extreme cases (with either monocots or dicots being more or less sensitive than the other). The main finding was that compared to the scatter of endpoints within a taxonomic group the differences between the two taxonomic groups were generally small. While this is only a preliminary evaluation that must be expanded once further aquatic macrophyte data are available for further active substances, the currently available information casts some doubt on the need of additional aquatic macrophyte testing based on the systematic

position of the terrestrial target plants.

**340 Are threshold derived from specific species sensitivity distributions protective for benthic diatom assemblage? The case of a four herbicides mixture** E. Larras; V. Gregorio, UNIL Anthropole; B. Montuelle, A. Bouchez, INRA; N. Chevre, Img / Faculty of Geosciences and Environment. Pollution of aquatic ecosystems is characterized by mixture of chemicals such as herbicides that are rejected by watershed runoff and urban discharge. Photosystem II inhibitors as atrazine, terbutryn, diuron and isoproturon are still found in European surface waters even if some are banned or restricted. These herbicides affect primary producers such as benthic diatoms which represent a considerable proportion of the fixed biomass. In the light of diatoms ecological significance and due to their sensitivity to herbicides, evaluating the risk of mixture of herbicides is therefore of great importance. Species sensitivity distributions (SSD) are used in risk assessment to derive protective thresholds (Hazardous Concentration, HC) from data obtained in laboratory. However, SSD are often based on model species because of the lack of data on environmental species. To assess if SSD based on environmental species are more adapted for herbicide mixture risk assessment, we compared the protectiveness of HC derived from generic SSD (G-SSD) built on literature sensitivity data, and specific SSD (S-SSD) built on the sensitivity of benthic diatoms typical of biofilm diversity in Lake Geneva. We built one S-SSD and one G-SSD for atrazine, terbutryn, diuron and isoproturon. On G-SSDs, diatoms species were more resistant while chlorophytes and cyanobacteria were sensitive except for terbutryn. As a result, G-HC thresholds were lower than S-HC. Then, we combined the 4 G-SSDs together, the 4 S-SSDs together and concentration addition model (CA), which predicts mixture toxicity when components share the same mode of action, for assessing the risk of mixture. We obtained one G-SSD and one S-SSD for a quaternary mixture and we derived HC<sup>5mix</sup> thresholds from each curve. In parallel, we exposed an artificial diatom community of 11 species to the same quaternary mixture to assess its sensitivity and compare it to both HC<sup>5mix</sup>. G-HC<sup>5mix</sup> over protected the artificial community while S-HC<sup>5mix</sup> was under protective. Heterotrophic diatoms appeared more resistant in benthic bioassays, suggesting that other parameters, for example concurrence, may influence their sensitivity to PSII inhibitors when species grow in benthic community. In the light of our results, it seems essential to represent as possible the whole diversity of species targeted in S-SSD to prevent any underestimation of protective thresholds for herbicide risk assessment for phytobenthos.

**341 Growth recovery of *Lemna gibba* and *Lemna minor* from a 7d diuron exposure** M. Burns, Hortsys Research Unit, CIRAD M.L. Hanson, University of Manitoba / Department of Environment and Geography; A. Crossan, I. Kennedy, Faculty of Agriculture and Environment, University of Sydney. In agricultural catchments, aquatic ecosystems can experience periodic exposure to varying concentrations of pesticides. After pulsed exposure and possible adverse impact, populations within an ecosystem that are not extirpated may recover. This paper investigates the potential for recovery of two duckweed species (*Lemna minor* and *L. gibba*) following a laboratory exposure to the herbicide diuron for seven days. Recovery potential was assessed for exposed plants by transferring duckweed to clean media and monitoring over seven days. Population growth and biomass production were measured in both exposure and recovery treatments. There was significant inhibition from the exposure to diuron under the initial 7d exposure treatment (EC<sub>50</sub> = 59 and 52 µg L<sup>-1</sup>; for *L. minor* and *L. gibba* frond numbers, respectively). Both duckweed species were able to recover their growth from diuron inhibition levels that were not significantly different from the control treatments after seven days. Specifically, the greatest exposure concentrations at which recovery were observed for *L. minor* and *L. gibba* were 60 and 208 µg L<sup>-1</sup>, respectively. These species were able to recover from exposure concentrations that are typically deemed significant in ecological risk assessments for diuron. These results suggest that in the case of exposure to this herbicide, a level of ecosystem resilience is definable

and should be considered in the assessment of ecological risk of diuron occurring in catchments.

**342 Lines of evidence for establishing an aquatic level of concern for the herbicide atrazine in North American surface water** R. Brain, Syngenta Crop Protection Inc / Department of Environmental Risk Characterization; A.J. Hosmer, Syngenta Crop Protection Inc; D. Campbell, Syngenta Crop Protection LLC. Atrazine, a triazine herbicide registered primarily for the control of broadleaf weeds in corn and sorghum, is estimated to be the second most heavily applied herbicide in the U.S. (~35 million kgs annually). Approximately 75% of the field corn acreage grown in the U.S. is treated with atrazine, which has been detected in surface waters from watersheds with vulnerable landscape characteristics as indicated by the Atrazine Ecological Monitoring Project (AEMP). The United States Environmental Protection Agency (EPA) is in the process of evaluating an aquatic level of concern (LOC) for atrazine. The Agency's approach for establishing an LOC involves calibrating a growth-based primary producer model using a set of ~40 micro/mesocosm (cosm) studies, which is highly sensitive to subjective binary cosm classification, delineated as 'effect' (scored as 1) and 'no effect' (scored as 0). A recent Scientific Advisory Panel (SAP) convened by the EPA indicated that most of the existing cosm studies with purported effects identified at atrazine concentrations less than 30 µg/L have design weaknesses rendering interpretation of results and scoring difficult and subjective. An independent cosm evaluation, which was reviewed by the SAP and designated as appropriate, was used to derive a 60-d LOC of 25 µg/L using the EPA model. Multiple additional lines of evidence support an LOC of 20 µg/L for 60 d, including a) the micro/mesocosm calibration dataset, b) species sensitivity distributions (SSDs) of the underlying laboratory toxicity data for primary producers used to populate the Agency's model, c) rapid recovery of primary producers from atrazine exposure in single-species laboratory tests and evaluations of native in-stream communities, d) two years of biomonitoring of algal communities in the Midwestern U.S., demonstrating no observed direct effects attributable to atrazine on community dynamics (e.g. species richness, composition, diversity etc.). These lines of evidence will be presented to provide context for an aquatic LOC for atrazine.

**343 The Effects of Mixtures of Herbicides in Use in Thailand on *Lemna minor*** R. Tagun, Environment. The aim of this study was to test the interactions of herbicide mixtures atrazine, 2,4-D, alachlor and paraquat on *Lemna minor*. Two mixture exposure scenarios were investigated: exposure to mixtures in combination; and exposure to different pesticides in sequence. For the combined exposure studies, the effects of single compounds and a range of binary mixtures of 2,4-D and atrazine and alachlor and paraquat on the growth of *L. minor*. The resulting data were analysed to determine EC50 values for the single substances and to concentration combinations causing a 50% inhibition of growth. For sequential studies, four treatments was assessed: atrazine followed by 2,4-D; 2,4-D then atrazine; alachlor then paraquat; paraquat then alachlor. The interactions were assessed at a number of concentrations and a simple study design was adopted, where plants were exposed to the first pesticide for 3.5 d (50% of the study duration) and then removed and exposed to the second pesticide for the remainder of the study. In order to determine the nature of any interaction, observed effects of the sequential exposures to the different compounds were compared to predicted effects obtained using a simple model. For the chemical analysis, atrazine and 2, 4-D was analysed by HPLC while alachlor and paraquat were determined by ELISA test kits. For the binary mixture, atrazine and 2,4D indicates that these substances interact antagonistically. In contrast, alachlor and paraquat, the resulting isobole indicates a possible synergistic effect. In term of sequential exposure, when *L. minor* were exposed to atrazine and then 2,4-D the effect of the mixture was lower than predicted using the simple model. On the other hand, when plants were exposed to 2,4-D and then atrazine, an enhancement of the predicted toxicity was seen. When plants were exposed to paraquat followed by alachlor, toxicity was lower than predicted but the effects of exposure to alachlor then paraquat seemed to

be highly concentration dependent with some mixture concentrations causing enhanced effects and others reduced effects. These results show that interactions of different pesticides can occur when organisms are exposed to mixtures in combination or sequentially. The interactive effects vary according to the modes of action of the pesticides studied, the sequence of exposure and the concentrations tested.

**344 Clotting time recovery and tissue residues following cessation of exposure to the anticoagulant rodenticide diphacinone in Eastern screech-owls** B.A. Rattner, USGSPatuxent Wildlife Research Ctr / USGS; R.S. Lazarus, USGS Patuxent Wildlife Research Center / USGS; K.E. Horak; S.F. Volker, U.S. Department of Agriculture / Animal and Plant Health Inspection Service, National Wildlife Research Center; D.A. Goldade, N. Hoffman, U.S. Department of Agriculture / Animal and Plant Health Inspection Service, National Wildlife Research Center; J.J. Johnston, U.S. Department of Agriculture / Food Safety and Inspection Service. Anticoagulant rodenticides are used for the control of vertebrate pests in urban and suburban settings, agriculture and in island restoration projects. New regulatory restrictions have been placed on the use of some second-generation anticoagulant rodenticides (SGARs) in the United States, and in some situations this action may be offset by expanded use of first-generation compounds (FGARs). We have demonstrated that the FGAR diphacinone (DPN) evokes overt signs of intoxication and lethality in raptors at exposure doses that are 20 to 30 times lower than reported for traditionally used wildlife test species (mallard, *Anas platyrhynchos* and Northern bobwhite, *Colinus virginianus*). Sublethal exposure of American kestrels (*Falco sparverius*) and Eastern screech-owls (*Megascops asio*) resulted in prolonged clotting time, reduced hematocrit, and/or gross and histological evidence of hemorrhage at doses as low as 0.16 mg DPN/kg bwt/day. Our most recent study examined clotting time, hematocrit and DPN liver and kidney residues in owls fed a diet of 10 ppm DPN for up to 7 days followed by untreated diet for up to 21 days. By day 3 of DPN exposure, Russell's viper venom time (RVVT) was prolonged, and by day 7 of DPN exposure, both RVVT and prothrombin time were prolonged and there was evidence of anemia in a few individuals. Upon termination of DPN exposure, coagulopathy and anemia were resolved to baseline values within 1 to 4 days. Surprisingly, DPN residues were consistently greater in kidney than in liver tissue (e.g., DPN on day 7 of exposure was 5.52 ?g/g ww kidney versus 0.96 ?g/g ww liver). Post-exposure concentrations decreased rapidly within 24 hours; within 1 week liver and kidney values were < 0.3 ?g/g ww, and within 3 weeks values were < 0.1 ?g/g ww. The terminal phase half-lives of DPN in liver and kidney were 7.8 days and 4.7 days, respectively. Both FGAR and SGAR exposure monitoring of free-ranging raptors has principally utilized liver tissue, but the present findings suggest that future monitoring efforts should also quantify concentrations in kidney. These data are being used to develop an adverse outcome pathway for anticoagulant rodenticides in avian species. In addition, our findings demonstrate that low level dietary exposure to DPN can evoke toxicity in raptors in a matter of days, but once exposure is terminated, recovery can occur rapidly.

**345 Does resistance to second generation anticoagulant rodenticides (SGARs) in rats increase secondary exposure of predators** R.E. Shore, CEH Lancaster; L.A. Walker, Centre for Ecology & Hydrology; A.D. Buckle, University of Reading; J.S. Chaplow, Centre for Ecology & Hydrology; L.J. Daniells; N.R. Llewellyn, M.G. Pereira, Centre for Ecology Hydrology; E. Potter, Centre for Ecology and Hydrology. Second-generation anticoagulant rodenticides (SGARs) are a key tool used in Britain and many other countries globally to control commensal rodents. However, use of SGARs is associated with widespread exposure of non-target wildlife through primary and secondary exposure. In Britain, long-term monitoring of SGAR exposure in a sentinel small mammal predator, the barn owl *Tyto alba*, has indicated an increase in secondary exposure to SGARs over the last 30 years. One potential reason is the spread of resistance in rats to difenacoum and bromadiolone. Barn owls generally only eat rats occasionally but are likely to be affected indirectly because resistance in rat populations

leads to subsequent greater deployment of bromadiolone and difenacoum outdoors in often ineffectual attempts to control populations. This is likely to enhance exposure in non-target rodents that are then eaten by owls. We hypothesised that barn owls from counties in Britain where SGAR resistance [in rats] has been documented would experience greater exposure than owls from elsewhere. We quantified the extent of exposure to SGARs in barn owls that died between 2000 and 2011, reporting (i) the proportion of owls with detectable (? 0.025 ?g/g wet weight) liver SGAR residues and (ii) the magnitude of those liver residues. We found that barn owls were more likely to have detectable liver summed SGAR residues if they were from counties where resistance to SGARs in rats has been detected ( $Z = 2.36$ ,  $P < 0.02$ ). The magnitude of summed SGAR residues in birds with detectable residues was also higher in owls from counties where resistance has been detected ( $F = 5.21$ ,  $P < 0.05$ ). This is the first study, as far as we are aware, to demonstrate that resistance in rats is associated with an increase in SGAR exposure, and potentially effects, even in predators that rarely eat rats. Our results emphasise the need to manage resistance in rats and/or ineffectual use of SGARs in resistance areas to limit exposure of non-target wildlife.

**346 Intentional and accidental poisoning of wild and domestic animals in Spain** R. Mateo, UCLMCSIC / Instituto de Investigacion en Recursos Cinegeticos; I.S. Sanchez-Barbudo, P.R. Camarero, U.C.L.M.-C.S.I.C.. In this study we have analyzed of 1,157 suspected cases of poisoning of wild and domestic animals in the natural environment (1,800 animals and 340 baits) from different Spanish regions during the period 2004-2010. We detected 41.2% of positive cases (40.8% of animals and 52.6% of baits). In domestic carnivores, detection of toxic reached 71.4%, indicating its usefulness as sentinels of the use of poison in the environment. In those animals positive for toxicological analysis, 78.3% have been considered as intentional poisonings. The diurnal raptors were most affected by poisoning (43.6% of positives), followed by carnivorous mammals (27.1%). The most frequently detected toxicants were anticholinesterase insecticides (baits/animals: 80.4%/65.8%), followed by anticoagulant rodenticides (5%/19.6%), strychnine (2.2%/6.5%) and arsenic (4.5%/2.3%). The differences observed between regions underlines the dominance in the use of strychnine in Asturias, anticoagulant rodenticides in Castilla y Leon, organophosphate insecticides in Aragon, carbamate insecticides in Castilla-La Mancha and Madrid, and the emergence of other poisons, such as ?-chloralose or barbiturates, in Catalonia. In summary, 82.3% of intentional poisonings were due to anticholinesterase pesticides and 85.5% of accidental anticoagulant rodenticides. Future regulations of pesticides and biocides should take into account the risk of illegal use in the preparation of poisoned baits which involves the marketing of formulations with high richness of active ingredients with low LD<sub>50</sub>.

**347 Compliance with the ban of lead ammunition in a Mediterranean wetland, the Ebro delta** N. Vallverdu-Coll, Instituto de Investigación en Recursos Cinegeticos; M.E. Ortiz-Santaliestra, J. Rodriguez-Estival, A. Lopez-Antia, M. Martinez-Haro, M.A. Taggart, Instituto de Investigación en Recursos Cinegeticos (IREC); R. Guitart, Universitat Autònoma de Barcelona; R. Mateo, Instituto de Investigación en Recursos Cinegeticos (IREC). The ingestion of lead (Pb) shot used for hunting continues being the main cause of Pb poisoning in waterfowl. In the Ebro delta (Spain), protected wetlands are surrounded by rice fields where waterbirds feed, and where Pb ammunition is still allowed. High Pb shot densities in sediments, and in turn high ingestion prevalence in waterfowl, have been detected. The use of Pb ammunition and the accumulation of Pb by birds after shot ingestion may pose a risk for human health due to consumption of contaminated meat. We assessed the degree of compliance with the ban on Pb ammunition in the Ebro delta wetlands, and studied the effect of the ban on the prevalence of Pb shot ingestion in waterbirds and on Pb levels in game meat. Waterfowl carcasses were collected and X-rayed from hunting bags (2007-2011) to determine the percentage of Pb and non-toxic embedded shot. Concentrations of Pb were analyzed in livers and muscles. In addition, gizzards were collected from hunting bags (2007-2012) and

examined to determine the percentage of Pb shot ingestion. During the first study season minimum hunter compliance, estimated as the percentage of waterbirds having only embedded steel shot (the non-toxic alternative) was 48.75%, while 26.88% of birds had only embedded Pb shot (minimum hunter noncompliance). These values changed in the subsequent seasons to 68.95% and 1.13%, respectively. The little compliance detected during the first study season led local administration to notify hunters that a total prohibition of hunting in protected wetlands would enter into force if the prohibition was not observed. Pb ingestion prevalence in 2007-2008 (28.6%) was not different from the pre-ban value (30.2%), but decreased significantly to values below 17.9% in the following seasons. Birds continue ingesting Pb shot at a relative high proportion, although their prohibition slowly contributes to reduce prevalence of ingestion. Pb muscle concentrations decreased significantly after the ban, in spite of which most species present individuals with Pb liver and muscle concentrations over the maximum safety limits. Whereas muscle Pb levels were determined by the presence of both ingested and embedded shot (all  $p < 0.001$ ), liver levels largely depended on ingested shot ( $p < 0.001$ ). Thus, besides restrictions in Pb ammunition use, additional mechanisms to reduce Pb ingestion prevalence in waterfowl are necessary to reduce risks for human consumers.

**348 Environmentally relevant concentrations of an antidepressant alter physiology and behaviour in wild birds** T. Bean, University of York / Environment; A. Boxall, University of York; J. Lane, The Food and Environment Research Agency; K. Herborn, University of Glasgow; K. Arnold, University of York. A growing and ageing population has led to increased pharmaceutical usage. Following ingestion, pharmaceuticals may not be completely metabolised so can be excreted to the wastewater system. Bird and bat species foraging on the invertebrates living on wastewater treatment works, and fields fertilised with sewage sludge, may therefore be exposed to a mixture of pharmaceuticals. Little is known about the risks to wildlife posed by environmentally relevant levels of pharmaceuticals. In this project, we investigated the effects of environmentally relevant concentrations of the antidepressant fluoxetine on wild caught starlings. Wild caught starlings housed in outdoor aviaries were allocated to either the treatment or the control group. To mimic foraging exposure, doses were injected into waxworms and hand fed to birds. We assayed relevant behaviours based on fluoxetine's effects on humans: lethargy was measured from general activity levels of birds and changes in anxiety were assessed via two behavioural responses to standardised stressors (exploration in a novel environment and boldness following human disturbance). Behaviour was measured during 2 trials over 2 days. After 16 weeks of treatment, all birds were more active during trials than at baseline but treated birds were significantly more active than controls, contrary to predictions. Controls showed low levels of exploration in the novel environment in trial 1 which increased in trial 2 as they became habituated to the new cage. In contrast, fluoxetine treated birds showed high risk exploratory activity in both trials. Similarly post-treatment, all birds became bolder but fluoxetine treated birds were relatively bold in both trials and showed considerably less habituation over the two days than pre-treatment. Moreover, at the end of the experiment, moving to the test cage caused a rise in stress hormones of controls which dropped after 24 hours. However, for the fluoxetine treated birds, stress hormones were not as elevated following the move to the test cage and stayed low for 24 hours. Thus, ingestion of low concentrations of fluoxetine may induce a 'therapeutic' effect in birds apparently reducing their anxiety levels. Environmentally relevant levels of fluoxetine could potentially reduce stress responsiveness of starlings, increasing the risks posed by predation and other dangers.

**349 Biomonitorization of birds under recovery: closing the gap between risk assessment and wildlife management** C.S. Santos, University of Aveiro / department of Biology & CESAM; M.S. Monteiro, Aveiro University / Department of Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; S. Loureiro, Universidade de

Aveiro / Biology. Birds, including waterbirds, have been used as bioindicators of environmental quality in a broad range of ecosystems. Amongst other attributes, their conspicuousness and sensibility to environmental contaminants makes them key species in environmental biomonitorization. Over the past years a significant part of the Portuguese biomonitoring studies has focused on organisms at lower trophic levels (e.g. crustacean and mollusks), but failed to address contaminants' effects upon organisms at higher trophic levels such as mammals or birds. The present study aims were to: (i) assess the exposure of Portuguese birds to environmental contaminants, in particular birds accepted for rehabilitation in wildlife recovery centres, (ii) clarify if these factors could lead to birds' illness and influence their recovery, and (iii) investigate the usefulness of ecotoxicological tools in the monitorization and recovery processes of birds. In order to address these issues, markers of neurotoxic and genotoxic exposure were assessed in aquatic birds from the orders Ciconiiformes and Charadriiformes. In the first part of this study, the analysis of neurotoxic markers, the reactivation of cholinesterase (ChE) activity on brain of the yellow-legged gull (*Larus michahellis*) was tested. In the second part of this work, the assessment of genotoxic effects, the frequency of micronucleus and other nuclear abnormalities was analyzed in erythrocytes of the white stork (*Ciconia ciconia*), grey heron (*Ardea cinerea*), purple heron (*Ardea purpurea*) and the little egret (*Egretta garzetta*). ChE activity in brain of *L. michahellis* was restored at a significant extent (increase in 50%), suggesting a previous exposure of these individuals to anti-ChE agents. High levels of genotoxic damage were also observed in the species of Ciconiiformes studied, with values varying significantly between years and geographical origins ( $p < 0.05$ ). These results suggest that Portuguese birds might be exposed to different levels of environmental contamination and that this contamination may impair birds' health. The use of ecotoxicological tools seems, therefore, to be a promising approach to monitor bird's recovery as it will probably allow the screening for early physiological signs of toxicity, enabling a more insightful evaluation of birds' health condition. Moreover, the use of these types of biomarkers may allow monitoring the potential rehabilitation of these birds.

**350 Environmental media induced changes of surface functionalisation of two titanium dioxide nanomaterials** C. Nickel, Institute of Energy and Environmental Technology eV IUTA / Air Quality & Sustainable Nanotechnology; B. Hellack, Institute of Energy and Environmental Technology e.V. - IUTA / Air Quality & Sustainable Nanotechnology; A. Nogowski, F. Babick, M. Stintz, Technical University Dresden / Institute of Process Engineering and Environmental Technology; T.A. Kuhlbusch, Institute of Energy and Environmental Technology e.V. - IUTA / Air Quality & Sustainable Nanotechnology. Titanium dioxide nanomaterials are already used in different products like in sunscreens. For their application in sunscreens the TiO<sub>2</sub> nanoparticles are coated to avoid adverse effects of the TiO<sub>2</sub> core. The aim of the investigation presented was to determine the possible degradation or change of the surface functionalisation of two different nanomaterials, UV Titan M262 and UV Titan M212 under varying conditions. Any change of the coating may affect the fate and behaviour of the nanomaterial in the environment which is very important for transport, bioavailability and risk assessment. Both tested materials are coated with aluminium oxide to avoid light induced generation of reactive oxygen species at the TiO<sub>2</sub> surface. A further coating with dimethicone (UV Titan M262) or glycerol (UV Titan M212) is applied to ensure hydrophobic and hydrophilic behaviour, respectively. Possible changes of the surface coating were studied by determination of the coating material in the supernatant and by the behaviour of the nanomaterials when environmental parameters like pH, CaCl<sub>2</sub> or humic acid (HA) concentrations were varied. Changes in the behaviour were used as indicators for possible aging of the coating. After suspension of the materials in deionised water both materials showed a comparable behaviour with a point of zero charge around pH 8.5. The analyses of the supernatant revealed that 86%  $\pm$  12% of the glycerol and 88%  $\pm$  8% of the dimethicone coating was removed from the surface. However, no release of the aluminium oxide coating was

observed, which agrees with the result that both materials did not show intrinsic hydroxyl radical generation even after severe treatment. The variation of  $\text{CaCl}_2$  concentration resulted in a destabilization of the suspension due to reduced double layer thickness, whereas HA resulted in an increased stabilization of the suspension, by electrostatic and steric repulsion. After HA adsorption both materials showed a negative zeta potential and no point of zero charge was observed (pH 4.5-10). Based on the results of this study it was shown that HA has a large stabilizing effect for the studied nanomaterials and tested conditions, but in natural environments additional conditions with opposite behaviour can occur which affected the fate and behaviour. *Acknowledgement: This work was sponsored by the Environmental Agency of Germany (UBA)*

**351 Occurrence, distribution and bioaccumulation of UV filters in water, sediment and fish from four Iberian river basins.** E. Gago Ferrero, IDAEACSIC / dep. of environmental chemistry; S. Diaz-Cruz, IDAEACSIC / Environmental Chemistry; D. Barcelo, IQABCSIC. Awareness regarding the harmful effects of sun-light on the human skin has recently increased the production and consumption of sunscreens. These products contain organic and inorganic ultraviolet filters (UV F) that absorb, reflect and/or scatter UV radiation, therefore preventing skin diseases such as skin cancer. UV filters are active ingredients added to sunscreens and to personal care products used daily, such as cosmetics, skin creams, body lotions, soaps, hair sprays, hair dyes, and shampoos. The concentrations used in these products are regulated and in general do not exceed 10% due to the fact that some UV filters show toxic effects. The increased production and use of UV F has led to increased and continuous inputs into the environment and this fact makes UV F to be considered environmental pseudo-persistent pollutants. These compounds and their active metabolites may enter the environment as complex mixtures through direct and indirect pathways. The direct sources are the washing off during bathing activities (sea, lake, river and swimming pool) as well as industrial wastewater discharges. Indirect inputs are related to urban wastewater discharges (showering, clothes washing and urine excretion) and via wastewater treatment plants. Aquatic pollution is particularly troublesome because aquatic biota is captive to continual life-cycle, multigenerational exposure. Despite several studies performed during the last decade on this topic, the knowledge on their occurrence, fate and effects in the environment remains highly fragmentary. Therefore, an important effort in this field is required to provide a global view of the risks for the ecosystems. The aim of this work was to develop analytical methodology and apply it to assess the occurrence of UV filters in surface waters and sediments collected along four Iberian rivers basins with Mediterranean regimen: Ebro, Llobregat, Guadalquivir and Júcar, all of them subjected to intensive anthropogenic activities. Beyond obtaining occurrence data, this study aimed also to assess the bioaccumulation and potential biomagnification processes through the trophic web. Individuals of different fish species collected along the four river basins, in the same locations as water and sediment, were also analyzed and evaluated.

**352 Micropollutant concentrations in sewage effluent from McMurdo Station and Scott Base and surrounding coastal waters, Ross Island, Antarctica** S.K. Gaw, University of Canterbury / Chemistry; P. Emnet, University of Canterbury; G. Northcott, Plant and Food Research; L. Graham, University of Canterbury. Antarctica is considered to be one of the last untouched wilderness areas on earth and as such is the focus of intense scientific investigation. The growing influx of science staff in the spring/summer season has increased focus on reducing the environmental impacts of scientific research activities in Antarctica. Many of the research stations are located in coastal areas into which they discharge sewage. Only 63% of the permanent bases and 31% of the summer stations have any kind of sewage treatment. In addition some research parties undertaking extended periods of fieldwork are allowed to dispose of raw sewage via tidal cracks in the sea ice. Sewage discharges are a recognised source of organic micropollutants entering the environment. Personal care products including soaps, sunscreens and toothpaste are a key source of organic micropollutants entering sewage treatment plants. A pilot study was

undertaken to determine if micropollutants from personal care products were present in treated sewage effluents from Antarctic Research Stations and were being released into Antarctic coastal waters. Wastewater samples from two Antarctic research stations, McMurdo Station (USA) and Scott Base (New Zealand) and seawater samples from the surrounding coastline were analysed for a suite of organic micropollutants. Organic micropollutants were detected in the WWTP effluents as well as at all of the sea water sampling locations including the reference site located up current of the research stations. Detected compounds include paraben preservatives, triclosan, octylphenol, bisphenol A, UV-filters, and the hormone estrone. Target analytes were detected in both wastewater and seawater at concentrations similar to those reported in temperate environments with higher population densities. The results of current research investigating temporal trends in micropollutant concentrations in the WWTP effluents and a more extensive spatial survey of micropollutants in Erebus Bay will also be presented. The environmental fate of the detected compounds will be discussed in terms of Antarctica's unique environmental conditions.

**353 Can organic contaminants in lake sediments bioaccumulate in *Daphnia* resting eggs?** A.C. Chiaia-Hernandez, Eawag / Environmental Chemistry; T. Hollingshaus, M. Moest, P. Spaak, Eawag; J. Hollender, Eawag / Dept Environmental Chemistry. Chemical contaminants can affect aquatic organisms such as natural *Daphnia* populations either by direct toxin uptake, or indirectly by e.g. ingestion of contaminated algae. In addition, chemicals in the sediment may bioaccumulate in *Daphnia* diapausing eggs (ephippia) influencing their fitness and hatching abilities. Since most probably not a single chemical has caused *Daphnia* to adapt but the combined sum of different compounds, it is important to screen for a broad range of organic contaminants. Sediments are excellent archives of environmental contamination if the chemicals persist over time and mostly under anaerobic conditions. After an appropriate extraction and LC combined with high resolution Orbitrap mass spectrometry (HR-MS), we screened for a broad range of organic compounds, including many PCPs in sediment samples. The results show that sediments can be integrators in time and space for emerging contaminants providing history of chemical deposition. In Lake Greifensee, the musk fragrance tonalide and the biocides triclosan and terbutryn have a similar input pattern with a maximum concentration in the late 1970s followed by a rapid decline. Their concentrations correlate with the total phosphorus concentration in the lake, which indicates an input to surface waters via waste water treatment plant (WWTP) discharge. Early layers from the lakes show the presence of PCPs such as octocrylene, and the transformation product of galaxolide (galaxolidone). In addition, suspect screening approaches allowed the detection of suspected compounds like the biocide triclocarban. This is a significant advantage compared to water samples, where usually no historical samples are available and emerging contaminants have not been studied in the past. The target analysis combined with the suspect screening of possible contaminants using HRMS provide a comprehensive picture of the overall contamination pattern, and PCP was found as an important class of the contamination. Based on our outcomes, a group of chemicals were selected to study their bioaccumulation and effect on ephippia. The results show that ephippia can take up contaminants from the pore water in the sediment and that the BCF for the neutral compounds correlate with the log K. The findings provide information about the past and present contamination of lakes as well as insights to understand the impact of habitat alteration on *Daphnia* and their reaction to contaminants.

**354 Metabolism of benzophenone-2 in novel in vitro and in vivo zebrafish bio-assays** V. LE FOL, INERIS/INRA TOXALIM / In vitro and in vivo ecotoxicology; S. AIT-AISSA, B. PICCINI, INERIS, National Institute of industrial environment and risk assessment / In vitro and in vivo ecotoxicology; A. HILLENWECK, INRA UMR 1331 TOXALIM, research centre in food toxicology / Metabolism of Xenobiotics; E. MAILLOT-MARECHAL, INERIS, National Institute of industrial environment and risk assessment / In vitro and in vivo ecotoxicology; E. PERDU, INRA UMR 1331 TOXALIM, research



centre in food toxicology / Metabolism of Xenobiotics; L. DEBRAUWER, INRA UMR 1331 TOXALIM, research centre in food toxicology / Axiom-Metatloul; F. BRION, INERIS, National Institute of industrial environment and risk assessment / In vitro and in vivo toxicology; D. ZALKO, INRA UMR 1331 TOXALIM, research centre in food toxicology / Metabolism of Xenobiotics. Contamination of aquatic environment by xeno-estrogens present in personal care products causes raising concerns. Benzophenones are used as UV filters in sunscreens. They can be detected in rivers, lakes, sewages and sediments with increasing concentrations during the summer season. A number of reports have demonstrated that rivers contamination with xeno-estrogens can result in hormonal dysfunctions in aquatic organisms, which have been characterized through histopathological changes at the level of gonads, developmental disorders and reproductive inability. In fish, benzophenone-2 (BP2) was reported to cause alterations such as the induction of plasmatic vitellogenin and decrease in spermatozoa production (males), as well as reduced spawning (females). Several bio-assays, including aquatic vertebrate models, have been designed over the last years with the aim to characterize the estrogeno-mimetic potential of chemicals. However, the characterization of the biotransformation capability of these biological models which allows to take into account bio-activation/detoxification processes in effect assessment, is rarely reported. We have recently developed *in vitro* bio-assays based on the expression of zebrafish estrogenic receptors in an hepatic cell line (ZFL), and an *in vivo* zebrafish assay based on the expression of the Green Fluorescent Protein (GFP) under the control of the *cyp19a1b* promoter (an estrogeno-regulated gene located in the brain). These bio-assays were used to investigate the estrogenic potency of benzophenone derivatives. Contrary to other chemicals, BP2 elicited a different response depending on the bio-assay we used. We hypothesized that this difference could rely on a different metabolic capability expressed by our models (i.e. detoxification/bio-activation ratio), which would explain the observed differences in the respective estrogenic responses. To examine this hypothesis, the *in vitro* and *in vivo* zebrafish systems were exposed to BP2 at various concentrations, using a tritium-labeled molecule (<sup>3</sup>H-BP2). BP2 metabolism was explored using radio-HPLC (metabolite profiling) and metabolite characterization was investigated using biochemical tools and high resolution mass spectrometry (HRMS).

**355 Determination of methyl siloxanes in soils and biota samples from the Antarctic region** M. Farre, J. Sanchis, C. Galban-Malagon, A. Cabrerizo, J. Dachs, D. Barcelo, IDAEA-CSIC. Low molecular weight cyclic (cVMS) and linear (lVMS) volatile methyl siloxanes are a class of synthetic compounds, which belong to the class of silicones. These compounds are very stable and are used in a plethora of applications, in particular in personal care products. cVMS are used as carriers/thinners for the more viscous siloxanes that are meant to remain on treated surfaces. In industry applications, are used as lubricants, slips, hydraulic, transmission fluids, moisturizers, anti-foaming agents and plasticizer in silicone joint sealants in the construction sector. In consumer products, are used on textiles and in polishes for its water repellent property and as an antifoaming agent in washing powder. Also in food, dimethyl siloxanes are used as antifoaming agents in the production of beer, jam, juices, deep-frying fats and oils and as anti-clotting agents in powdered food. Many of these compounds are also used in personal care products including cosmetics, deodorants and soaps among others (Horii and Kannan, 2008; Foster, 2008; Lewis, 1999; Lodén et al., 2011; Mills and Showell, 2004). These compounds are also present in the industrial releases associated with the manufacture of high molecular weight silicon polymers. As a result of their wide use, siloxanes are presumably spread into the environment (~10×10<sup>6</sup> kg year<sup>-1</sup> in the US) according to Dow Corning estimation (Navea et al., 2011), by both via point sources and via diffuse sources and may be found everywhere in the environment. Recent studies have suggested that siloxanes may have direct or indirect toxic effects on various biological processes. Consequently, a number of cVMS are currently under review for priority pollutant classification in North America and Europe. Therefore, the occurrence, fate and behaviour of linear and cyclic methyl siloxanes should be assessed and characterized in the environment. In this work

we have investigated the presence of eight compounds (hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4), Decamethylcyclopentasiloxane (D5), dodecamethylcyclohexasiloxane (D6), octamethyltrisiloxane (MDM), decamethyltetrasiloxane (MD2M), dodecamethylpentasiloxane (MD3M), and tetradecamethylhexasiloxane (MD4M)) in soils, phytoplankton and krill from the Antarctic region.

**356 Background and future prospects for the Global Monitoring Plan under the SC** R. Guardans, MARM. The historical background and future prospects for the GMP under the SC are briefly described with particular attention to the need of international stable and effective monitoring strategies to establish and maintain QA/QC standards in the sampling, analytical and data management procedures and to make effective use in science and policy making of the best available data

**357 Air Measurements under the GAPS Network in Support of the Global Monitoring Plan of the Stockholm Convention on POPs** L. Harner, Environment Canada / Atmospheric Science & Technology Directorate; L. Ahrens, Swedish University of Agricultural Sciences SLU / Dept of Aquatic Sciences and Assessment; S. Lee, Environment Canada / Atmospheric Science and Technology Directorate; E. Sverko, Environment Canada / Science and Technology; J.K. Schuster, Atmospheric Science & Technology Directorate; J. Charland, Environment Canada. The Global Atmospheric Passive Sampling (GAPS) Network has been operating since 2004 at more than 50 sites measuring persistent organic pollutants (POPs) in air. Data from GAPS made a significant contribution to the first Global Monitoring Plan (GMP1) report that was accepted by Parties at COP4 of the Stockholm Convention on POPs in 2009. The first GMP highlighted challenges for future reporting that included: i.) addressing the lack of data in some regions through capacity building, technology transfer; and implementation of new programs; ii.) interpreting air monitoring data in an integrated approach that includes models, emissions information, meteorology, and the context of a changing environment; and iii.) generating data on the next generation of priority chemicals to inform risk assessment. Since GMP1, the GAPS network has generated data on new classes of chemicals *inter alia* alternative flame retardants, per- and polyfluoroalkyl substances, siloxanes, penta- and hexachlorobenzene. In some cases, these measurements required modifications to the conventional passive samplers used under GAPS and/or improved characterization of existing samplers. For instance, the sorbent-impregnated polyurethane foam (PUF) or SIP disk samplers were developed for capturing a broader range of target compounds that includes the more volatile chemicals; and recent field calibration of the PUF disk sampler indicates that both gas- and particle-phase chemicals are captured at similar sampling rates indicating that the sampling chamber does not discriminate particle-phase chemicals. The GAPS Network has also worked with partners in the GRULAC (Group of Latin American and Caribbean countries) region to generate data for dioxins/furans in air across the region that will contribute to GMP2, due in 2015. Starting in January 2011, PUF disk samplers were deployed for 4 consecutive 6 month periods, at up to 14 sites in the GRULAC region and samples were analyzed for a suite of 17 PCDD/Fs. Highest levels of PCDD/Fs are associated with urban sites and the agricultural site, where biomass burning may be a factor. The air profile is dominated by OCDD, hepta-DD and several PCDFs. Concentrations of PCDD/Fs in air at background sites are about an order of magnitude lower compared to the source-type sites. Temporal trends are also available for some sites for the first 3 consecutive sampling periods and exhibit similar levels and profiles of PCDD/Fs at each site, indicating consistency in source contributions.

**358 Persistent organic compounds in ambient air of Africa** J. Klanova, P. Kukucka, Masaryk University / RECETOX; P. Pribylova, J. Boruvkova, Masaryk University; R. Prokes, Masaryk University / RECETOX, Faculty of Science. Application of persistent organic pollutants (POPs) in the African countries is closely connected to pesticides. They were used in the agricultural production of food crops such as maize, sorghum and millet as well as cash crops for export such

as cocoa, rubber, cotton and timber. They were applied also for disease vector control, especially for mosquito (malaria) and tsetse fly (trypanosomiasis). POP pesticides have been generally imported and not produced in Africa, but pesticide formulation plants exist in many countries. DDT, endosulfan, chlordane, lindane (HCH), heptachlor, toxaphene, HCB and aldrin were identified as most frequently used pesticides. The most serious problem the African region faces is the issue of stocks and reservoirs of obsolete discarded and banned POP pesticides. In addition, banned pesticides continue to be used in practice without any control of the authorities. The air samples from 13 sampling sites in 12 countries were collected during two years of the African study. Sampling sites ranged from continental through rural to urban backgrounds. Levels of various chemicals ranged over several orders of magnitude. Congo, Sudan and Senegal had the highest levels of PCDDs/Fs (up to 262, 120 and 80 pg/sample, i.e. 0.45, 0.2 and 0.13 pg/m<sup>3</sup>, of 17 EPA congeners, respectively) while they never exceeded 1 pg/sample at Mount Kenya. The highest concentrations of PCBs were found in Sudan (4.2 ng/sample, 21 pg/m<sup>3</sup>) and highest concentrations of PBDEs in Sudan (14.3 ng/sample, 24 pg/m<sup>3</sup>), Congo (5.8 ng/sample, 10 pg/m<sup>3</sup>), Canary Islands and Mauritius (both up to 4 ng/sample, 7 pg/m<sup>3</sup>). For pesticides, the highest levels of DDTs were determined in Ethiopia (78 ng/sample, 390 pg/m<sup>3</sup>), HCHs in Togo (2.4 ng/sample, 12 pg/m<sup>3</sup>), and endosulfane in Ethiopia (75 ng/sample, 120 pg/m<sup>3</sup>). This study is an important contribution to our current knowledge on the levels and distribution of POPs in the African continent. As such, it also significantly contributes to successful realization of the Global Monitoring Plan, an important tool for effectiveness evaluation of the Stockholm Convention.

**359 POPs in the Oceans – spatial, temporal trends and air-water exchange** R. Lohmann, University of Rhode Island / Graduate School of Oceanography; J. Klanova, Masaryk University / RECETOX. The fate of persistent organic pollutants (POPs) in the open Oceans is under debate. In 1989/1990, a first global study covering the world's oceans reported polychlorinated biphenyls (PCBs) and other POPs mostly being taken up via air-to-water exchange. This was backed up by theoretical work that predicted air-water exchange (via net deposition) to be the main delivery pathway for PCBs into the Atlantic Ocean. The initial transfer of PCBs into the mixed surface ocean layer would lead to removal fluxes to deep oceans and sediments, probably by coupling to the 'biological' pump. Shelf sediments were identified as major repositories of PCBs, but the exact pathway of how the PCBs reached the sediments was not investigated. Removal of PCBs by deep water formation seems to be of regional importance, but represents only a small fraction of total PCB losses from the oceans. Most previously published ship-based transects that reported on POPs and their cycling were performed on North-South transects on the eastern side of the Atlantic Ocean. Yet the sampling regime was invariably affected by continental emissions off Europe and Africa, making extrapolations across the entire Atlantic Ocean difficult and fraught with uncertainties. In addition, oceans are not homogeneously well-mixed water bodies, but display strong gradients in temperature, productivity, and influx of terrestrial materials, including POPs. Numerous currents move water around and induce mixing both horizontally and, less efficiently, vertically. Recently, cruises that covered both sides of the Atlantic Ocean transect have shown important variations in concentrations as a function of ocean currents or river plumes, especially for perfluorinated compounds, chlorinated pesticides, PCBs and to a lesser degree also for polycyclic aromatic hydrocarbons (PAHs). Air-water exchange gradients for PCBs and OCPs (but not for PAHs) suggest that the major oligotrophic oceans display net volatilization. Similar results were also observed in the Pacific Ocean. As was previously concluded for PCBs, there is little indication that concentrations of PAHs in the surface water of the Atlantic Ocean have changed strongly over the last decade. Overall, the picture of POPs in the global oceans is complicated, but more details are emerging which support a scenario in which major parts of the oceans are returning POPs to the atmosphere.

**360 Biogeochemical controls on the remobilization and reservoirs of**

**polychlorinated biphenyls (PCBs) in Antarctica** A. Cabrerizo, IDAEA-CSIC / Department of Environmental Chemistry; J. Dachs, IDAEA-CSIC / Environmental Chemistry; D. Barcelo, IQABCSIC; K.C. Jones, Lancaster University / Lancaster Environment Centre. After decades of primary emissions, reservoirs of persistent organic pollutants (POPs) have accumulated in soils and snow/ice in Polar Regions. Even though once considered a final sink for these chemicals, several studies have shown evidence that historical burdens of POPs are currently being remobilized from retreating glaciers/ice cover in the Arctic and Antarctica. This remobilization may be enhanced under climatic change scenarios (changes in temperature, retreating ice,...) once primary emissions have decreased due to regulation actions, increasing their availability for exchange with the atmosphere, and therefore increasing the ecosystem's exposure to POPs. While an increase of 1 °C in ambient temperature due to climate change would increase current Antarctic atmospheric inventories of PCBs by 21-45 %, a concurrent increase of 0.5% SOM would counteract the influence of warming by reducing the POP fugacity in soil. A 1 °C increase in Antarctic temperatures will induce an increase of the soil-vegetation organic carbon and POP pools by 25%, becoming a net sink of POPs, and trapping up to 70 times more POPs than those remobilized to the atmosphere. Here we show, for the first time, field measurements proving that polychlorinated biphenyls (PCBs) are currently re-volatilizing from Antarctic snow/ice and soils. This remobilization is driven not only by temperature and ice-cover changes, but also by changes in soil organic matter (SOM) and other biogeochemical processes.

**361 WHO/UNEP-coordinated global surveys on concentrations of Persistent Organic Pollutants (POPs) in human milk** R. Malisch, K. Kypke, A. Kotz, State Institute for Chemical and Veterinary Analysis of Food; A. Tritscher, WHO GEMS / Food Programme, Food Safety and Zoonoses, World Health Organization; K. Magulova, United Nations Environment Programme (UNEP), Stockholm Convention Secretariat, International Environment House; H. Fiedler, UNEPDTIE Chemicals Branch. Since 1987 the World Health Organization (WHO) has carried out global surveys on the concentrations of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) in human milk. The first WHO-coordinated exposure study took place in 1987-1988, the second round in 1992-1993 and the third round in 2000-2003. These studies fulfil important requirements for biomonitoring as requested by the Stockholm Convention on Persistent Organic Pollutants (signed in 2001 and entered into force in 2004). The objective of this Stockholm Convention is to protect human health and environment from POPs by reducing or eliminating their releases into the environment. For the evaluation of the effectiveness, human milk was chosen as one of the two core matrices to be monitored under the Stockholm Convention. Therefore, it was agreed expand the studies for inclusion of the Stockholm Convention POPs and to perform the WHO human milk surveys in close collaboration with United Nations Environment Programme (UNEP) and the Stockholm Convention Secretariat. Thus, the fourth round in 2005-2007 and the since 2008 following continuous studies were organized as joint WHO/UNEP studies. A comprehensive protocol was developed for collection of representative samples, handling and analysis samples. 66 countries participated between 2000 and 2011 submitting 84 pooled (= mixed representative) samples. Large global and regional differences with respect to contamination of human milk with different POPs were found. Time trends can be derived for countries with repeated participation over time. Selected results will be presented to give an overview of the complex picture. References <sup>1</sup> Environmental Health Series No 34 (1989): Levels of PCBs, PCDDs, and PCDFs in breast milk, WHO Regional Office for Europe, Copenhagen, Denmark. <sup>2</sup> Environmental Health in Europe No. 3 (1996): Levels of PCBs, PCDDs and PCDFs in human milk: Second round of WHO-coordinated exposure study, WHO Regional Office for Europe, Copenhagen, Denmark. <sup>3</sup> WHO (2000) Quality Assessment of Levels of PCBs, PCDDs and PCDFs in Human Milk and Blood Plasma – fourth round of WHO-coordinated study (2000), EUR/00/5020352, WHO Regional Office for Europe, Copenhagen, Denmark. <sup>4</sup> Rainer Malisch and FX Rolaf van Leeuwen

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### 362 Bioanalytical tools to measure endocrine active compounds

E.D. Leusch, Griffith University Smart Water Research Centre / School of Environment; A. Hebert, Veolia Environnement Recherche et Innovation SNC; M. Schriks, KWR Watercycle Research Institute. Chemicals present in the environment have been conclusively linked to endocrine dysfunction in exposed wildlife. While the evidence from wildlife is overwhelming and conclusive, the evidence for human effect is less so. Nevertheless, the precautionary principle would dictate that we take endocrine disruption as a potentially serious threat to human life even at low concentrations, and it is important to understand the contribution from water and its relevance. Methods that rely on biological activity are finding increasing utility as screening tools, because the chemical nature of complex water samples may be unknown and/or difficult to quantify/identify, and the biological method may be the best (or only) indicator of biological activity. Due to the complex nature of the endocrine system it is important to assess multiple endpoints when investigating endocrine active compounds. Much of the work on endocrine disruption from exposure to environmental contaminants has been carried out on the estrogenic axis. In the last decade substantial effort has been put into the development of bioassays to assess the estrogenicity of various waters. But the endocrine system is not simply about estrogenic activity, and several other hormonal systems play a crucial role in the maintenance of homeostasis, sexual development, metabolism, growth and behaviour. Substantially less information is available on those other endocrine endpoints, although it is becoming clear that these pathways can also be disrupted by exposure to environmental contaminants. The Global Water Research Coalition (GWRC) project “bioanalytical tools to analyse hormonal activity in environmental waters” continues and expands on previous GWRC efforts to develop and validate methods to measure estrogenic activity in water to include a range of substantially less well-studied endocrine endpoints. Our review specifically focused on androgens, progestagens, steroidogenesis, glucocorticoids, thyroid hormone, PPAR and retinoid acid receptors, and collected information on: production, function and molecular and cellular mode of action of the different hormones; overview of chemicals identified as EDCs for each particular endpoint;

review of *in vitro* methods currently available for each endpoint; current status of interassay comparisons and interlaboratory validations; reported activity in environmental waters; and an expert evaluation of the different *in vitro* methods currently available.

### 363 Transcriptomics and/or functional genomics tools for effect based analysis of environmental water

S. Van der Linden, B. Pieterse, Biodetection Systems BV; R. Cavill, Maastricht University / Department of Toxicogenomics; B. Brouwer, VU University Amsterdam / Department of Animal Ecology; B. van der Burg, Biodetection Systems BV. Many chemicals that are intentionally or unintentionally produced finally end up in the aquatic environment, where they may build up or can be degraded to a multitude of unknown breakdown products. As some of these compounds can withstand environmental degradation or sometimes even water purification treatment steps, animals and humans may run the risk of continuous exposure to complex mixtures of unknown composition and unknown effects. Chemical analysis alone will not suffice to determine the presence of (compounds with) adverse effects. Partly because it is complicated to - cost effectively - identify all components present in complex mixtures like an environmental extract, but also because knowledge regarding their biological effects is largely lacking. One way to tackle this problem is by using *in vitro* bioassays that respond specifically to groups of compounds with a known mode of action. By focusing on essential pathways that are known to be linked to adverse effects (i.e. adverse outcome pathways (AOPs), the presence of compounds that can disrupt these key AOPs can be determined. We have developed a panel of bioassays focusing on several AOPs that are related to different aspects of human and animal health, most importantly pathways related to endocrine disruption and genotoxicity. These allow for a fast quantification of a multitude of effects in environmental samples. A more holistic way of analyzing the presence of adverse effects qualitatively, might be by measuring the effects on a multitude of genes simultaneously, using transcriptomics tools. The gene expression pattern, e.g. analyzed by using microarrays, may be indicative of the types of activity present in the sample. We have analyzed a range of pure compounds and environmental samples for activity on a large set of nuclear (hormone) receptors. Simultaneously, we have analyzed gene expression pattern from cells that are exposed to the same set of samples, using microarrays for quantification of the genes expressed. An overview of these analyses on a range of pathways will be presented and the results from reporter gene assays and gene expression analysis will be compared. As many of AOPs are thought to be of key importance for human and animal health and many of these pathways are relatively conserved, specific effects detected on these pathways are thought to be of high relevance for hazard and quality assessment of environmental water and related matrices.

### 364 Endocrine Disruption Potentials and Related Mechanisms of Several Organophosphate Flame Retardants

X. Liu, Seoul National University / School of Public Health; K. Ji, Seoul National University; H. Moon, Hanyang University / Marine Environment Research Division; K. Choi, Seoul National University / School of Public Health. Organophosphate flame retardants (OPFRs) have been widely used as alternatives to polybrominated diphenyl ethers (PBDE). However, knowledge on their potential toxicities is limited. In this study, cell lines and a zebrafish (*Danio rerio*) model were employed to investigate the endocrine disruption potency and underlying mechanisms of several OPFRs. Firstly, two human cell lines (H295R and MVLN) were employed to screen the endocrine disrupting potentials of six OPFRs, i.e., tris-(2-chlorethyl) phosphate (TCEP), tris-2-chloroisopropyl phosphate (TCPP), tris-(1,3-dichloro-2-propyl) phosphate (TDCPP), tris-(2-butoxyethyl) phosphate (TBEP), triphenyl phosphate (TPP), and tricresylphosphate (TCP). By all six OPFRs, both 17 $\beta$ -estradiol (E2) and testosterone (T) concentrations increased in H295R cells, and transcription of major steroidogenic genes was upregulated but sulfotransferase genes were downregulated. In MVLN cells, TDCPP, TPP, and TCP acted as ER antagonists. Then, a 21 d reproduction test was conducted to study their effects on reproduction

performances. In this test, adult fish pairs were exposed to TDCPP or TPP (0, 0.04, 0.2, and 1.0 mg/L) for 21 d. The results showed that fecundities were significantly decreased, which were accompanied with significant increases of plasma E2, vitellogenin (VTG), E2/T and E2/11-KT, and decreases of T and 11-KT. Altered transcription of genes along hypothalamus-pituitary-gonad (HPG) axis was sex-dependent. Finally, a long term exposure was conducted to assess developmental toxicity and cross-talks among the HPG, hypothalamus-pituitary-thyroid (HPT) and hypothalamic-pituitary-adrenal (HPA) axes. In this test, fish eggs were exposed to 0.005, 0.05 and 0.5 mg/L TDCPP or TPP for 120 d. After exposure, conditional factor (CF), gonad somatic index (GSI), and liver somatic index (LSI) were significantly decreased. In females, exposure to TDCPP or TPP led to increase of cortisol, follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroxine (T4), triiodothyronine (T3), and E2, and lesser concentration of 11-KT. In males, decreased plasma cortisol, FSH, LH, T4, T3, T, and 11-KT were observed. Transcription of genes along HPG, HPA and HPT axis were mostly up-regulated in female, while down-regulated in males. The results of a series of experiments showed that several OPFRs are endocrine disrupting even at levels environmentally occurring. Ecological implications of these observations deserve further investigation.

**365 Glucocorticoid-like compounds detected in wastewater and river water samples from the Czech Republic and Switzerland.** Macikova, Masaryk University / Faculty of Science, RECETOX; A.A. Ammann, K.J. Groh, K. Schirmer, M.J. Suter, Eawag. Many contaminants found in surface waters and wastewater can disturb endocrine functions, for example via interaction with the endogenous hormone receptors. Glucocorticoids are among the hormones that regulate key physiological processes such as immune response, energy metabolism and stress response. Disruption of glucocorticoid signaling has been associated with several adverse effects and diseases including teratogenicity, obesity, type 2 diabetes or inflammatory and autoimmune diseases. A number of chemicals known to interfere with glucocorticoid-hormone signaling pathway were detected in river water and wastewater extracts using HPLC-MS/MS. Pure compounds as well as extracts of environmental samples were further assessed for their ability to interfere with glucocorticoid receptor using GR-CALUX assay. Relative potencies of pure compounds to elicit GR-mediated activity were calculated compared to dexamethasone (Dex) as the standard. Glucocorticoid-like activity was detected in untreated hospital wastewater effluents as well as in treated municipal WWTP effluents, and reached up to 542 ng Dex-equivalents/L (hospital WW), resp. 37 ng Dex-EQ/L (WWTP effluent). GR-mediated activity of river water samples was very low and could not have been calculated from the bioassay, however, concentration of GCs in rivers were not negligible. Furthermore, anti-glucocorticoid activity was found in some river water samples suggesting that glucocorticoid-like effects of the whole mixture could be masked.

**366 Identification of Thyroid Hormone-Disrupting Compounds in Polar Bear Plasma by Effect-Directed Analysis (EDA)** E. Simon, IVM VU University; E. Lie, The Norwegian School of Veterinary Science (NVH); K. Loken, The Norwegian School of Veterinary Science; J. Bytingsvik, B.M. Jenssen, Norwegian University of Science and Technology; M. van Velzen, J. de Boer, T. Hamers, M. Lamoree, Inst. for Environmental Studies (IVM) - VU University. Recent studies demonstrated that in addition to other endocrine systems the thyroid hormone (TH) system is also vulnerable for environmental contaminants. TH-disrupting compounds can bioaccumulate in adipose tissue and blood of top predators and are capable of competitive binding to transthyretin (TTR), which is one of the transport proteins of the TH, thyroxine (T4). Effect-Directed Analysis (EDA) was performed on polar bear plasma samples to identify the compounds responsible for the elevated TH-disrupting potency measured in the radioligand T4\*-TTR binding assay. A set of initially target analyzed TTR-binding compounds explained ~40% of the measured activities. In order to detect the compounds causing the remaining activity, a generic

identification strategy was developed. The extracts were analyzed by high resolution LC-ToF-MS. Library-based identification was applied to the full-scan data screening for accurate mass- and isotope pattern-match between the compound lists and the data files. The libraries were compiled based on different selection criteria, such as TTR-binding-, blood accumulating potency and environmental occurrence of the compounds. Out of the tentatively identified 23 suspects nonylphenol was confirmed chemically by evaluating its chromatographic and mass spectrometric behaviour after simultaneous injection of its analytical standard and the sample extracts on the LC-TOF-MS. Then, Isotope Cluster Analysis (ICA) was applied to the original LC-ToF-MS data enabling specific screening of halogenated isotope patterns. This strategy led us to the successful identification and confirmation of three (di-)hydroxylated octachlorinated biphenyls. As no pure standards were available, these compounds were successfully synthesized from their corresponding methoxylated form prior to analytical and toxicological confirmation. All analytically confirmed, identified compounds (i.e. nonylphenol and (di)OH-octaCBs) showed TTR-binding potency in the bioassay and could explain another ~35% of the total measured TTR-binding activities. However, the contribution to the total measured activity of nonylphenol compared to the (di)OH-octaCBs is negligible due to its weaker TTR-binding affinity its plasma concentration level is remarkable (2.5-6.2 µg/L). A major but very trivial obstacle for the confirmation of toxicants that is the often limited availability of pure standards that are required for the confirmation of the identified compounds.

**367 Can PNEC for estrogenic in vitro assays be derived?** B. Jarosova, Masaryk University / Faculty of Science, RECETOX; K. Hilscherova, Masaryk University Faculty of Science RECETOX / Faculty of Science, RECETOX; L. Blaha, Masaryk University, Faculty of Science, RECETOX / Research Centre for Toxic Compounds in the Environment (RECETOX). Since *in vivo* potential of estrogenic substances differs from their *in vitro* potential, results of *in vitro* bioassays alone cannot be directly used in environmental assessment. However, estrogenic *in vitro* bioassays are so sensitive, relatively inexpensive and rapid that they would be a very good tool for environmental risk assessment if we could better interpret their results. Therefore we decided to review the literature and gather information about: i) compounds which were responsible for measured estrogenic activity in European waste waters ii) relative *in vitro* potency (relative to standard estrogen (17β-estradiol, E2)) of these substances iii) the range of ratios in which these substances might occur in waste water samples and iv) *in vivo* derived PNECs for these substances. Based on this review, we would like in the platform presentation to derive possible ranges of PNECs for different *in vitro* bioassays and discuss uncertainties of their possible use.

**368 Which are the synergists? And are they of any importance from a risk assessment perspective?** N. Cedergreen, Royal Veterinary Agricultural University / Department of Plant and Environmental Sciences; M.A. Bjergager, University of Copenhagen / Faculty of Science. The past decades of research have shown that joint toxicity of non-interacting chemical mixtures can be well predicted by the reference models of Concentration Addition and Independent Action. Despite this, little has yet been done to incorporate this knowledge in legislation and risk assessment of chemical mixtures. One reason often given is the concern of synergy. Synergy is defined as chemical interactions leading to larger biological effects than those predicted by a reference model. The question is, however, how often significant synergism actually occurs under natural conditions? And how severe it is? And which chemicals are likely to induce synergy? To answer these questions we reviewed more than 1000 chemical combinations reported in the literature, picking out the studies where synergy was well described and the ratio between the predicted and observed effect concentrations, the Synergy Ratio (SR), was larger than 2. We focused on four well investigated groups of chemicals likely to co-occur in the environment: pesticides, pharmaceuticals, metals and antifoulants. For the mixtures of pesticides, pharmaceuticals and metals, the frequency of synergy was

remarcably stable around 5%. The average synergy factor for the pesticides and pharmaceuticals was  $5.01 \pm 4.30$  ( $n=50$ ), whereas for the metals it was  $2.89 \pm 1.05$  ( $n=4$ ) and for the antifoulants it was  $6.98 \pm 8.18$  ( $n=27$ ). For the 50 synergistic pesticide combinations either azole fungicides, organophosphates or cabamate insecticides were involved. These groups of pesticides are known to interact with either P450 monooxygenases or esterases known to be involved in the degradation of xenobiotics. The synergy could therefore be explained by toxicokinetic interactions in all cases. The few metal combinations showing synergy did not reveal any specific trends in terms of synergists. The modes of action of the antifoulants are very diverse, and no pattern relating which of the compounds acted as synergists could be distinguished. Approximately 95% of the tested mixtures show additivity or antagonism. This is an important take-home message, when it comes to risk assessment. For the synergistic pesticide mixtures, synergy is not occurring randomly but seem to be associated with pesticides already known to interact with the metabolism of other pesticides. Hence, a more focussed regulation for these compounds could be made by for example adding extra safetyfactors.

**369 Independent, additive, synergistic and complex response behaviour of microorganisms on toxic mixtures characterized by micro fluid segment technique** J. cao, Ilmenau University of Technology / Inst. for Chemistry and Biotechnology; A. Knauer, Ilmenau University of Technology; m. köhler University / Phys ChemMicro Reaction Technology. The determination of dose/response functions for combination of drugs in different concentrations is expensive and time-consuming by use of conventional methods. The micro fluid segment technique allows the complete screening of two-dimensional concentration spaces at a very low consumption of chemicals. Here, it is shown that this technique is well suited for distinguishing different characteristics of combinatorial effects. Segments were formed by mixing the cell suspension with the three effector solutions and additional buffer. Fluid segments with single volumes of about 0.5  $\mu$ L have been formed. An automated evaluation of toxic effects can easily be realized by multi-channel micro flow-through photometry and fluorimetry. This arrangement allows the measuring of cell density by optical transmission as well as the detection of coloured cell populations and the measurement of autofluorescence or under the addition of fluorescent sensor particles. The investigation of the behaviour of *E. Coli* and *Chlorella vulgaris* on combinations of different frequently applied drugs (antibiotics and substances for blood pressure control), food components, a widely used herbicide as well as metallic nanoparticles resulted in very different characteristics of cellular response: Independent, additive, synergistic as well as complex response behaviour of microorganisms on the different binary and ternary mixtures have been found. In particular it was found that the toxicity of silver nitrate solution and silver nanoparticles on *chlorella* is comparable for the pure substances, but leads to a significant different behaviour in case of combination with atrazine. A strong activation of the growth of *E. coli* in the presence of ampicillin was found in dependence of the concentrations of caffeine and the blood pressure drug. The investigations show that all principle types of combinatorial behaviour can be observed in microtoxicological screenings by the applied microfluidic method. In addition, it was found that non-monotonous functions have to be expected and that complex response pattern can be found including cellular growth behind the critical thresholds of concentration of a single substance if binary or ternary mixtures are applied. It can be concluded that there is an urgent need for the use and further development of micro fluidic techniques in order to realize such studies with a reasonable effort.

**370 How low can you go?** M.A. Bjergager, University of Copenhagen / Faculty of Science; K. Norgaard, Roskilde University; A. Kretschmann, University of Copenhagen / Faculty of Science; P. Mayer, Aarhus University / Department of Environmental Science; N. Cedergreen, Royal Veterinary Agricultural University / Department of Plant and Environmental Sciences. Several studies have shown synergistic interaction between the azole fungicides and pyrethroid

insecticides towards aquatic and terrestrial organisms both in controlled laboratory and in outdoor microcosm studies. Both types of pesticides are among the most widely used groups of pesticides and may occur in the environment together. We could expect, however, that there is a lower threshold concentration below which the azole fungicides do not enhance the toxic effect of the pyrethroids. To test this hypothesis, laboratory experiments with *Daphnia magna* were carried out testing the synergising potential of the three azole fungicides propiconazole, epoxiconazole and prochloraz on the pyrethroid insecticide alpha-cypermethrin. Two types of experiments were conducted. In the first experiments, full concentration-response setups with alpha-cypermethrin were conducted with six varying constant concentrations of each of the fungicides. Pesticide concentrations in these setups could, however, not be confirmed analytically, hence, a second approach using passive dosing techniques was tested. In this setup, a dilution series ranging from 1.56  $\mu$ g/L to 200  $\mu$ g/L fungicide was tested in combination with two fixed alpha-cypermethrin concentrations of 0.01  $\mu$ g/L and 0.1  $\mu$ g/L corresponding to EC<sub>50</sub> and EC<sub>10</sub>, respectively. All pesticides were also assessed singly and untreated controls were included. The results showed that there was indeed a threshold for the three fungicides, below which less than two-fold synergy occurred. Prochloraz was observed to decrease the alpha-cypermethrin EC<sub>50</sub> more than a factor of two at concentrations of  $34 \pm 14$   $\mu$ g/L and above. At the concentrations tested, prochloraz in itself is not expected to have any significant acute effect at *D. magna* as its EC<sub>50</sub>-value has previously been estimated to 3500  $\mu$ g/L. Propiconazole and epoxiconazole showed similar synergising patterns as prochloraz. To be able to discuss the results in an environmental context we need measured and not only nominal concentrations. The passive dosing experiments including chemical analyses of the test media will add to the information about the threshold concentration. Based on these experiments, the range of the threshold values found for the three fungicides will be discussed in relation to fungicide concentrations measured in the environment. Also the consequences of test system choice for the evaluation of synergy will be discussed.

**371 Non-toxic chemicals become toxic in a mixture – “solubility addition” of solid hydrophobic chemicals increases exposure.** Mayer, Technical University of Denmark / Department of Environmental Engineering; K.E. Smith, Aarhus University / Department of Environmental Science; S.N. Norgaard Schmidt, Aarhus University / Environmental Chemistry and Microbiology; N. Dom, University of Antwerp / Biology; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology. This study addresses the question whether hydrophobic organic chemicals that exert no toxicity at their solubility limit (saturation) can form a toxic mixture. Spiking methods generally do not allow testing exactly at saturation without introducing microcrystals. Passive dosing was thus applied to test the acute toxicity of several high melting point polycyclic aromatic hydrocarbons (PAHs) and their mixtures at the respective saturation levels to *Daphnia magna*. Anthracene, chrysene and benzo(a)pyrene exerted no or only limited acute toxicity (0-20%), whereas binary and tertiary mixtures of these resulted in significant acute toxicity (70-88%). It is in such cases not possible to describe and predict mixture toxicity using the toxic unit approach, since there are no toxicity data to calculate the toxic units. The toxicity of five PAHs and their mixtures were instead fitted with one (sum) chemical activity-response curve indicating a similar mode of toxic action (i.e., concentration addition). The effective chemical activity (Ea-50) of 0.029 and the effective concentration on a lipid basis (EC<sub>50</sub><sup>lipid</sup>, eq.-50) of 95.7 mM were well within the range for baseline toxicity. These results raise questions about the focus of risk assessment schemes and toxicity testing guidelines on individual substances, since apparently non-toxic chemicals might become toxic in a mixture.

**372 Neuroprobabilistic Integrated Risk Index of Chemical Aquatic Pollution (IRICAP): Case studies in Spanish river basins.** fabrega, Universitat Rovira i Virgili / Chemical engineering department; s. kumar; A. Ginebreda, IDAEACSIC; M. Schuhmacher, Rovira i Virgili University; J. Domingo, Universitat Rovira i Virgili; M. Nadal,

University Rovira i Virgili. Because of technical advances, an increasing number of chemicals are found in the environment in general, and in aquatic compartments in particular. Those substances create a notable concern since many of them may pose an important risk for the human health. Because of this large number of pollutants, reliable prioritization methods are clearly necessary. One of the most extended methods to prioritize the hazard of different compounds is related to their properties of persistence, bioaccumulation and toxicity, also known as PBT. Furthermore, these data can be clustered by using Self-organizing Maps (SOM), a special kind of neural networks with the capability to cluster data depending on PBT similarities. In previous works, a SOM-based Hazard Index (HI) was successfully applied in soils, being afterwards improved taking into account neuroprobabilistic aspects. The objective of the present study was to develop a neuroprobabilistic Integrated Risk Index of Chemical Aquatic Pollution (IRICAP), whose aim is to evaluate the human health risk associated to the exposure of chemical mixtures contained in river water. For the IRICAP development, a new HI was created by considering nearly 200 compounds, including pharmaceutical products, illicit drugs, estrogens, detergents, pesticides, perfluorinated compounds, endocrine disruptors, and persistent organic pollutants. According to the HI outcomes, the most hazardous compounds were perfluorinated compounds, pesticides, and illicit drugs. In the second step, a neuroprobabilistic IRICAP was developed and applied to four river basins in Spain: Llobregat, Ebro, Júcar and Guadalquivir. An IRICAP value was calculated in those sampling sites where monitoring data were available. For that purpose, the HI corresponding to each chemical was multiplied by the concentration of that pollutant, and the results of all substances were aggregated. Subsequently, a risk profile along each river basin was obtained. Finally, a Pareto distribution was applied to prioritize those chemicals with the most significant incidence on the IRICAP. Therefore, those pollutants posing the highest risk were identified, making easier the implementation by regulatory organizations.

**373 Retrospective RA for almost 400 analytes – challenges and lessons learned from applying current assessment schemes for mixture toxicity under the WFD** M. Junghans, Swiss Centre for Applied Ecotoxicology EAWAG EPF / Ecotox Centre; P. Kunz; R. Gauch, Oekotoxzentrum / EPFL; S. von Arb, Swiss Centre for Applied Ecotoxicology Eawag-EPFL; C. Moschet, Eawag / Uchem; A. Piazzoli, ETH; I. Wittmer, Eawag; H. Singer, Eawag - aquatic research; C. Stamm, Eawag; J. Hollender, Eawag / Dept Environmental Chemistry; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy, Physiology and Cell Biology. Modern chemical analytics are able to detect increasing numbers of chemical substances in a single run. Measurement campaigns in surface waters can yield analytical results for several hundred substances per sample. For assessing the chemical status of a water body under the Water Framework Directive (WFD) based on such measurements, only single substances are currently evaluated for their compliance with Environmental Quality Standards (EQSs). With more than 100 substances detected in a single sample however, the need for an assessment of the combined risk of these substances becomes pressing. Recently, a decision tree for prioritising samples regarding mixture risk assessment was proposed, that combines the mixture toxicity framework proposed by the WHO with the decision tree for mixture toxicity assessment proposed by EU scientific committees. This decision tree was applied to analytical results from a recent measurement campaign in Swiss surface waters, in which 379 organic substances were analysed by high resolution mass spectrometry. These analytes comprised biocides, plant-protection products, pharmaceuticals and their transformation products as well as some substances from other applications. The number of substances quantified in 34 individual samples ranged from 42 to 138 (median = 105). Of these substances 214 were detected in at least one sample. Most EQS could be found for plant protection products. For transformation products as well as for pharmaceuticals the EQS availability was generally poor. The samples are classified into 3 classes: I) a risk quotient (RQ=measured concentration/EQS) greater than 1 already for single substances in the sample II) no risk identified and III) risk

identified only if combined risk is assessed. Subsequently, risk assessment is further refined for samples in classes I and III, by analyzing the risk for the different taxonomic groups. The study shows that the bottlenecks of mixture risk assessment under the WFD are: identification of mixture components and availability of EQSs and/or toxicity data especially for pharmaceuticals and transformation products. In addition the presence of the insecticide synergist piperonyl butoxide in 7 samples proved to be a challenge for mixture risk assessments.

**374 What if Science follows Policies?** B. Cheze, IFP Energies nouvelles; L. Patouillard, IFP Energies nouvelles / Economics and environmental evaluation department. One of the drivers for the development of advanced biofuels (i.e. biomass-based fuels produced from lignocellulosic materials (G2) and microalgae (G3)) is the will of policy makers to increase the share of biofuels in the transport sector. For instance, the United States (US) and the European Union (EU) have recently developed regulations inciting biofuel production. Both regulations set minimum thresholds for life cycle greenhouse gas (GHG) emission savings compared to fossil fuel as the most important environmental criteria that should be met to be eligible as sustainable biofuel. Despite a substantial literature on advanced biofuel, there is no consensus about their Life Cycle Analysis (LCA) GHG emission benefits compared to fossil fuels and the fact that they can meet legal GHG emission targets remains unclear. Conversely, the extent to which literature that meets those targets are more likely to be published has to be examined. This paper investigates the potential influence of biofuel policies on LCA GHG emission results of advanced biofuels by identifying publication biases thanks to specific set of analytical techniques (Funnel graphs and Galbraith plots). Then an harmonisation is performed using specific econometrics method (meta-regression analysis), corrected from publication biases. The purpose of this harmonisation is to identify and quantify key factors that influence LCA GHG emission results of advanced biofuels in order to be more conclusive on its real performances and to support policy makers. This quantitative literature review is based on 47 LCA studies providing 593 observations. Results reveal the existence of an asymmetrical publication bias of North American (NA) vs. EU studies, highlighting the influence of public policy designs on the very scientific publication process. After identifying the main determinants of the variability of advanced biofuel LCA GHG emission results, we perform harmonisation correcting from identified biases. Our results indicates a hierarchy between advanced biofuels. Mean value and Confidence Interval of LCA GHG emissions weighted by the influence of its main drivers for BtL, Ethanol and G3 biofuel are estimated to 19.5 [16.7;22.2], 19.7 [17.4;22.0] and 60.0 [43.3;76.7] gCO<sub>2</sub> eq/MJ of biofuel respectively. Even if this range of values is lower than fossil reference (about 83.8 in gCO<sub>2</sub> eq/MJ), only G2 Ethanol and BtL do comply with the GHG emission reduction thresholds defined in the US and EU regulations.

**375 Geopolitical Implications of Life Cycle Assessment** G. Sonnemann, . Life Cycle Assessment (LCA) has been developed as a tool for the systematic evaluation of the environmental impacts of a product through all stages of its life cycle. By addressing both the emissions to the environment and the resource use of a product system LCA has a number of geopolitical implications. On the one side it accounts for the emissions covered under the Multilateral Environmental Agreements that are legal instruments for environmental protection, governed by international law. On the other hand, it accounts for resource uses like water, land & soil, fossil fuels and minerals, including the so-called critical materials, and in this way it is also used as a risk assessment tool, addressing among other issues geopolitical aspects in the supply chain. Emissions related to the UN Framework Convention on Climate Change are taken into account by assessing the Global Warming Potential. The Ozone Layer Depletion Potential assesses ozone-depleting chemicals covered under the Vienna Convention. Chemicals included in the Stockholm and Rotterdam Conventions are part of various Life Cycle Toxicity Impact Models such as USEtox. These Conventions can assist companies in identifying the emissions of

which a sound management is a globally agreed priority. The challenge of ensuring the Earth's ecosystem services that more and more companies are trying to take adequately into account has also geopolitical implications and is in the hands of the Convention on Biological Diversity. With regard to resource uses the increased use of geographic information in life cycle assessment allows to better address biodiversity loss and to map the supply chain of companies for their products and hence to identify potential future supply constraints for instance for water due to risks related to changing weather patterns and for minerals due to the risk of conflicts. The further development of advanced LCA methods using geographic information system will facilitate a growth in using LCA for this type of applications. Hence, overall, it can be expected that LCA, which was developed as a tool for assessing environmental impacts, will be used increasingly as an instrument to address geopolitical aspects and to identify related risks throughout the life cycle of a product.

### **376 UNEP Capacity Building Approach towards Mainstreaming Life Cycle Thinking in the Emerging and Rapidly Growing Economies**

**A. Quiros, ECO GLOBAL ALCALA; S. Valdivia, UNEP / Secretariat ILCI; T. Mermer, UNEP.** The main limiting factor in the developing world to advance more LCA based policies and applications is the lack of necessary capacity to understand, develop and use life cycle (LC) based approaches towards their use for strategic purposes. Thus, capacity building activities for the implementation of recommended policies and tools by the public and private sectors are fundamental. The 3-year project on *'Enabling companies and consumers to benefit from information on life cycle environmental performance of products choices'* started in March 2012 and is being implemented by UNEP. Its aim is to promote quantification and assessment methods of LC impacts and a management system and their use and to facilitate the agreement on product sustainability communication principles. The project will be implemented in Latin America, Africa and Asia through the following: a) Capacity building on LC based quantification and assessment tools; b) Capacity building for the development of life-cycle data for the main export commodities and products; c) Pilot cases on LCM in business and governments in three countries; and d) consensus building and dissemination of internationally recognised principles for communication. The target groups include: i) governments to create the relevant enabling policy framework, ii) standard-setters to participate in international negotiations, iii) business and industry to implement such tools; and iii) academics and other training institutions to continue building capacity.

### **377 The role of consequential LCA for private and public decision-making: status-quo and perspectives**

**E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies (CRTE); M. Guiton, Resource Centre for Environmental Technologies (CRTE); A. Marvuglia, CRP Henri Tudor / Cork Constraint Computation Centre; I. Vázquez-Rowe, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE; E. Igos, S. Rege, CRP Henri Tudor / Resource Centre for Environmental Technologies (CRTE); E. Popovici, Public Research Centre Henri Tudor / Resource Centre for Environmental Technologies (CRTE); J. Garcia, SCORE LCA.** Since a decade, consequential life cycle assessment (cLCA) approach is preconized to comprehensively assess the consequences, in terms of induced environmental impacts, of human driven actions, most often related to policy or strategic decisions. cLCA has been defined as "the attempt to describe how environmentally relevant flows will change in response to possible decisions" or "how micro-economic decisions do influence macro-economic scenarios". While in principle cLCA adopts a completely different perspective than conventional (attributational) LCA, in practice, the type of questions which can be answered by cLCA, as well as the operational approach, data needs, limitations, uncertainties and finally its practicability remain dramatically unclear to most of practitioners in industry and to policy makers. At the current stage of development of cLCA, there is clearly a gap between academic research on one side and stakeholders needs and understanding on the other side. The SCORE LCA association, including leading industry players (EDF,

GDF SUEZ, Renault, Saint-Gobain, Total, Veolia) and the French Environmental Protection Agency (ADEME), together with the CRP Henri Tudor initiated a research study aiming at contributing to fill these gaps. The first step of the study was to draw a comprehensive and independent status quo of cLCA, as compared to the other LCA perspective, based on a literature review and on the position of cLCA experts interviewed via a detailed questionnaire. The aim of the status-quo was to position the different LCA perspectives around a number of criteria (pertaining e.g. to the decision context, the modelling approaches, ...) and thus highlighting methodological inconsistencies, gaps and further research challenges. The executive synthesis and overall conclusions are currently being harmonized. However, it can already be inferred from this study that cLCA is certainly a meaningful concept to address large scale decision processes in the studied sectors (at industrial and policy level) but, at the present state of development, lacks of operability and harmonization. The main research challenges to be addressed are the explicit definition of the decision context (and its boundaries), the position of the change(s) to be analysed along the time horizon, the availability, transparency and reliability of consequential inventory data and finally the communication of the results, eventually in relation to aLCAs of the same product system.

### **378 Application of three independent consequential LCA approaches to the agricultural sector in Luxembourg**

**S. Vázquez-Rowe, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE; A. Marvuglia, CRP Henri Tudor / Cork Constraint Computation Centre; J. Thenie, A. Haurie, ORDECSYS; S. Rege, E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies (CRTE).** Consequential LCA (C-LCA) is widely used to link micro-economic actions with macro-economic consequences, by identifying the marginal suppliers and technologies prone to be affected by large scale changes in the demand of a specific product. Moreover, it has shown to be useful to monitor environmental consequences linked to bioenergy production. However, detecting the marginal technologies subject to suffer indirect effects due to changes in the production system is not an easy task. Hence, to provide a C-LCA assessment, researchers have combined LCA with different econometric models, by adapting the principle of C-LCA to the specific characteristics of the analysed system. For instance, some studies suggest the use of dynamic economic models to identify possible consequences occurring at the agricultural stage of a given production system. On the contrary, other perspectives advocate for developing a simplified model of the related effects in C-LCA to obtain manageable systems, based on default assumptions and decision nodes. The main aim of this study is to assess one single case study linked to biocrops cultivation using three different C-LCA model approaches. Firstly, an economic modelling tool, the partial equilibrium (PE) model is integrated with C-LCA to detect changes in land cultivation based on the farmers' revenue maximization. This approach considers the agricultural sector as a closed system, allowing detailed representations of agricultural and land use restrictions. Secondly, another PE model perspective is proposed, which aims at minimizing a total adaptation cost (so called opportunity cost) to satisfy a given new demand of domestically produced biofuel. Finally, the consequential system delimitation for agricultural LCA approach, which seeks expert criteria to model the economic assumptions, was modelled. The chosen case study aims at assessing the environmental changes in the agricultural sector in Luxembourg due to a predicted increase in maize cultivation to produce biomethane (projected based on the 2020 target set by the Luxembourgish Renewable Energy Action Plan). Obtained results are used to perform a discussion on the strengths and weaknesses of applying the different approaches, as well as identifying the consequences they may engender in the bioenergy policy. Discussion will also focus on the main drawbacks and assumptions that were needed in each approach to adapt their characteristics to those of the selected case study.

**379 What is the right pathway to be sustainable? Case of biofuels and bioproducts in Europe** **S. Belboom, Department of Chemical Engineering - Processes and Sustainable Development; A. Leonard,**



University of Liege / Departement of Chemical Engineering - Processes and Sustainable Development. Due to subsidies and the European promotion of biofuels, crops areas used for the production of bioethanol were increased through years inducing direct and indirect land use change. A lot of studies about the sustainability of biofuels were carried out and results are mitigated depending on methodological assumptions and used feedstock. Biofuels for transportation is the most common application of the bioethanol but the chemical field is also easily accessible. Indeed, bioethanol can be catalytically dehydrated to obtain bioethylene which is a monomer allowing the production of a multitude of plastics. This study presents the comparison of two applications of the same product, bioethanol, using the LCA methodology with the ReCiPe 2008 method. Bioethanol is produced in Europe, using sugar beet or wheat, cultivated on the already available land. No direct or indirect land use change was taken into account. This study tends to evaluate the best use of the already accessible crops, not the expansion of the market. The final products taken into account are the biofuel E5 (5% of bioethanol in volume) and the High Density PolyEthylene. Agricultural areas in Western Europe are limited and then it is very important to dedicate their use to the best application, i.e. with the highest gain for climate change and fossil fuel reduction. The functional unit is then the use of one hectare of cultivated land. Results are shown as the difference between the impact of the life cycle of the amount of fossil fuels that can be replaced by the cultivation of one hectare of land and the impact of the life cycle of their agricultural equivalent. The comparison for climate change and fossil fuel depletion categories between both crops gives an advantage to sugar beet when the comparison is performed by hectare. This is due to the lowest yield of wheat per hectare. When comparing both applications, the gain for climate change and for fossil fuel is higher in both cases when using HDPE. This study showed, with a short example, that LCA methodology can be a powerful tool to assess the best application of the same product. In our case, the policy used for the promotion of biofuels permits to achieve a reduction of GHG emissions and resources consumptions but there is a more performant application which is the chemical use. As it was already the case for the waste field, a hierarchy of the use of land and agricultural products should be developed.

**380 Metals-induced temperature sensitivity in natural waters: Will chronic pollution amplify the effects of global warming on aquatic organisms?** M.L. Brooks, Aquatic Ecology Environmental Chemistry Lab / Dept of Zoology; T. Hallman, Oregon State University - Department of Fisheries and Wildlife. Evaluating the combined impacts of climate change and other anthropogenic disturbances on aquatic organisms is a tremendous ecological challenge. Metabolic oxygen demand from pollutants (e.g. elimination, sequestration) compounds the elevated metabolism of ectotherms at higher temperatures. Animals stressed by commonly observed pollution levels will have lower pejus temperatures ( $T_p$ ). Temperatures beyond  $T_p$  inhibit growth because costs from cellular oxygen demand (maximal aerobic efficiency) exceed oxygen availability. **Methods.** To consider risks of range contractions, Geographic Information System (GIS) coverages of the eastern United States (USA) combined current summer temperatures, current legally impaired waters (US EPA 303(d) listing), and  $2.5^\circ\text{C}$  temperature projections (IPCC A2 scenario). In outdoor mesocosms and filtered lake water, we measured energetics (e.g. growth rates, size at age), metals bioaccumulation, and body condition of Cope's grey tree frogs (*Hyla chrysoscelis*) from Gosner stage 25 to 42: 3 temperatures: ambient,  $+1.5^\circ\text{C}$ ,  $+2.5^\circ\text{C}$  x, 6 levels of Cd, Cu, and Pb mixtures x 4 replicates = 72 aquaria. Mixtures were bioavailable cumulative criterion units (BCCU)—the sum of free ionic activity per the criterion value as free ionic activity. Thus, BCCU are multiples of the protective criterion; herein 3.7 to 30 BCCU—10 BCCU is below USA drinking water standards. The 3.7 BCCU “control” was collected within a nature preserve. Adult frogs from the control and 30 BCCU treatments underwent 20-d, post-metamorphosis feeding trials. **Results.** In waters  $>10$ -fold BCCU. In  $>10$  BCCU, temperature explained 44% of variance and tissue Pb explained another 18%. Initial size at metamorphosis did not differ between 3.7 and 30 BCCU metamorphs. After 20-d feeding

trials, body condition of adults experiencing 30 BCCU and  $+2.5^\circ\text{C}$  as larvae declined by 200% relative to those raised in 30 BCCU without thermal stress as larvae. Control adults did not significantly differ among treatments. **Implications.** We consistently observed lower  $T_p$  as altered growth for animals stressed by metals  $>10$  BCCU. Thus, animals had different temperature tolerances *depending on water quality*. Data indicate that pollution will compound the energetic costs of global warming, causing range contractions among aquatic organisms in the geographic center of the Midwestern USA.

**381 Effects of competition and repeated exposure to a pesticide on populations of *Daphnia magna*** L. Dolciotti, UFZ Leipzig / System Ecotoxicology; K. Foit, UFZ Helmholtz Centre for Environmental Research; M. Liess, UFZ Center for Environmental Research / Department of System Ecotoxicology. In the evaluation of the toxicity of substances on aquatic non target animals, test organisms are exposed either to a short contamination of 24 or 48 hours or to a chronic one in which the test system is renewed every day to maintain a constant concentration. However, standard toxicity tests do not represent entirely the way aquatic organisms are exposed to contaminants in situ. In the field we rather observe sequential pulses of contaminants due to drift or run off. Large amounts of pesticide are applied to agriculture area each year with much of it washed into adjacent water bodies. Moreover populations in a community context experience intra and interspecific competition, interactions that influence the way a population is shaped and likely also the way a population respond to a contaminant. In our experiment we studied how repeated insecticide pulses affect populations of *Daphnia magna* in the presence of the important ecological factor competition. Populations of *Daphnia magna* were exposed to two pulses of the insecticide pirimicarb (3, 10, 24  $\mu\text{g/L}$ ). Each pulse was followed by a recovery period of 28 days. In half of the test-systems, mosquito larvae (*Culex pipiens*) were added to exert a strong competition pressure. Population abundance and biomass were quantified by image analysis. *Daphnia magna* showed an acute mortality up to 80% at the highest treatment concentration while *Culex pipiens* larvae were insensitive at all concentration levels. The acute mortality of *Daphnia* was not increasing from the first to the second pulse. The sufficiency of recovery time after each pulse was depending on the presence of competition: without *Culex* larvae, populations of *Daphnia* recovered fast and abundance repeatedly reached control level within the recovery time of 28 days. With *Culex* larvae, populations of *Daphnia* failed to recover, after the second pulse and one population became extinct. Our experiment revealed that competition might delay the recovery of *Daphnia magna* populations, a common test organism in ecotoxicology that is known to recover fast. We conclude that a high competition pressure combined with repeated pulses of pesticides might explain unexpected long-term effects observed in the field.

**382 Effects of silver nanoparticles on the functioning of estuarine bacterial communities** V. Echavarrri, HeriotWatt University / Centre for Marine Biodiversity and Biotechnology, School of Life Sciences; L. Paterson, Heriot-Watt University / School of Engineering and Physical Sciences; T.J. Aspray, Heriot-Watt University / School of Life Sciences; M. Hartl, HeriotWatt University / Centre for Marine Biodiversity and Biotechnology, School of Life Sciences. The rise of nanotechnology has led to an increase in the manufacturing and use of new materials that, at the nano-scale, exhibit different characteristics compared to their respective bulk material. Nano-silver is one of these materials, and, owing to its antimicrobial properties, is being incorporated in a wide variety of health and personal care products. As much of the silver is disposed of through domestic waste water, the question arises whether accumulation in the receiving estuarine environment could negatively affect the functioning of resident bacterial communities that play an important role in biogeochemical processes, providing important ecosystems services. The fate of AgNPs and their impact on highly dynamic estuarine ecosystems is still unclear. In the present study a microcosm approach was established in order to model the estuarine conditions to carry out a more realistic study of the effects of AgNPs on bacterial communities. It focused on the effects of AgNPs on bacterial

community functioning in water and sediments. The viability and abundance of bacteria was analysed using plate counts. Effects on the community metabolic profile was assessed based on the utilisation of different substrates and oxygen uptake. To assess the effects on the water quality, inorganic nitrogen and chemical oxygen demand (COD) were monitored. The results obtained revealed a significant reduction in the bacterial abundance was observed owing to the exposure to AgNPs which was reflected on the oxygen uptake rate. However the quality of the water was not affected based on the analysis of COD, nitrate and nitrite. The AgNPs used exhibited greater toxicity than those used in similar studies. This toxicity depended on their physicochemical characteristics, their interactions with the components of the environment in which they were released and the organism targeted. Understanding these aspects is essential for meaningful environmental risk assessment and regulation of an emerging technology. For this reason the AgNPs used in the exposures have been characterized under different salinity gradients. We conclude that, even though a negative impact on the abundance and viability of bacteria and also in the oxygen uptake was detected, this did not lead to a decrease in the water quality. This approach could enable the investigation of the acute toxicity of similar nanoparticles on estuarine and coastal bacterial communities in a cost-effective way.

**383 Microecosystems "bryophyte-microorganisms": a good tool for ecosystemic ecotoxicology** C. Meyer, Laboratory of ChronoEnvironment; D. GILBERT, N. BERNARD, University of Franche-Comte / Laboratory of Chrono-Environment. A new challenge for modern society is to understand the effect of pollutants on ecological integrity of ecosystems. This concept is used in several legal agreements on environmental protection and is defined by the ability of an ecosystem to support and maintain a community of organisms that as species composition, diversity and functional organisation (Parrish et al., 2003). Because the study of the impact of pollutants on ecosystems is complicated, microecosystems "bryophyte-microorganisms" could be good realistic models to understand the impacts of contaminants on ecosystems. Bryosystems constitute whole ecosystems integrating above- and belowground linkages that can be used in the study of environmental pollution on biodiversity and ecosystem functioning. For many years, we highlighted the potential of these microecosystems to assist in the study of the response of the microbial communities' structure, functional groups and the species composition of top predators to environmental disturbances i.e. particulate, particulate + NO and PAH atmospheric pollutions *in situ* and under controlled conditions. Our results showed that atmospheric contaminants have a direct effect on microbial species (decrease of biomass) and indicated that indirect effects exist through modifications of the interactions between microbial communities or between microbial communities and mosses. Among predators, testate amoebae (TA) are more affected (decrease of diversity or biomass of TA with superior trophic level) suggesting a decrease of the pressure of predation on lower trophic level, that is confirmed by the modification of the microbial communities' structure. Bryosystems are a good tool to evaluate the impacts of atmospheric pollutants on community ecology and it is now important to well understand the effects of these pollutants on microecosystem ecology (fluxes of energy and material among functional groups and their environment) and on food web ecology (structure and dynamics of species' feeding relationships). Thus, the use of such microecosystems could be reveal central and relevant information on anthropogenic disturbances at ecosystem scale.

**384 Comparison of abundance and trait responses of aquatic macroinvertebrate exposed to fungicide or hydrocarbon mixture in stream and pond mesocosms** Y. Bayona, INRAAgrocampus Ouest / UMR 985; M. Roucaute, INRA / UMR ESE; K. Cailleaud, Total Petrochemicals France / PERL; A. Basseres, TotalFinaElf; L.L. Lagadic, INRA / UMR INRAAgrocampus Ouest Ecology and Ecosystem Health; T. Caquet, INRA / UMR ESE 0985. Complex responses may be observed at the community level following exposure to chemicals due to a combination of direct and indirect effects affecting structural features

and functional processes. The nature and intensity of these effects depend on various factors including the characteristics of the ecosystem and the traits of exposed taxa. Community recovery may be more rapid in lotic than in lentic systems due to drifting of individuals from upstream. Conversely, lentic invertebrate species may be more adapted to stressful conditions such as low dissolved oxygen concentration. To address all these effects Ecological Risk Assessment (ERA) has to include endpoints that are more closely linked to ecosystem functioning than taxa abundances such as those involving biological and ecological traits. The purpose of the present study was to compare the effects of a dithiocarbamate fungicide (thiram) and a hydrocarbon mixture on pond and stream mesocosms. In both types of mesocosm, nominal exposure concentrations were of 35 and 170 µg/L for thiram whereas nominal hydrocarbon mixture concentrations were 0.01, 0.4, 2 and 20 mg/L. All treatments were performed in duplicates and four systems were kept as controls. Flow-through outdoor streams (length: 40 m, depth: 50 cm) were continuously treated for 3 weeks. Outdoor pond mesocosms (7.5 m<sup>3</sup>) were treated weekly for 4 weeks. The exposure period was followed by a 2- or 10-month recovery phase for stream and pond mesocosm, respectively. Macroinvertebrates were sampled using artificial substrates weekly or every 3 weeks in stream and pond mesocosms, respectively. Alder litter breakdown rate was measured using litter bags. Changes in the structure of the invertebrate community were analyzed by the PRC method and the ones in the trait structure were analyzed using the RLQ method. Hydrocarbon effect on communities was assessed by abundance and biological traits of macroinvertebrates and by litter breakdown rate, with recovery in most case. In hydrocarbon exposed mesocosm, effects only occur for concentrations above the toxic threshold assessed in MSDS. Fungicide impacted all measured endpoints without recovery in streams whereas only effect on biological traits was assessed in pond mesocosms. Finally, recovery depends of mesocosms and type of chemicals. Both differences in term of impact measurement and recovery potential (structure and traits) in dynamic and static mesocosm will be discussed in presentation.

**385 Ionic silver can directly and indirectly impairs the functioning of detritus-based aquatic ecosystems** J. Arce Funck, Laboratoire Interdisciplinaire des Environnements Continentaux; V. Felten, H. Clivot, F. Guerold, M. Danger, Université de Lorraine / Laboratoire Interdisciplinaire des Environnements Continentaux. Silver (Ag) is a very potent biocide found in natural water at concentrations ranging from 0.03 to 500 ng.L<sup>-1</sup>, but it has insufficiently been studied compared to other metals. The increasingly use of Ag nanoparticles can raise the risks for aquatic system as they can salt out ionic Ag, potentially affecting the organisms and in turn the whole ecosystem functioning. Forested headwater streams rely mainly on the decomposition of allochthonous organic matter as a source of nutrient and energy. In these ecosystems, initial stages of detritus decomposition are mainly ensured by aquatic hyphomycetes, followed by the degradation mediated by detritivores invertebrate (e.g. *Gammarus fossarum*). The successful detritus processing by both trophic levels is essential for the optimal functioning of detritus-based ecosystems. While the interactions between leaf litter, decomposers, and detritivores can be impaired by some metals, very little is known about Ag impact. We conducted two microcosm experiments to evaluate: (1) Ag effect on fungal decomposition process, and (2) its indirect impact on *G. fossarum* feeding rate, but also (3) *G. fossarum* Ag LC50s (according to gender and development status) and (4) Ag effect on *G. fossarum* physiological and behavioural biomarkers. Decomposition process, fungal community structure and gammarids feeding rate were significantly impacted only at the highest [Ag] (100 µg.L<sup>-1</sup>). 96h-LC50s indicated that juvenile gammarids are extremely sensitive (1.01 µg Ag.L<sup>-1</sup>). Strong physiological and behavioural disturbances were registered on male *G. fossarum*, as soon as 96h-exposure to 0.5 µg Ag.L<sup>-1</sup>. Compared to control, Ag exposure lead to higher mortality, Ag bioconcentration, Se-Gpx activity and lipid peroxydation, but also to strong iono-/osmo-regulation disruption (lower haemolymph osmolality, [Na<sup>+</sup>] and [Cl<sup>-</sup>]) and behaviour disturbance (lower ventilation and locomotion). This study shows that Ag could significantly affect the functioning of

forested headwater streams. In natural water, it seems unrealistic that dissolved Ag directly affects microbial detritus processing (significant effect only at 100 µg Ag.L<sup>-1</sup>) but realistic [Ag] had direct effects on the physiology, the behaviour and the survival of a key detritivore, suggesting a probable indirect negative impact on detritus processing at realistic concentrations. The release of ionic Ag from nanoparticles increasingly used should thus certainly deserve more attention in the next few years.

**386 Detection, Monitoring, and Control of Tributyltin - An Almost Complete Success Story** P. Matthiessen, Independent Consultant.

**387 Top three myths about TBT** M. Waldock, Cefas.

**388 Not running afoul with antifouling paints - the next generation of protection** K. Long, Regulatory Compliance Ltd / Bilston Glen Business Centre.

**389 Panel discussion** E.M. Mihaich.

**390 Estrogens in the aquatic environment: From science to regulation** J.P. Sumpter, Brunel University.

**391 Aquatic environments and endocrine activity: The regulatory approach in Japan** T. Iguchi, National Institute for Basic Biology / Molecular Environmental Endocrinology.

**392 Endocrine active chemicals in the environment - can we determine acceptable risks?** S. Marshall, Unilever / SEAC.

**393 Panel discussion** E.M. Mihaich.

**394 Effects of Carbo-Iron nanomaterial on the metabolome of the chlorophyte *Scenedesmus vacuolatus*** F. Sans Piche, Helmholtz - Centre for Environmental Research – UFZ / Dept. Bioanalytical Ecotoxicology; O. Frank, W. Busch, Helmholtz - Centre for Environmental Research – UFZ; D. Kühnel, Helmholtz Centre for Environmental Research; M. Schmitt-Jansen, UFZ Helmholtz Ctr Environm Research / Dept. Bioanalytical Ecotoxicology. Carbo-Iron nanomaterial has been developed as resource-saving and effective groundwater and sewage remediation technology. Before usage of Carbo-Iron nanomaterial in the environment, their ecotoxicological potential should be assessed. Therefore, the aim of this study was to assess the effects of Carbo-Iron on the metabolome of the unicellular microalga *Scenedesmus vacuolatus* by applying a metabolomics approach modified after Kluender et al. (2009). Next to modifications of the protocol for testing nanoparticles, two types of materials, Carbo-Iron (CI) and activated carbon particles (AC) were tested. The results revealed that the particles do not interfere with the metabolomics protocol. The two particles tested showed comparable decrease of light intensities during exposure (shading) and growth inhibition of algae. Principal component analysis performed on polar metabolite extracts revealed concentration-dependent metabolic changes induced by both particles and a relatively lower amount of most metabolites compared to controls but did not show a difference between CI and AC. Sugars such as sucrose, ribose and mannose decreased indicating a disturbance of the energy metabolism of the cells. The results will be discussed in perspective of the biochemical changes induced by CI beyond a shading effect.

**395 Effects of iron nanoparticles on *Physcomitrella patens* (Hedw.) Bruch & Schimp.** L. Canivet, University of Lille; P. Dubot, 2MCMC – ICMPE UMR 7182; G. Garcon, R. Courtcuisse, University of Lille 2; F. Denayer, Université de Lille Droit Sante. With the emergence of nanotechnology and the increasing use of nanoparticles, a question emerges: “what are the effects of this nanoparticles on ecosystems?” To try to answer to this question, the objective of our study was to evaluate the genotoxic and cytotoxic effects and the modulation of gene expression in a bryophyte, *Physcomitrella patens*, (Hedw.) Bruch & Schimp. exposed to iron engineered nanoparticles. Nanoparticles can be

dispersed and transported by wind over long distances. Plants are the first exposed to nanoparticles. To date, plants are widely used as biological model to study impacts of pollution on ecosystems and studies on nanotoxicity in plants develop in recent years. But no study has been made on the impact of nanoparticles on bryophytes. However, bryophytes are specific organisms that could be used to evaluate the impact of atmospheric pollutants on the environment. So, to study the impact of nanoparticles on ecosystems, we interested to these effects on *Physcomitrella patens*. Moreover, *Physcomitrella patens* has a particular interest because its genome has been completely sequenced. Then, the chemical composition of nanoparticles plays an important role in their ecotoxicity. The novel properties of nanoparticles such as their smaller size, large surface area and higher reactivity are a major point of concern. So, an essential step was the characterization of iron nanoparticles in their pristine state but also in their dispersion medium. In our study, we exposed plants of *Physcomitrella patens* to iron-engineering nanoparticles, dispersed by atmospheric way, under the most realistic conditions. Before studying the effects of iron-engineering nanoparticles, we verified the uptake capabilities of these nanoparticles in bryophyte cells by confocal microscopy. Then, a wide range of chemical and biological variable was analysed to determine the nanoparticles effects: the viability, the cytotoxicity, and the genotoxicity. Moreover, to explain the nanoparticles effects further upstream, we studied the modulation of the expression of some genes involved in oxidative stress. Perspective, with best knowledge of engineering nanoparticles impacts, we will continue this project by focusing on environmental nanoparticles collected on an industrial site, whose characterization will be as complete as possible. Key words: *Physcomitrella patens*, nanoparticles, ecotoxicity, realistic conditions

**396 Effect of a set of different TiO<sub>2</sub> and ZrO<sub>2</sub> (nano)particles in soils** S.I. Gomes, University of Aveiro / department of Biology & CESAM; N. Pinna, University of Aveiro / Department of Chemistry & CICECO; J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience – Terrestrial Ecology; M.J. Amorim, Universidade de Aveiro / Department of Biology and CESAM. Titanium dioxide (TiO<sub>2</sub>) particles is one of the most used nanomaterials worldwide, used e.g. in sun lotion and tooth paste. It is also used because its photocatalitical activity enhanced by UV is being explored for diverse applications. Zirconium dioxide nanoparticles (ZrO<sub>2</sub>-NPs) are also widely used, e.g. in catalysis and ceramics. Given this, the release of these NPs in the enviroment will occur and organisms may be exposed. However, little is known about the effects of these particles on terrestrial organisms. Enchytraeids (Oligochaeta) are important members of the soil community, there is a standard (OECD, 2004) test-protocol for these organisms, and they are able to survive both in soils and water, which makes them important and usefull organisms for comparing exposure routes. In this study we assessed the effects - on the enchytraeid *Enchytraeus crypticus* (Oligochaeta) - of i) 4 different TiO<sub>2</sub> NPs (with various sizes, coatings and crystal structures) in comparison with bulk TiO<sub>2</sub>, and ii) one ZrO<sub>2</sub>-NPs in comparison with the respective bulk (micro) and ZrCl<sub>4</sub> (salt). Effects were assessed via soil exposure (OECD, 2004) at survival and reproduction level and via reconstituted water and soil extracts. The effects of UV irradiation on TiO<sub>2</sub> toxicity were measured by the combined exposure of the organisms to several TiO<sub>2</sub> concentrations and UV irradiation (tested to be non-toxic alone). TiO<sub>2</sub> and ZrO<sub>2</sub> nanoparticles did not affect *E. crypticus* survival and affected reproduction only to a imited extent, when exposed in soil. An approach with possible increased bioavailability (water exposure) revealed similar results for survival. Realistic worse case scenario of UV exposure did not potentiate (increase) the toxicity of TiO<sub>2</sub>-NPs. Keywords: nanoparticles, Ti, Zr, Ecotoxicity, soil organisms

**397 ZnO nanoparticle toxicity at different soil pHs – survival and biomass change in the isopod *Porcellionides pruinosus* P.d. Tourinho**, University of Aveiro / department of Biology & CESAM; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; S. Loureiro,

Universidade de Aveiro / Biology. Environmental conditions are well known to affect the stability of nanoparticles (NPs) in soils. When it comes to soil properties, pH is one of the most important factors as it can change NP surface charge and zeta potential. The aim of this study was to evaluate the effects of soil pH on the toxicity of ZnO NPs to the terrestrial isopod *Porcellionides pruinosus*, using a natural soil with three different pH values and a natural standard soil (Lufa 2.2 soil). The toxicity test was also conducted with other two Zn forms: a microsize ZnO (non-nano ZnO) and ZnCl<sub>2</sub>. A forest soil was collected and amended with calcium carbonate to adjust pH to approximately 4.5 (Soil 1), 5.9 (Soil 2), and 7.3 (Soil 3). The Lufa 2.2 soil was also used in the experiment with a pH of 5.1. Soils were spiked at nominal concentrations of 250, 500, 1000, 2000 and 4000 mg Zn/kg dry soil. Adult isopods were exposed individually, in 10 replicates per treatment and a control for each pH set. Animals were feed with alder leaves *ad libitum*. After 14 days, survival, feeding inhibition and biomass gained were determined. LC50 and EC50 on biomass gained were calculated with a logistic curve. LC50 values were compared by a generalized likelihood ratio test. Zinc body content in isopods were analysed by a two-way analysis of variance (ANOVA). Soil pH, calcium and zinc levels in pore water changed in a dose-related manner, showing a great difference between soils spiked with ZnO particles (ZnO NPs and non-nano ZnO) and ZnCl<sub>2</sub>. LC50 values differed significantly between soils only for ZnO NPs and ZnCl<sub>2</sub>. Food consumption decreased in isopods exposed to ZnO NPs and non-nano ZnO only in Soil 3. For ZnCl<sub>2</sub>, consumption ratio decreased in all tested soils. Effects on biomass (EC50) showed a wide range across soils, with higher values in Soil 2, suggesting that soil properties do affect the Zn toxicity in a similar manner for all Zn forms. Zinc body content in the isopods was affected by Zn concentration in soil for ZnO NPs and ZnO non-nano, but did not differ between soils. For ZnCl<sub>2</sub>, zinc body content was significantly affected by both Zn soil concentration and soil type. These results indicate that isopods exposed to ZnO NPs were able to store similar Zn quantities independent on the soil, but still the effects occurred at different soil concentrations. Toxicity outcomes were quite comparable between Zn forms, indicating that ZnO NPs toxicity could not be caused only by Zn<sup>2+</sup> ions from NPs dissolution.

**398 Transcriptome analysis of zebrafish embryos exposed to dendrimers** E. Oliveira, IDAEA CSIC / Environmental Chemistry; M. Casado, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) / Environmental Chemistry; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; J. Navas, INIA - Madrid; C. Barata, CSIC / Environmental Chemistry; B. Pina, IDAEACSCIC / Environmental Chemistry. The recent application of dendrimers into nanomedicine field for diagnostic imaging, drug delivery and gene transfection results in an increased release of those biomaterials into the environment. Therefore, the development of appropriate methodologies for their monitoring and evaluation of their potential toxicity becomes increasingly important. Poly(amidoamine) dendrimers have a well-defined, mono dispersive and stable molecular architecture, composed of an ethylenediamine core initiator and successive layers of radially repeating units attached to the core and outer surface of terminal functional units. Dendrimers are envisioned as attractive drug carrier systems, largely considered non-toxic, although there is little information about their health risks in humans and other vertebrates. We present here a toxicological and transcriptomic approach using zebrafish as a model organism to the study of PAMAM third-generation (G3) and fourth-generation (G4), -NH<sub>2</sub> terminated, water soluble dendrimers. The data reveals similar toxicity profiles for both molecules, and suggests that the nervous system may be particularly sensitive to these molecules. In addition, a more generic foreign body reaction pattern appeared also activated, suggesting the potential for inflammatory and/or allergenic responses. The development of a system to monitoring toxicity of newly developed macromolecules is of great interest to assess the possible effects to developing vertebrates, including humans. New regulations may be introduced in terms of safety and efficacy of these new products, since they affect important metabolic pathways concerned to development

and also immunological system.

**399 Modelling plant uptake of organic compounds using molecular properties** M. Limmer, G. Morrison, Missouri University of Science and Technology; J.G. Burken, Missouri University of Science and Technology / Civil Architectural and Environmental. Uptake of xenobiotics such as pharmaceuticals by plants has received recent attention. Traditional models to explain plant uptake of organic compounds have utilized octanol-water partitioning (log K<sub>ow</sub>) to explain empirically the transpiration stream concentration factor (TSCF). However, these single-parameter models vary between researchers and provide little insight into the fundamental mechanisms underlying plant uptake of organic chemicals. This presentation will describe recent, fundamental work by our lab to develop rule-based and predictive models that can better explain the variability in measured TSCFs for a wide variety of compounds such as pharmaceuticals and pesticides. We found that compounds translocated by plants followed Lipinski's Rule of 5. (His "Rule of Five" states such a compound generally has 5 or fewer hydrogen bond donors, 10 or fewer hydrogen bond donors, a molecular mass of less than 500 Daltons and a log octanol-water partitioning coefficient (log K<sub>ow</sub>) of less than 5.) A series resistance model of symplastic and apoplastic transport of contaminants by plants was developed to quantify a compound's ability to enter a plant using molecular properties such as polar surface area, molecular volume and hydrophobicity. These models indicate that a wide variety of compounds, such as pharmaceuticals, could enter plant roots and be translocated above-ground.

**400 Uptake of organic compounds by submerged, freshwater, sediment-rooted macrophytes** N. Diepens, Wageningen University; A. De Agustin Camacho, Alterra; G. Arts, Alterra Wageningen University and Research Centre / Environmental Risk Assessment; A.A. Koelmans, Wageningen University / Environment. Historically, plant-chemical interaction was mainly focused on terrestrial and emergent plants while submerged aquatic macrophytes received less attention. Furthermore, only the water phase was considered. The AMRAP (Aquatic Macrophyte Risk Assessment) workshop identified a lack of knowledge about the relative importance of sediment exposure for the uptake of toxicants by sediment-rooted macrophytes. Translocation of contaminants from sediment into the water column via the macrophytes may be a significant pathway and might be important in hazard assessment. Despite the fact that several simulation models have been developed, none has considered all relevant aquatic compartments including sediment, water and plants. The aim of the current work is to provide estimates of parameters that drive uptake, translocation and elimination of organic compounds in sediment-rooted, submerged aquatic macrophytes, and to apply these parameters in chemical uptake models for macrophytes. The approach includes the generation of experimental datasets in combination with modelling of the processes mentioned above. The results from the previous water only uptake studies can be used as extra data in the model and interpretation of the data in an ecological context. Optimum growth tests were done using *Myriophyllum spicatum* and *Elodea canadensis* following the AMRAP set-up. These species differ in their traits such as leaf area and transport mechanisms. Furthermore, uptake, elimination and translocation of sediment-spiked chemicals via roots and shoots was studied during 28 day sediment toxicity tests. The spiked chemical included a mixture of PCBs and an insecticide (chlorpyrifos). For *Elodea canadensis* total dry weight did not markedly differ among the OM treatments. For *Myriophyllum spicatum* biomass showed a tendency to be higher at lower OM percentages (Fig. 1). The same pattern was observed in the total length of the *Myriophyllum* plants. The higher nutrient concentrations of N:P 3:1 and 6:1 showed a higher Chlorophyll-a content in the water layer (more algae), less growth in macrophyte shoots, less side shoots and no or less developed macrophyte roots in the sediment. Growth on sediment without nutrients was lowest, with the other nutrient levels in between. Furthermore, results of the uptake, translocation and elimination of PCBs and chlorpyrifos by the aquatic macrophytes will be presented.

**401 Hg bioaccumulation in shoots of the macrophyte *Elodea nuttallii*: an opportunity for phytoremediation or a risk for the trophic chain?** C. Cosio, Geneva University. Previous studies suggest that macrophytes might participate in bioaccumulation and biomagnification of toxic mercury (Hg) in aquatic environment. Hg bioaccumulation and uptake mechanisms in macrophytes need therefore to be studied. Amongst several macrophytes collected in an Hg contaminated reservoir in Romania, *Elodea nuttallii* showed a high organic and inorganic Hg accumulation and was then further studied in the laboratory. Tolerance and accumulation of Hg this plant was also high in the microcosm. Basipetal transport of inorganic Hg was predominant, whereas acropetal transport of methyl-Hg was observed with apparently negligible methylation or demethylation *in planta*. Hg concentrations were higher in roots>leaves>stems and in top>middle>bottom of shoots. In shoots, more than 60% Hg was found intracellularly where it is believed to be highly available to predators. Accumulation of inorganic and methyl-Hg in shoots was highly reduced by cold, death and by competition with  $\text{Cu}^+$  for inorganic Hg. Hg in *E. nuttallii* shoots seems to mainly originate from the water column, but methyl-Hg can also be remobilized from the sediments and might drive in part its entry in the food web. At the cellular level, uptake of Hg into the cell sap of shoots was linked to the metabolism, in particular to copper transporters. The present work highlights an important breakthrough in our understanding of Hg accumulation and biomagnifications: the remobilization of methyl-Hg from sediments to aquatic plants and differences in uptake mechanisms of inorganic and methyl-Hg in a macrophyte. Our results revealed that shoots of *E. nuttallii* are extremely tolerant to Hg and show huge bioaccumulation ability. Both might be interesting for phytoremediation of contaminated water. Based on densities previously reported in the field ( $350\text{--}2800\text{ g DW m}^{-3}$ ) and concentration of Hg found in Babeni reservoir, it can be estimated that  $0.7\text{ to }5.6\text{ mg Hg m}^{-3}$  could be removed with these plants. On the other hand, the Hg uptake by shoots might be an important pathway of Hg from the water to organisms feeding on the macrophytes. In conclusion, the presence of these plants should not be overlooked in contaminated sites.

**402 Use of the *Lemna minor* growth inhibition test to study dose dependent effects of uranium in plants.** n. Horemans, Belgian Nuclear Research Centre SCKCEN; M. Van Hees, Belgian Nuclear research centre (SCK-CEN); A. Van Hoeck, Belgian Nuclear Research Centre (SCK-CEN); H. Vandenhove, Belgian Nuclear Research Centre, SCK-CEN / Biosphere Impact Studies. Anthropogenic activities can lead to an enhancement of radionuclides and radioactive radiation in the environment. To assess the impact of uranium on plants, a growth inhibition test was set up in which *Lemna minor* was exposed to different uranium concentrations ranging from  $0.05\text{ }\mu\text{M}$  up to  $500\text{ }\mu\text{M}$  for 7 days. The test was performed as described in the OECD guidelines (OECD 221, 2006) with some modifications towards the growth medium. The growth medium was selected so that uranium was predominantly present as soluble uranyl kations (modelled using Geochemist's Workbench<sup>®</sup>) and so that it was sufficiently rich to sustain the growth of the *Lemna* plants. For the different media tested it was shown that adapted K-medium as described by Cedergreen et al. (2007 Environm Toxic Chem 26:149-156) with low phosphate concentrations ( $0.05\text{mg/L}$ ) best fulfilled the constraints for growth and uranium bioavailability. Uptake of uranium into the plants increased linearly with uranium dose. Growth of the *Lemna* plants was analysed as the total area of the plant fronds and the number of fronds. A clear discrepancy between these two endpoints assessed was noticed. For the frond area the dose response curve followed a typical sigmoidal shape with an  $\text{EC}_{50}$  around  $75\text{ }\mu\text{M}$ . For the number of fronds, however, growth inhibition stabilised between  $20$  and  $100\text{ }\mu\text{M}$  uranium indicating the number of new fronds appearing was stable but new fronds were smaller. Above  $100\text{ }\mu\text{M}$  growth rate inhibition based on frond number caught up with that expressed on frond area. Following the pH of the medium during the growth inhibition test it was noticed that plants were able to increase the pH with more than 2 pH units. As uranium

speciation and hence its toxicity strongly depends on the pH experiments with and without MES ( $2\text{mM}$ ) as buffer were compared. It was shown that in the presence of this MES concentration the pH maximally increased with  $0.3\text{ to }0.5$  pH unit indeed resulting in a higher U toxicity ( $\text{EC}_{50}$  shifted to about  $30\text{ }\mu\text{M}$ ). These results clearly indicate the dependency of the toxicity of uranium on the presence the uranyl kations which are the most prevalent uranium species at lower pH levels.

**403 Developing good practice on the management of aquatic plants in watercourses – taking a risk-based approach.** Tarrant, Environment Agency / Flood Risk. In many watercourses the control of aquatic plants is an essential part of their effective management. However, we feel that there is currently insufficient guidance to support this good practice approach to the management of aquatic plants and riparian vegetation in and alongside watercourses. Over the last two decades – since the last published guidance document on this subject – there has been significant advances in our understanding of the relative effectiveness of the techniques for aquatic plant control alongside significant changes in legislation particularly, the need to comply with the Water Framework Directive (WFD). Also there have been many calls from the operational flood risk management authorities, including the Internal Drainage Boards (IDBs) for further research and guidance particularly, to provide advice on herbicide application. We are aiming to synthesise the latest research to develop good practice guidance on the management of aquatic plants in, and vegetation alongside watercourses, through the comparison of a number of management techniques in different watercourses. This work will ensure that those who manage aquatic plants and vegetation in and alongside watercourses can carry out their work in a way that is cost effective, minimises negative impacts on the environment and is compliant with the governing legislation. The use of herbicide for aquatic plant management will be a strong research focus and will be placed within the wider context of the other available plant management techniques. The project is in its early stages so is yet to report in full. However, this paper offers a timely update for the Environmental Toxicology and Chemistry community on the objectives and progress with this work. **Session:** Risk assessment, regulation, policy and public perception **Key words:** aquatic plants, flood, management, herbicide **Presentation preference:** Platform

**404 FLOATING PENNYWORT IN THE RIVER SOAR, LEICESTERSHIRE** P. Harding; r. Brunt, Environment Agency. The invasive non-native floating pennywort (*Hydrocotyle ranunculoides*) first appeared in the River Soar near Leicester City Centre in 2004, and has continued to spread downstream through Leicestershire and into Nottinghamshire. It has now become established in the River Trent, with the potential to spread more widely in the catchment. Monitoring by fixed-point and aerial photography has enabled semi-quantitative comparison of pennywort beds to track the invasion and assess the impact of control measures and natural factors. Efforts to manage the invasion in the River Soar have focussed on sustainable management through partnership in line with the GB Framework Strategy for invasive non-native species. Early management was largely by mechanical removal, which proved successful but resource-intensive. Using this approach Leicester City Council has progressively removed the plant in Leicester, demonstrating that top-down eradication is achievable. Downstream of Leicester the Canal & Rivers Trust (formerly British Waterways) and the Environment Agency have progressively replaced mechanical control with herbicide use, and this has proved both effective and cost-effective. Growth of floating pennywort is influenced significantly by temperature and flow. In a mild winter rafts may remain intact and consequently become displaced as large masses, potentially posing an increased risk of flooding. Conversely, severe air frosts cause fragmentation of the beds into small pieces which wash downstream as thousands of potentially viable 'cuttings'. This scenario may have led to the sudden appearance of floating pennywort in the River Trent in 2009. Monitoring in 2010 did not indicate growth suppression following a cold winter, but exceptionally low temperatures in December 2010 appeared to eliminate

floating pennywort from several parts of the river until 2012.

**405 Nanosilver effects in marine ectotherms: linking low and high informational levels to assess adaptation under realistic conditions**

F. Dondero, DiSIT; I. Saggese, University of Piemonte Orientale Amedeo Avogadro / DiSIT; V. Palmeri, Università degli Studi di Palermo. The rising use of nanomaterials in several applications - including health care products and domestic uses - is posing new ecotoxicological concerns also for the marine environment. Here we propose a framework of analysis for the assessment of biological effects of engineered nano-objects (ENO) in the marine ectothermic organism *Mytilus galloprovincialis*, a filter feeding mollusk with great ability to accumulate trace contaminants. The framework included high and low order level effect determination, spanning from bioaccumulation, molecular and biochemical responses, bioenergetics, life trait history and fitness. Different nano-silver preparations (5 nm and 50 nm) were selected and further used in acute, chronic and microcosm exposures aimed to assess ecotoxicological endpoints and mechanistic effects of nano-silver. We provided short-term toxicity endpoints using actual doses for silver in marine bivalves; long-term microcosm study under realistic conditions; mechanistic effects of (nano)-silver linking low and high informational levels. In conclusion, our data underpin the hypothesis that the environmental risk due to silver released from engineered nanomaterial tends to be underestimated.

**406 Antibacterial effect of Ag-nanoparticles: modulation of the dissolution at the particle-cell interface**

A. Kahru, Environmental Toxicology; O. Bondarenko, National Institute of Chemical Physics and Biophysics; A. Ivask, Nat Inst of Chemical Physics and Biophysics / Environmental Toxicology. Silver nanoparticles (AgNPs) are currently used as broad-spectrum antimicrobials in over 300 consumer products including cosmetics, clothing, detergents, dietary supplements, water filters and children's toys. It is generally acknowledged that the toxic properties of AgNPs to various aquatic organisms and bacteria are largely determined by their dissolution, i.e., release of toxic Ag-ions. The effective concentration of released Ag-ions, however, depends on various biotic and abiotic factors and is difficult to predict. In this study we thoroughly characterized three types of AgNPs with different coatings (uncoated AgNPs (nAg); casein-coated colloidal AgNPs (nAg-Col) and polyvinylpyrrolidone-coated AgNPs (nAg-PVP) for their size, stability, z-potential, dissolution and antibacterial efficiency. Using three different techniques to determine the dissolution of AgNPs, we showed that the antibacterial effects of these AgNPs correlated with their dissolution. Specifically, using six bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fluorescens*, *P. putida* and *P. aeruginosa*) we demonstrated that the minimal inhibitory concentration, MIC; mg Ag/l, for uncoated AgNPs towards all bacteria was >100, for nAg-PVP from 20-40 (for *S. aureus* >100), for nAg-Col from 5 (*P. aeruginosa*) to 100, for AgNO<sub>3</sub> from 2.5 to 40. To all bacteria most inhibitory was AgNO<sub>3</sub> (100% soluble), followed by nAg-PVP (solubilized fraction 278%), nAg-Col (1.874%) and, finally, uncoated nAg (practically insoluble). The obtained results, however, revealed the specific role of particles in enhancement of toxicity of Ag-ions. Specifically, the nanoparticulate formulations of silver improved the delivery of Ag-ions into bacterial cells whereas the efficiency was strain-specific, being highest for human pathogen *P. aeruginosa* intrinsically resistant to various conventional antibiotics. The separation of bacterial cells from Ag-NPs by particle-impermeable membrane (cut-off 20 kDa, ~4 nm) remarkably reduced the toxicity of Ag NPs, confirming the importance of direct contact between Ag-NPs and bacterial cells for enhanced delivery of Ag-ions. We assume that in the close vicinity of cell envelope the direct cell-particle contact lead to the enhanced solubilisation of AgNPs facilitating the enhanced internalisation of Ag-ions and we showed that this effect could not be always predicted from the conventional dissolution studies.

**407 Species specific toxicity of copper nanoparticles: responses explained by particles or ions?**

M.G. Vijver, CML Leiden University; J. Hua, L. Song, Leiden University; W.J. Peijnenburg,

RIVM / Laboratory for Ecological Risk Assessment. Various studies showed that CuNPs can cause a diversity of toxic effects to biological systems. CuNPs showed a size- and concentration-dependent toxicity. It is recently known that the toxicity caused by micro copper is lower than the toxicity of CuNPs and the toxicity caused by copper ions in CuNPs media and the toxicity of copper oxide NPs cannot be simply explained by Cu ions released to the cell medium. However, little attention has been paid to species-specific NP toxicity and only a limited number of studies have quantified the toxicity contribution of the particle form of NPs and released ions to the total toxicity of particle suspensions for varying NP-sizes. Therefore in this contribution, the basic research question was: what is the relative toxic contribution of Cu ions and particles inducing toxic effects in different aquatic organisms? We present experimental data on dose-responses of different sized copper nanoparticles among different cell lines. And we have preliminary findings on data for different fresh water invertebrates and on zebrafish development. Our results show that both chemical and physical characteristics of NPs contribute to their toxicity to living cell lines and that sensitivity is species-specific. For mammalian cell lines both the ion and particle forms of CuNPs in suspensions contribute significantly to total toxicity, while particulate forms are most important for piscine cell lines. Experiments using zebrafish developments (preliminary data) showed that all sizes of Cu suspensions had inhibitory effects on hatching success, behavioural responses, and malformations. Different kinds of abnormalities were observed in zebrafish embryos depending on the size of Cu suspensions. MP colloids induced 77% three-combined malformations at lower 0.5 mg/L nominal concentrations. This study also revealed that CuNPs contributed to the toxicity of copper suspensions mainly (71%-100%), contribution of CuNPs increased with decreasing concentration. Therefore our study shows that species sensitivity is likely to be found and that the relative contribution of ions versus particles differs among organisms groups.

**408 Titanium dioxide nanoparticles increase sensitivity in the next generation of the water flea *Daphnia magna***

M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; F. Seitz, Inst for Environmental Sciences / Institute for Environmental Sciences; R.R. Rosenfeldt, University of KoblenzLandau Institute for Environmental Sciences / Institute for Environmental Sciences; R. Schulz, University of KoblenzLandau / Institute for Environmental Sciences. The nanoparticle industry is expected to become a trillion dollar business in the near future. Therefore, the unintentional introduction of nanoparticles into the environment is increasingly likely. However, currently applied risk-assessment practices require further adaptation to accommodate the intrinsic nature of engineered nanoparticles. Combining a chronic flow-through exposure system with subsequent acute toxicity tests for the standard test organism *Daphnia magna*, we found that juvenile offspring of adults that were previously exposed to titanium dioxide nanoparticles exhibit a significantly increased sensitivity to titanium dioxide nanoparticles compared with the offspring of unexposed adults, as displayed by lower 96 h-EC<sub>50</sub> values. This observation is particularly remarkable because adults exhibited no differences among treatments in terms of typically assessed endpoints, such as sensitivity, number of offspring, or energy reserves. Hence, the present study suggests that ecotoxicological research requires further development to include the assessment of the environmental risks of nanoparticles for the next and hence not directly exposed generation, which is currently not included in standard test protocols.

**409 Extrapolating population level effects from nanoparticle toxicodynamics**

A. Gergs, Roskilde University / Department of Environmental Social and Spatial Change; M. Pettitt, University of Birmingham, School of Geography, Earth and Environmental Sciences; T. Dalkvist, Eawag / Department of Environmental Toxicology; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research; R. Ashauer, University of York / Environment; E. Valsami-Jones, Natural History Museum, University of Birmingham; A.

Palmqvist, Roskilde University / Department of Environmental Social and Spatial Change. Lethal and sub-lethal toxic effects are commonly determined in standard laboratory tests on the level of individual organisms or the sub-individual level. What these (sub-) individual-level effects mean at higher levels of biological organisation remains an open question. For bridging the gap, toxicodynamics as observed on the individual level have to be extrapolated to population level, which is the primary protection goal for environmental risk assessment. Toxicants differ in their biochemical and physiological modes of action which may translate into specific temporal patterns of growth and reproduction in individual organisms. In the present study we therefore tested the ultimate consequences of different theoretical physiological modes of action for population dynamics by means of an individual based population model (IBM). Within the IBM, assimilation of food and subsequent allocation of energy to growth, reproduction and survival in individual organisms is modelled based on formulations provided by the dynamic energy budget (DEB) theory. In response to a toxicant, energy allocation patterns change in particular ways according to the toxicant's physiological mode(s) of action. Similar to real life, modeled population dynamics emerge from individual life histories, toxicodynamics and intraspecific competition for food and space. To unravel the physiological mode(s) of action and investigate lethal effects of silver nanoparticles (Ag-NP) and titanium dioxide nanoparticles (TiO<sub>2</sub>-NP) in *Daphnia magna*, we conducted a literature search on acute and chronic effects caused by the different nanoparticles and parameterized the DEB model accordingly. In order to simulate lethal effects we additionally implemented the general unified threshold model of survival (GUTS) and parameterized the model based on Ag-NP data available in the literature. Finally, by application of the IBM, we explored population level responses of sub-lethal and lethal effects found for individual *D. magna* exposed to Ag-NPs and TiO<sub>2</sub>-NPs. We will discuss the implications that different chemical modes of action bear for environmental risk assessment as well as the current potential for use of mechanistic effect models in toxicity assessment of engineered nanoparticles.

**410 Toxicity of tungsten carbide nanoparticles with cobalt doping (WC-Co) investigated as a binary mixture of xenobiotics** S. Reynaldi, Dept Agronomia Medellin / Cs Agronomias; D. Kuehnelt, Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology; R. Altenburger, UFC Centre for Environmental Research / Department of Bioanalytical Ecotoxicology. Tungsten carbide nanoparticles (WC) with cobalt doping (WC-Co) were considered as a mixture of xenobiotics, and its toxicity was investigated in *Daphnia magna*. After 72 and 96h exposure, observed toxicity of WC-Co was compared with predictions from concentration addition (CA) and independent action (IA) models. These models predict mixture toxicity based on effect concentrations (CA) or effects (IA) of individual components of mixtures. WC and cobalt released from cobalt chloride (CoCl<sub>2</sub>·6H<sub>2</sub>O) were considered individual components of WC-Co. After 72h, observed EC<sub>50</sub> for WC-Co was 0.18 mM, and predicted EC<sub>50</sub> was 0.66 mM and 0.72 mM for CA and IA, respectively. After 96h, observed EC<sub>50</sub> for WC-Co was 0.11 mM, and predicted EC<sub>50</sub> by CA was 0.58 mM and by IA was 0.49 mM. Thus, the observed EC<sub>50</sub> clearly exceeded those predicted by models. Moreover, the differences between observed and predicted EC<sub>50</sub> became larger from 72 to 96h exposure. WC-Co toxicity may result from a synergistic interaction between individual components and this interaction may increase over exposure time.

**411 A DNA metabarcoding approach to understand trophic transfers of pollutants** S. Drouhot, UMR 6249 Chrono-environnement; C. Tougaard, University of Montpellier II / CNRS / UMR 5554 Institut des Sciences de l'Evolution de Montpellier; F. Pompanon, University Joseph Fourier / CNRS / UMR 5553 Laboratoire d'Ecologie Alpine; O. Faure, Ecole Nationale Supérieure des Mines de Saint-Etienne Centre SPIN / CNRS / UMR 5600 Environnement-Ville-Société; C. Druart, University of Franche-Comté / CNRS / UMR 6249 Chrono-environnement; D. Rioux, F. Boyer, University Joseph Fourier /

CNRS / UMR 5553 Laboratoire d'Ecologie Alpine; A. Prudent, A. Goydadin, N. Capelli, P. Giraudoux, R. Scheffler, F. Raoul, University of Franche-Comté / CNRS / UMR 6249 Chrono-environnement. Wildlife is considered to be mainly exposed to environmental contaminants via oral route. Food web analysis is therefore crucial in environmental risk assessment. In this context, diet information is commonly obtained from literature and little attention has been given to site-specific considerations (habitat, season...), which might considerably affect diet composition and thus wildlife exposure assessment. By associating next-generation sequencing and DNA barcoding techniques, the metabarcoding molecular approach of diet analysis could achieve a better taxonomic identification of food items with lower time investment than traditional micro- and macro-histological observations of food remains. In this context, the aim of this study is to evaluate the use of this new molecular method to investigate the diet of wild small mammals exposed to arsenic and eventually to better understand and model its transfers. On a partially remediated former gold mine in southern France, small mammals were captured in spring and autumn in zones differing by their remediation treatment and in a control site. Botanical surveys were also performed on the polluted site. DNA was extracted from stomach content (*n*=96) and faeces (*n*=19) and then amplified with 3 primers sets, allowing to get plants and invertebrates (molluscs, arthropods and earthworms) DNA. Finally, amplified DNA was sequenced on next-generation sequencer. Preliminary results on plant DNA showed that 95% of the sequences were at least identified to family level and among them 13% were identified up to species level. This attests the possibility to reach a precise taxonomic level with this molecular method. Stomach contents and faeces from a given small mammal specimen gave complementary information, probably because they correspond to different meals. The results also suggested different food patterns among small mammal species, probably related to their foraging ecology. Comparisons between plant taxa identified in the diet and local botanical survey showed some mismatches, which may be related to small mammal mobility during their foraging activity. As a conclusion, the recent DNA metabarcoding is a promising approach to relate local food web structure to resource availability and thus to better understand pollutant trophic transfer in ecosystems.

**412 Spatial and Temporal Patterns and Trends of Perfluorinated Carboxylates and Sulfonates in Seabird eggs from the Pacific Coast of Canada** J.E. Elliott, Environment Canada. John E. Elliott<sup>1</sup>, Kyle H. Elliott<sup>2</sup>, Melanie F. Guigueno<sup>3</sup>, Laurie K. Wilson<sup>1</sup>, Sandi Lee<sup>1</sup>, Francois Cyr<sup>3</sup> <sup>1</sup> Environment Canada, Delta, BC, Canada; <sup>2</sup> University of Manitoba, Winnipeg, MB, Canada; <sup>3</sup> Environment Canada, Ottawa, ON, Canada There is a continuing need to monitor contamination of the marine environment by xenobiotic compounds, particularly those which are persistent and accumulate in food chains. Eggs of marine birds have proven to be an efficient and effective means of measuring and tracking substances, such as persistent organic pollutants (POPs) and mercury which are transferred from the female bird to the egg via yolk lipids or proteins. Here we report and discuss data from long term monitoring of POPs and mercury in seabird eggs from the northeast Pacific. For this program, the marine system was divided, and representative species selected. The nearshore subsurface is monitored using two cormorant, *Phalacrocorax*, species, *auritus* and *pelagicus*, both feed on a variety of benthic and pelagic fish. The offshore subsurface is monitored using the rhinoceros auklet, *Cerorhinca monocerata*, a feeder mainly on small pelagic fishes, with the offshore surface species, the Leach's storm-petrel, *Oceanodroma leucorhoa*, which feeds mainly on surface plankton and larval fishes. At three breeding colonies each along the Pacific coast of Canada and at four year intervals 15 eggs are collected and analyzed as five pools of 3 eggs each. Among the chemicals measured in this long term study are the perfluorinated carboxylates and sulfonates. Data from a recent retrospective study, using archived samples collected from 1990 to 2011, shows, as reported for more polluted environments, that PFOS (perfluorooctane sulfonate) increased in continental shelf ranging auklet eggs until the late 1990s and have declined since then. In contrast, another compound, PFUDA (perfluoroundecanoate) increased steadily in eggs of both near and



offshore species. Stable isotopes will be used to examine the possible role of dietary variation, possibly related to marine regime shifts, in variation in contaminant levels in these monitored seabirds.

**413 Lifetime PCB 153 bioaccumulation and pharmacokinetics in pilot whales: Bayesian population PBPK modelling and Markov chain Monte Carlo simulations** L. Weijts, University of Antwerp Dept Biology; D. Tibax, University of Antwerp; A. Roach, Office of Environment and Heritage; R. Yang, Colorado State University; R. McDougall, AEGIS Technologies Group; M. Lyons, Colorado State University; C. Housand, AEGIS Technologies Group; T. Manning, J. Chapman, K. Edge, Office of Environment and Heritage; D. Pemberton, Dept of Primary Industries, Parks, Water and Environment; A. Covaci, University of Antwerp / Toxicological Centre; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology. Physiologically based pharmacokinetic (PBPK) models are mathematical representations of the ADME processes of chemicals in organisms. The 'experiments' involved in PBPK modeling can be minimally- or non-invasive; thus, this approach is of particular interest for assessing pollution in wild marine mammals since all toxicological experiments are prohibited or ethically undesirable. PBPK models for chemicals in marine mammals have previously been parameterized by adopting those from other organisms and validated using results measured in tissue samples from dead, stranded animals. However, such an approach entails a fair amount of model uncertainty. Bayesian methods and Markov chain Monte Carlo (MCMC) simulations are currently the best statistical approaches to address this uncertainty. The goal of the present study was therefore to develop a PBPK model for lifetime bioaccumulation of PCB 153, one of the most persistent PCBs in marine mammals, in male long-finned pilot whales and to evaluate that model using the Bayesian approach and MCMC simulations. The structural PBPK model consisted of 2 flow-limited compartments: blubber and the 'rest of the body'. Physiological and chemical parameters in this model were primarily taken from the literature. In the statistical model, a Bayesian approach with MCMC analysis, was applied in order to evaluate or update the parameters taken from the literature (priors) with regard to pilot whale data taken from our own analyses. The model predicts a steep increase in PCB 153 level from birth until the age of about 1.5 years followed by a rapid decline until the age of 3 years due to the growth dilution effect and a further slow decline caused by other factors such as decreasing PCB 153 levels in the environment. This method starts with estimated or known parameters from the literature (priors) and uses the available dataset to derive new parameter values (posteriors). These posteriors can accordingly be used further as "priors" as soon as new datasets are available resulting in more updated parameters. Such an approach provides progressively more robust parameters so that the updated parameters are better representing the bioaccumulation in populations in the wild. Therefore, this study is the first step towards the production of reliable parameter values for pilot whales.

**414 Squamous epithelial proliferation as a biomarker of exposure to AhR-active compounds for ecological monitoring using wild mink as a sentinel species** M.J. Zwiernik, Michigan State University / Animal Science Vet Med; S.D. Fitzgerald, Michigan State University / Department of Pathobiology and Diagnostic Investigation; J.L. Newsted, Cardno Entrix; J.N. Moore, U.S. Fish and Wildlife Service / Ecological Services; J.E. Link, E.M. Koppel, S.J. Bursian, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science. Squamous epithelial proliferation (SEP) in the mandible and maxilla of wild mink has been proposed as a sentinel biomarker for mammalian wildlife exposure to dioxin-like compounds (DLCs) including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). The proposal originates from the fact that mink are very sensitive to the effects of DLCs, exquisitely sensitive to DLC induced SEP, they are widely distributed throughout Europe and the Americas, and can be used in controlled laboratory exposure studies. The SEP biomarker is abnormal tissue consisting of nests and cords of epithelial cells that

extend into the alveolar bone causing skull and jawbone osteolysis. Its occurrence is easily and inexpensively identified histologically at lesser severity or grossly at great severity. Herein we provide additional support for the use of SEP as a sentinel endpoint in wild mink for the assessment of risk from DLC exposure. The additional support includes a review of the literature pertaining to gingival and alveolar bone abnormalities in mammals exposed to DLC as well as a summary of recently performed laboratory mink dosing studies utilizing individual DLC compounds or environmental mixtures of DLC compounds. In short, SEP and SEP symptoms have been noted in a wide range of mammalian species exposed to DLC, the incidence and severity of SEP and SEP associated symptoms, increases in severity with increased DLC dose, and in controlled studies jaw lesions always occurred at doses lesser than those for which adverse individual or reproductive health effects were observed. Given that the assessment of the ecological risk of DLC is often a complex and expensive undertaking, the availability of a low cost, easily observed biomarker that can be used to screen for biologically significant exposures of DLCs in individuals or populations would be of great utility for environmental monitoring, source identification, and effectiveness of remedial activities.

**415 Influence of individual parameters, habitat diversity, and trace metal contamination on tick-borne disease prevalence in wild wood mice** N. TETE, Laboratoire Chrono-environnement; E. AFONSO, C. FRITSCH, R. SCHEIFLER, University of Franche-Comte / Laboratoire Chrono-environnement. Recent studies have shown that parasitic infections in aquatic animals may be related to environmental pollution. However, to our knowledge, this link has received little attention in terrestrial ecosystems. Thus, the aim of this study was to test the hypothesis that pollution by trace metals (TM) influences prevalence to bacterial infection transmitted by vectors in a rodent species. The field work took place around the site of an ancient lead (Pb) and zinc (Zn) smelter. After more than 100 years of activity, this area is drastically polluted, mainly by three TM: cadmium (Cd), Pb, and Zn. Categories of environmental contamination (low, medium, high) were determined for both Cd and Pb. During the autumn 2010, 227 wood mice were trapped along a pollution gradient. Four bacteria were studied by PCR: *Borrelia* spp., *Coxiella burnetii*, *Anaplasma phagocytophilum*, and *Bartonella* spp. To study the influence of the habitat on bacteria prevalence a Shannon habitat diversity index was calculated around the trapping site of each animal. Over the four bacteria tested, *Borrelia* spp. was not detected; *C. burnetii* was detected in only one individual (0.4%), *A. phagocytophilum* in 22 individuals (9.7%), and *Bartonella* spp. in 125 individuals (55.1%). No differences in *A. phagocytophilum* and *Bartonella* spp. prevalence were observed nor depending on age (logistic regression, LRT  $p = 0.372$  and  $p = 0.622$ , respectively), neither depending on gender (LRT  $p = 0.678$  and  $p = 0.461$ , respectively). *A. phagocytophilum* prevalence were positively influenced by the habitat diversity (LRT,  $p = 0.008$ ) while *Bartonella* spp. were not (LRT,  $p = 0.569$ ). Once the habitat diversity taken into account, *A. phagocytophilum* prevalence was positively though marginally influenced by Cd contamination in the environment (LRT,  $p = 0.054$ ) and, to a lower extent, by Pb contamination (LRT,  $p = 0.087$ ). However, *Bartonella* spp. prevalence was not influenced by Cd and Pb levels of contamination (LRT,  $p = 0.434$  and  $p = 0.569$ , respectively). None of Cd or Pb internal concentrations in the liver or in kidneys had any influence on these two bacteria prevalence. Due to the possible synergistic effects of pollution and parasitism on wildlife conservation, further studies based on such a systemic approach including pollution and other stressors should be developed in ecotoxicology.

**416 Long time trends of concentrations of POPs and Mercury in Scottish wild birds** M.G. Pereira, Centre for Ecology Hydrology; E. Potter, Centre for Ecology and Hydrology; L. Walker, Centre for Ecology Hydrology; J. Crosse, Centre for Ecology and Hydrology; R.F. Shore, CEH Lancaster. The Predatory Bird Monitoring Scheme (PBMS) is the umbrella project that encompasses long-term contaminant monitoring work in avian predators in the UK. The PBMS monitors sentinel avian species to detect and quantify current and emerging

chemical threats. It also provides scientific evidence on how chemical risk varies over time and space. This may occur due to market-led or regulatory changes in chemical use and may also be associated with larger-scale phenomena, such as global environmental change. The PBMS has monitored long-term trends in pollution in the eggs of a range of terrestrial and marine feeding predatory birds from Scotland. Some (merlin- *Falco columbarius*, gannet- *Morus bassanus*) have been studied as sentinels of terrestrial and marine contamination, respectively, while others (golden eagle *Aquila chrysaetos*, white-tailed sea eagle- *Haliaeetus albicilla*) have been monitored because of their high conservation status. In this paper we report long-term trends in concentrations of old and new POPs and mercury (Hg) in the eggs of these species. From this long term data sets we conclude that: Concentrations of PCBs and Hg did not show a consistent temporal trend for all the species studied and the legacy PCBs do not show a significant temporal decline in all species as may have been expected given the long-term prohibition of their use. PBDE concentrations in gannet eggs declined after the legislation banning the use of these compounds was introduced. Although it is not certain that DDE or PCBs were a contributory cause of the reproductive failure observed in white-tailed eagles from Scotland, the residues in some eggs were of a magnitude that may be expected to have adverse effects.

**417 High-resolution fractionation of complete GC chromatograms for Effect Directed Analysis** L. Kool, VU University / BioMolecular Analysis group. Effect-directed analysis (EDA) is an important tool in studying the biological effect of individual compounds in a mixture, e.g., toxic compounds in the aquatic environment. At present, for environmental EDA, complex environmental samples showing toxic activity are fractionated with liquid chromatography (LC) into a limited number of fractions. Each fraction is tested for toxic activity in a bioassay. Further fractionation steps may be necessary to find and identify the toxic compounds. Although post-column fractionation by capturing an LC eluent is straightforward and LC fractionation setups are widespread, many compound classes relevant in environmental EDA are preferably separated and analyzed by gas chromatography (GC) rather than LC, e.g., polycyclic aromatic hydrocarbons (PAHs), many pesticides, and mineral oils. Moreover, many methodologies are certified in this area are GC rather than LC methods and considered the standard. Fractionation of compounds after GC is not often performed because of the complicated setups required, and GC fractionation only allows automated collection of a very limited number of fractions, usually only 6, utilizing complicated cryo-trapping or adsorbent collection vessels. As GC offers a very high resolution in the separation of compound classes like non-polar organics and volatiles, a short collection time per fraction (second range) is necessary for fractionation after GC without losing (too much of) the separation efficiency. In this presentation, we describe the development, optimization, validation, and application of a novel GC fractionation technology that allows complete GC chromatograms to be fractionated in high yield and high resolution. The number of fractions collected is drastically increased over the currently available technology. The GC fractionation technology developed was applied to environmental extracts which were successfully analyzed with GC combined with high-resolution EDA. Complete GC chromatograms were collected in second range fractions prior to straightforward EDA. Endocrine disrupting pollutants in these mixtures analyzed were identified as bioactives towards the dioxin receptor by using a mammalian cellular gene reporter assay as readout. For this, another novelty was introduced in which cells were seeded and grown post-fractionation, directly onto the collected fractions, which additionally eliminates handling steps and circumvents compound dilution.

**418 Hormone receptor-affinity extraction coupled to high-resolution mass spectrometry for activity-directed analysis of EDCs in complex mixtures** L. Ferguson, Pratt School of Engineering / Department of Civil and Environmental Engineering; K.A. Stencel, Duke University / Nicholas School of the Environment; G.J. Getzinger, Duke University / Civil and Env Engineering; L.K. Shaw, University of South Carolina /

Department of Chemistry & Biochemistry. We report the development of a new method, hormone receptor-affinity extraction, for isolating xenoestrogenic environmental contaminants from complex water and wastewater samples prior to analysis by high resolution mass spectrometry. This technique utilizes the specificity of hexahistidine-tagged, recombinant human estrogen receptor ( $\alpha$  isoform) ligand binding domain (ER $\alpha$ -LBD) to bind trace estrogenic compounds in solution prior to co-purification of the ER $\alpha$ -LBD-xenoestrogen complex using immobilized metal-affinity chromatography. The method reduces sample complexity and enriches for estrogen receptor-relevant endocrine disruptors. Xenoestrogens in municipal wastewater effluent and in surface waters impacted by irrigation runoff from land-application of wastewater effluent were isolated by receptor-affinity extraction and quantified by HPLC coupled to high-resolution mass spectrometry using stable-isotope dilution. Synthetic xenoestrogens (nonylphenol, bisphenol A, and octylphenol) were detected in water and wastewater at concentrations up to approximately 350 ng·L<sup>-1</sup>, while biogenic estrogens (17 $\beta$ -estradiol, estrone, and estriol) were present in these samples at lower concentrations (typically below 10 ng·L<sup>-1</sup>). The pharmaceutical contraceptive estrogen 17 $\beta$ -ethynylestradiol was not measured in water or wastewater samples above detection limits (< 1 ng·L<sup>-1</sup>). Qualitative analysis of non-target xenoestrogens in wastewater receptor-affinity extracts was performed by HPLC-QTOF and HPLC-Orbitrap MS/MS, and the weak xenoestrogen 2-(2-(4-nonylphenoxy)ethoxy)acetic acid (NP2EC) was identified by accurate mass measurement and high resolution MS/MS. Identification of this compound (an oxidative degradation product of nonylphenol polyethoxylate surfactants) in estrogen-receptor affinity isolates of wastewater effluent demonstrates the utility of this method for characterizing trace xenoestrogens in complex mixtures without prior knowledge of their identities.

**419 Identification and confirmation of contaminants in marine waters using passive samplers, high resolution fractionation and mass spectrometry** P. Booij, P. Leonards, Institute for Environmental Studies; P. de Voogt, S. Sjollem, University of Amsterdam; D. Vethaak, DELTARES; M. Lamoree, Institute for Environmental Studies. Anthropogenic contaminants and natural toxins in estuarine and coastal ecosystems can affect primary production at the basis of the marine food chain. These chemical stressors are hypothesized to disturb the photosynthesis of phytoplankton and, therefore, the carrying capacity of coastal and estuarine waters. In our study we aim to identify and confirm the contaminants that have an effect on algae in order to quantify the toxic pressure in the Dutch coastal and estuarine waters. To identify contaminants Effect-Directed Analysis was performed with passive samplers (POCIS and silicone rubber sheets) at three estuarine and coastal locations in The Netherlands. The passive sampler extracts were tested for toxicity on algae using the Pulse Amplitude Modulation (PAM) fluorescence assay. To reduce the complexity of the extracts high resolution fractionation in 96 well plates was performed. The well plate was directly used to perform the PAM assay after evaporation of the LC solvent. In general, there was a significant overlap in active fractions between the POCIS and silicone sheets for all three locations. This is an indication that both passive samplers are able to sample the same types of compounds that are responsible for the effect on algae. Compounds were identified using a stepwise approach. A selection of compounds was purchased and analytically confirmed by using LC-ESI-MicroTOF-MS. Confirmed compounds were quantified with LC-ESI-MicroTOF, and the PAM assay was performed to determine the effect concentrations on the  $\alpha$ -PSII. Twenty-six compounds could be identified. Five of these compounds (all pesticides) could be confirmed for retention time and response in the PAM assay. These five pesticides are probably the main contributors to the photosystem II inhibition in microalgae for the Dutch coastal waters. *Acknowledgement* - This project is financed by DELTARES, NL.

**420 Effect-Directed Analysis approach (EDA) for the identification of active compounds in pig manure** C. Gardia Parege, EPOCLPTC / EPOC-LPTC UMR 5805 CNRS; M. Devier, University of Bordeaux /

UMR 5805 CNRS EPOC-LPTC; G. Hernandez Raquet, INRA / LISBP INSA UMR INSA/CNRS 5504 - UMR INSA/INRA 792; P. Balaguer, University of Montpellier 1 / INSERM U896; H. Budzinski, University of Bordeaux. Animal breeding activities are identified as a major source of hormones in environment. In addition to veterinary drugs used in feed and water to improve the growth performance or to prevent infection in intensive animal production, animals may also be in contact with many endocrine disrupting compounds (EDCs) as PAHs, dioxins, phthalates, detergents coming from farm activities. These EDCs will be concentrated in manure. Considering this and the use of manure as agricultural fertilizer, manure is considered as potential source of contamination. In this study, the assessment of the potential endocrine disrupting activity of pig manure was performed using *in vitro* bioassays. In order to isolate the active chemicals and to identify them, Effect-Directed Analysis (EDA) method was used. This approach combining bioassays, fractionation procedures and chemical analytical methods (LC-MS/MS, LC-QToF) aims to establish the cause-effect relationships by sequential reduction of the complexity of environmental mixtures to individual toxicants. Pig manure was extracted by Accelerated Solvent Extraction (ASE). Then, the crude extract was fractionated using sequential procedure based on a preliminary fractionation by Solid-Phase Extraction (SPE) followed by a finest fractionation by Reverse Phase – High Performance Liquid Chromatography (RP-HPLC). Each fraction was tested using 2 cell lines based on luciferase gene under the control of estrogen receptor  $\alpha$  and aryl hydrocarbon receptor allowing the specific and integrative detection of a wide range of active chemicals. The most active fractions were identified. On the basis of RP-HPLC fractionation calibration, targeted chemical analyses were performed on several fractions but the detected compounds poorly explained the observed biological activities. So for all selected active fractions, compounds responsible for these activities remained unknown. The identification of active compounds was performed using a LC-HRMS system (LC-QToF) and several drugs, metabolic drugs and pesticides were found in several fractions. These results demonstrated the usefulness of EDA-based strategy for the identification of active compounds in environmental complex samples when mass balance analysis (MBA) approach was not satisfactory.

#### 421 Identification of polar mutagenic contaminants in river water: Improving candidate structure selection

**M. Krauss**, Helmholtz Centre for Environmental Research / Effect-Directed Analysis; C.M. Gallampois, Linköping University / Department of Clinical and Experimental Medicine; A. Bahlmann, Helmholtz Zentrum für Umweltforschung UFZ; E. Schymanski, Eawag Swiss Federal Institute of Aquatic Science; U. Nadin, Helmholtz centre for environmental research - UFZ; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis. To identify mutagenic compounds, effect-directed analysis (EDA) using a combination of biotesting, fractionation and mass spectrometry (MS)-based structure elucidation has proved to be a valuable approach. A major challenge in the workflow is the assignment of a candidate structure to the molecular formula derived from MS data. Our objective was to improve candidate selection on the basis of liquid chromatography-high resolution tandem mass spectrometry. Blue rayon passive sampler extracts from a location at the Elbe river were separated into acidic, neutral and basic fractions and fractionated further by LC using a sequence of two different columns. The Ames fluctuation assay with strain TA98 was used to screen samples for mutagenic compounds with and without metabolic activation. Fractions showing the highest mutagenicity were analyzed using a high resolution/high accuracy LTQ Orbitrap MS and additionally tested using the YG1024 and YG1041 strains. High mutagenicity was found predominantly in the neutral and acidic fractions. For one selected sub-fraction of the neutral fraction showing the highest mutagenicity, the effects were about five times higher in the Ames test using strains YG1041 and YG1024 than for strain TA98 (all with metabolic activation), suggesting that aromatic amines contributed considerably to the total mutagenicity. In this subfraction 17 peaks of interest could be detected and a molecular formula assigned to these. Application of MetFrag involving candidate selection via a comparison

of measured vs. predicted spectra enabled us to propose a list of 92 candidate structures. After comparison of retention times measured with those predicted using a linear solvation energy relationship approach, the list of candidates was decreased to 22. As aromatic amines were likely responsible for the mutagenic effects, we performed a mutagenicity prediction of the 46 aromatic amine candidates on the basis of the stability of corresponding nitrenium ions as ultimate electrophiles attacking the DNA. This approach identified nine aromatic amine candidates as likely mutagens. In combining the results of these two methods, only for two candidates the predictions of retention and mutagenicity were positive. This work showed that the predictions of retention, MS fragmentation and mutagenicity can be valuable tools in structure elucidation by significantly reducing the candidate structure list.

#### 422 Characterization of the toxic constituents of oil sands process affected water by Orbitrap-MS

**A. dos Santos Pereira**, University of Alberta / Division of Analytical and Environmental Toxicology; S.B. Wiseman, University of Saskatchewan / Toxicology Centre; R. Mankidy, University of Saskatchewan / Toxicology; Y. He, H. Alharbi, J.P. Giesy, University of Saskatchewan / Toxicology Centre; J.W. Martin, University of Alberta / Laboratory Medicine and Pathology. Oil sands deposits in Northern Alberta, Canada, represent the world's third largest crude oil deposit (in the form of bitumen), estimated at 176.8 billion barrels. Environmental challenges in the surface mining oil sands industry arise from the fact that the bitumen is separated from sand and fine clay using alkaline hot water, resulting in large volumes of toxic oil sands process affected water (OSPW). This OSPW must be contained in tailings ponds due to its toxicity, and a zero-discharge policy. Tailings ponds are growing in volume and in number, and in 2011 covered approximately 170 km<sup>2</sup> despite heavy recycling of OSPW into the extraction process. OSPW contains a very complex mixture of organic compounds and is acutely and chronically toxic to diverse organisms including zooplankton, phytoplankton, invertebrates, fishes, mammals, birds and plants. The acute toxicity of OSPW has been attributed to a complex mixture of dissolved organic acids, however the specific chemicals that contribute to the acute toxicity, let alone the chronic, reproductive, or developmental toxicity, remain unknown. Approximately 2000 organic compound groups were detected in the OSPW fractions, including compounds matching the formulae: C<sub>x</sub>H<sub>y</sub>O<sub>z</sub> (z = 1 to 6); C<sub>x</sub>H<sub>y</sub>ON (z = 0 to 4); C<sub>x</sub>H<sub>y</sub>OS (z = 1 to 4) and C<sub>x</sub>H<sub>y</sub>ONS (z = 1 to 2). The O species (C<sub>x</sub>H<sub>y</sub>O<sub>z</sub>) were the more abundant species detected in the neutral fraction under both ionization conditions; and this fraction had the greatest estrogenicity and embryotoxicity among all primary fractions (neutral, acid and basic). The O species detected in negative mode (Figure 1A) are predominantly of classic naphthenic acids (NAs), however the analysis in positive mode showed the presence of an additional homologous family of non-acid O species (probably hydroxy-keto compounds) constituting 100 different species ranging from 10 to 25 carbon atoms and with double bond equivalents (DBE) ranging from 2 to 16. These new O species should also be considered as candidate molecules for the adverse effects of OSPW.

#### 423 Identifying new Persistent Organic Pollutants under the Stockholm Convention

**M. Scheringer**, ETH Zurich / Institute for Chemical and Bioengineering; S. Stempel, S. Hukari, C.A. Ng, ETH Zurich; M. Blepp, Öko-Institut; K. Hungerbühler, ETH Zurich. The Stockholm Convention on Persistent Organic Pollutants (POPs) is an important Multilateral Environmental Agreement (MEA) that helps to identify POPs and to regulate them at the global level. As of 2012, 22 POPs are regulated under the Convention. These POPs are listed in Annex A (elimination; 18 chemicals), B (restriction; two chemicals), or C (unintentional production; five chemicals); three chemicals are listed in both Annex A and C. Before a chemical can be regulated under the Convention, it is assessed by the POP Review Committee (POPRC). In this assessment, a potential POP candidate is first evaluated against screening criteria defined in Annex D of the Convention; these criteria cover persistence (P), bioaccumulation potential (B), toxicity (T), and

long-range transport (LRT). When the POPRC has agreed that a chemical fulfills the Annex D criteria, the next steps are a fuller evaluation of the environmental hazard and risk caused by the chemical according to Annex E (risk profile) and a socio-economic assessment (Annex F). For each chemical, the entire process takes several years. Yet, there are many thousands of unevaluated chemicals on the market and, therefore, a key question for the future identification and regulation of POPs under the Stockholm Convention is how many potential POPs exist and how they can be identified. To address this question, we compiled a list of 93 000 chemicals specified by CAS registry number and SMILES code. For each of the chemicals on this list, we collected data on biodegradation half-life, bioconcentration and bioaccumulation factor (BCF, BAF), acute and chronic toxicity in aquatic species, and degradation half-life for reaction with OH radicals. These data include both measured and estimated values; because measured data are scarce, most data points had to be estimated from the chemical structure. Next, we compared the property data to the Annex D criteria: 60 days for the degradation half-life in water, 5000 for BCF and BAF, and 2 days for the degradation half-life in air. For acute toxicity, we used the threshold defined for PBT assessment under the European chemicals regulation, REACH (0.1 mg/L). We found 574 chemicals that exceed all four thresholds and can be considered potential POPs; most of these chemicals are halogenated. We further prioritized the chemicals by means of production volume and found 12 potential POPs that are high-production volume chemicals or have been registered under REACH.

**424 Analytical challenges for new and candidate POPs** J. de Boer, VU University / IVM; H.A. Leslie, Institute for Environmental Studies VU Amsterdam. The Stockholm Convention has listed eighteen persistent organic pollutants (POPs) for elimination (Annex A), two for restriction (Annex B) and five for reduction of unintentional production (Annex C). Several of these POPs consist of mixtures and all are halogenated aromatic or aliphatic hydrocarbons. A Global Monitoring Program (GMP) has been designed to follow the concentrations of these POPs in the environment and in human milk and blood. All countries that have signed the Convention are obliged to contribute to the GMP. However, many countries have no or little experience in analyzing POPs. Apart from instrumentation such as gas (GC) and liquid chromatography (LC) and mass spectrometry (MS), trained staff is essential for a successful monitoring program per country. Therefore, United Nations Environment Program (UNEP) is investing in capacity building. Key activities are a bi-ennial interlaboratory study, on-site training of laboratory staff, provision of consumables, instruction films and several specific training activities using expert laboratories. Recent results of these training programs show several common factors that slow down progress in participating laboratories, for example: a lack of daily routine in analyzing POPs, bureaucracy (which slows down ordering processes), limited access to the scientific literature, no maintenance of instruments, power failures, dust in laboratories, etc. On top of that, there are the usual challenges of developing analytical methods for existing and candidate POPs. Difficulties are particularly met for the analysis of perfluorinated alkyl substances, brominated diphenylethers and toxaphene. No doubt, chlorinated paraffins will cause serious problems when they would be listed as POPs. There is insufficient capacity for the analysis of chlorinated dioxins, furans and dioxin-like PCBs. The first bi-ennial interlaboratory assessment on POPs showed substantial discrepancies between laboratory results, at least comparable to the results obtained by European and North-American laboratories in the mid-1980s.

**425 Hexabromocyclododecane (HBCD) - A Hazardous Flame Retardant Used in Polystyrene Insulation Products** A. Jensen, NIPSECT. Hexabromocyclododecane (HBCDD or HBCD) is a brominated flame retardant mainly used in polystyrene-based building insulation products (EPS and XPS) but is also used in electronics as a substitute for banned flame retardants. This lipophilic and persistent organic pollutant accumulates in natural organisms and magnifies in the food chain, leading to progressively increasing background levels in human tissues and in wildlife, especially of the most stable a-isomer.

The extent of this accumulation correlates directly with its ever-more prevalent use. Limited toxicological information is available to assess the long-term implications for health or the environment of the HBCDD contamination. HBCDD is an endocrine disruptor in animals. In 2008 The European Chemicals Agency identified HBCDD as 1 of 14 substances of "Very High Concern", and in September 2010 HBCDD was added to REACH's Authorization List. In February 2011 HBCDD was selected to be phased out by EU REACH Regulation before 2015. In October 2010, 2011 and 2012 the POP Review Committee under the Stockholm Convention has assessed the risks from HBCDD, and it had concluded that HBCDD fulfills the criteria of a persistent organic pollutant (POP), and the committee recommended a global ban of HBCDD use (Annex A). In January 2012 HBCDD it was among 15 new priority substances proposed by the European Commission to be regulated by the EU Water Framework Directive. This presentation will provide a succinct up-to date overview of HBCDD's properties, uses, regulation and discusses the risks associated with its prevalence in our homes and immediate environment.

**426 Recent decreases of some perfluoroalkyl substances and brominated flame retardants in the Arctic** K. Vorkamp; F.F. Riget, R. Bossi, Aarhus University; C. Sonne, R. Dietz, Aarhus University / Department of Arctic Environment. Documentation of the ubiquitous presence of Polybrominated diphenyl ethers (PBDEs) in the environment and in humans has led to bans of the technical products PentaBDE and OctaBDE through the Stockholm Convention on Persistent Organic Pollutants (POPs). The perfluoroalkyl substances (PFAS) PFOS and perfluorooctane sulfonyl fluorid are included in Annex B (restriction) of the Stockholm Convention. PBDEs and PFAS are systematically monitored in Greenland biota as part of the AMAP Monitoring and Assessment Programme (AMAP), including ringed seals from East and West Greenland and polar bears from East Greenland. The most recent international AMAP assessment concluded that levels of PFOS and other PFAS increased in ringed seal and polar bear from Greenland. With regard to PBDEs, the international AMAP assessment showed a significant increase of BDE-47 in ringed seals from West Greenland, but no trend was reported for PBDEs in ringed seals from East Greenland. The updated temporal trends of PFOS in West Greenland and East Greenland ringed seals showed a marked increase from 2003 to 2006, followed by a sharp decrease. In polar bears, the PFOS concentrations were considerably higher than in ringed seals, however, the temporal trend of PFOS in polar bears showed a similar pattern to that in ringed. This recent decrease is different from the conclusion in the latest AMAP assessment and might indicate effects of international regulations. In juvenile ringed seals from West Greenland, BDE-47 is the only BDE congener consistently above detection limits. BDE-47 continues to increase in blubber of these ringed seals, at an annual rate of 4.8%. No time trend is apparent in ringed seals from East Greenland: While a relatively constant concentration is observed in juvenile seals, concentrations decrease in adult seals, although not significantly. These results confirm the latest international AMAP assessment. If global regulations under the Stockholm Convention have the effect on decreasing levels in Central East Greenland, the current significant trend in Central West Greenland should be temporary. In subadult polar bears, significant increases were found BDE-100 and BDE-153, at average annual rates of 5.0%. BDE-47, BDE-99 and ?PBDE, however, showed a significant non-linear trend and peaks in 2000 and 2004, respectively, which agrees with indications of declines in ringed seals from the same area.

**427 Prioritization, screening and identification of organosilicon contaminants in the environment** A.H. Kierkegaard, M.S. McLachlan, Stockholm University; K. Breivik, Norwegian Inst for Air Research; J.A. Arnot, ARC Arnot Research Consulting / Department of Physical Environmental Science; F. Wania, University of Toronto at Scarborough / Department of Physical & Environmental Sciences. A mass balance model of chemical fate and bioaccumulation in the environment was used to rank 287 high- and low-production volume organosilicon compounds for their concentration in the environment and

in top predators. Key physical chemical properties of each chemical were estimated using quantitative structure-activity relationships (QSARs) and a total emission estimate of each chemical was made using information, which included amounts entering commerce and emission factors. Based on the model predicted concentrations in air, sediment and human tissue, chemicals were selected for screening through environmental sampling and analysis. Known environmental organosilicon contaminants such as the cyclic and linear volatile methyl siloxanes (VMS) were excluded as well as structures subject to rapid hydrolysis, a feature which was not taken into account in the model simulations because of current limitations in predicting hydrolysis half-lives with QSARs. Analytical standards were only commercially available for half of the remaining 30 organosilicon compounds. Ten of these were not stable in solution, which left 5 organosilicon compounds eligible for environmental screening. These were tetrakis(trimethylsilyloxy)silane, phenyl-tris(trimethylsilyloxy)silane, trifluoropropyltrimethylcyclotrisiloxane, trifluoropropylmethylcyclotetrasiloxane and tetraphenyltrisiloxane. Four of these chemicals were identified in sewage sludge, in sediment from Stockholm harbor, and in Stockholm ambient air samples. The trifluoropropyl-substituted siloxanes were analysed with UPLC-MS/MS, the others with GC-MS. Trifluoropropyltrimethylcyclotrisiloxane was solely detected as its corresponding linear diol. To date it is unclear whether the diol is present in the environment as such or formed during extraction or cleanup. The concentrations of the chemicals ranged from  $\text{pg m}^{-3}$  in air up to  $\text{ng g}^{-1}$  d.w. in sewage sludge, which are orders of magnitude below the levels of cyclic VMS (such as D5) in the same matrices.

#### 428 Forensic Techniques to Age Date Human Exposure to PCBs

D. Megson, Plymouth University; G. O'Sullivan, Department of Environmental Science, Mount Royal University; S. Comber, P. Worsfold, M. Lohan, School of Geography Earth and Environmental Science, Plymouth University. Polychlorinated Biphenyls (PCBs), a group of 209 chlorinated organic compounds, were first synthesised for industrial purposes in 1929. They were widely used until the 1980s when their use was phased out due to environmental and human health risks. Due to the persistence and toxicity of PCBs their concentrations in the US population are routinely monitored within the United States National Health and Nutrition Examination Survey (NHANES). This work presents a multivariate statistical analysis of the NHANES 2003-04 dataset to identify steady state (persistent in the body) and episodic (identified in humans transiently) congeners from background concentrations of PCBs present within the US population. These results were used in conjunction with other reviews on PCB metabolism to identify how the structure of PCBs relate to rates of biotransformation in humans. Congeners with chlorine atoms in the 2,5- and 2,3,6- positions appear to be more susceptible to biotransformation whereas congeners with chlorine bonds in the 2,3,4- 2,4,5- 3,4,5- and 2,3,4,5- positions appear to be more persistent. This work identifies a subset of 23 key PCBs which have the best potential to age date human exposure to PCBs. A summary of on-going method development using GC-GC-MS to accurately quantify these 23 key congeners will also be presented.

#### 429 EDC testing in the future: Exploring roles of pathway-based in silico, in vitro and in vivo methods

G.T. Ankley, US EPA / National Health and Environmental Effects Research Laboratory; D.L. Villeneuve, US EPA / Mid-Continent Ecology Division; T. Hutchinson, School of Biological Sciences Plymouth University. Current methods for screening, testing and monitoring endocrine-disrupting chemicals (EDCs) rely substantially upon moderate- to long-term assays that can, in some instances, require significant resources, including numbers of animals. Recent developments in the areas of short-term *in vivo* tests/endpoints (including genomics), *in vitro* assays (e.g., high throughput technologies), and computational biology (including bioinformatics) could provide the basis for efficiently detecting chemicals with the potential to perturb specific biological pathways within the hypothalamic-pituitary-gonadal and -thyroidal axes. Adverse outcome pathways (AOPs) offer a useful organizational framework for

identifying and implementing these types of approaches. Pathway-based *in vivo*, *in vitro* and computational approaches, while not yet capable of wholly replacing longer-term whole-organism tests, nonetheless could fill roles related to screening/prioritization of large numbers of test chemicals for endocrine activities, or rapid broad-scale environmental monitoring of EDCs. This presentation will provide an overview of the AOP framework in the context of EDCs, and explore some of the newer pathway-based technologies being considered for EDC testing and monitoring, including their potential advantages and drawbacks.

#### 430 Mechanism-based testing strategy using in vitro approaches for identification of thyroid hormone disrupting chemicals

A. Murk, Wageningen Agricultural University / Dept. of Toxicology. The thyroid hormone (TH) system is responsible for several important physiological processes, including regulation of energy metabolism, growth and differentiation, development and maintenance of brain function, thermoregulation, osmo-regulation, and axis of regulation of other endocrine systems, sexual behaviour and fertility, cardiovascular function. Therefore, strategies are being developed to identify TH disrupting (THD) chemicals (THDC). Information on THD potency of chemicals still is derived from animal studies. For the majority of chemicals, however, this information is hardly available. It is unlikely that animal experiments will be performed for all THD relevant chemicals in the near future for ethical, financial and practical reasons. In addition, typical animal experiments often do not provide information on the mechanism of action of THDC, making it harder to extrapolate results across species. Relevant effects may not be identified in animal studies when the effects are delayed, life stage specific, not assessed by the experimental paradigm (e.g., behaviour) or only occur when an organism has to adapt to environmental factors by modulating TH levels. Therefore, *in vitro* and *in silico* alternatives to identify THDC and quantify their potency are needed. THDC have many potential mechanisms of action, including altered hormone production, transport, metabolism, receptor activation and disruption of several feed-back mechanisms. *In vitro* assays are available for many of these endpoints, and the application of modern '-omics' technologies, applicable for *in vivo* studies can help to reveal relevant and possibly new endpoints for inclusion in a targeted THDC *in vitro* test battery. An international group of experts in the areas of thyroid endocrinology, toxicology of endocrine disruption, high-throughput screening, computational biology, and regulatory affairs has reviewed the state of science for 1) known mechanisms for THD plus examples of THDC and 2) *in vitro* THD tests currently available or under development related to these mechanisms. Based on this scientific review, the panel has recommended a battery of test methods to be able to classify chemicals as of less or high concern for further hazard and risk assessment for THD. Research gaps are identified to be able to optimise and validate the targeted THD *in vitro* test battery for a mechanism-based strategy to decide to opt out or to proceed with further testing for THD.

#### 431 Müllerian duct differentiation – a sensitive target for endocrine disrupters in amphibians

C. Berg, Dept of Environmental Toxicology. The present project aims to develop methods to investigate developmental and reproductive toxicity of endocrine disrupters using the model frog *Xenopus tropicalis* test system. Here I review our findings regarding developmental reproductive toxicity involving disruption of Müllerian duct differentiation. Müllerian ducts are the precursors of the female reproductive tract in most vertebrates but they are absent in teleost fish. Developmental reproductive effects were characterised using two types of endocrine disrupters present in the aquatic environment; progestins and estrogens. The estrogen ethynylestradiol EE<sub>2</sub> is a well studied reproductive toxicant. Progestins (synthetic progesterone) were recently shown to present a risk to female reproduction in fish and amphibians. Progestins are extensively used in human and veterinary medicine and they are ubiquitous in surface and ground waters. *Xenopus tropicalis* were exposed to EE<sub>2</sub> or the progestin levonorgestrel (LNG) via the water during the entire larval period. Nominal exposure concentrations were verified by chemical analysis at regular time intervals. Exposure was discontinued at metamorphosis,

and a subset of frogs was raised to adult age for fertility evaluation i.e. gametogenesis, fecundity, fertility success, mating behaviour, and sperm quality. We found that low environmental concentrations of ethinylestradiol EE (as low as 1.8 ng/L) caused infertility by disrupting the differentiation of Müllerian ducts and testicles. Developmental levonorgestrel exposure inhibited oogenesis and caused a complete lack of developed oviducts rendering the females sterile. No effects of levonorgestrel were seen in the males. In summary, 1) testicular and Müllerian duct differentiation are targets for developmental estrogen exposure 2) egg development and Müllerian duct differentiation are sensitive targets for developmental progestin exposure, 3) development of the female reproductive system is more susceptible to progestin toxicity than the male system. In conclusion, Müllerian duct differentiation is susceptible to disruption by different types of endocrine disruptors resulting in severe reproductive toxicity.

**432 Development and validation of OECD test guidelines on mollusc reproductive toxicity tests** V. Ducrot, INRA / Ecotoxicology and Quality of Aquatic Ecosystems; D. Azam, INRA; R. Brown, AstraZeneca; S. Charles, University Lyon / Laboratory of Biometry and Evolutionary Biology; H. Holbech, University of Southern Denmark / Department of Biology; T. Hutchinson, School of Biological Sciences Plymouth University; L.L. Lagadic, INRA / UMR INRAAgrocampus Ouest Ecology and Ecosystem Health; J. Oehlmann, Johann Wolfgang Goethe Universität Frankfurt am / Aquatic Ecotoxicology; L. Weltje, BASF SE / Agricultural Centre; P. Matthiessen, Independent Consultant. Validated guidelines in line with the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals (EDTA) have been developed for rodents, amphibians, fish, aquatic insects and crustaceans. Only aquatic arthropods have been considered in this test battery although the comparison of endpoints relevant for reproduction in invertebrates often shows a much higher sensitivity in molluscs vs. e.g. daphnids. The OECD test guideline programme has thus been extended to cover reproduction effects of chemicals in molluscs. Existing mollusc toxicity test protocols have been reviewed in an OECD Detailed Review Paper that identifies two relevant candidate species for developing freshwater tests: *Potamopyrgus antipodarum* and *Lymnaea stagnalis*. However, this review did not clarify which toxicity test design/conditions are the most appropriate for chemicals assessment. Therefore, a mollusc reproduction test guideline will be developed describing partial- and full- life-cycle test protocols in these species, so as to propose a balanced suite of apical mollusc toxicity tests applicable for the assessment of any type of chemical, including endocrine disruptors, as level 4 and 5 assays of the EDTA Framework. The guideline project is led by a consortium of experts (Germany/United-Kingdom/France/Denmark) from academia, industry and government stakeholders. To date, expert knowledge has been gathered and formed the basis of draft standard operating procedures (SOPs) for the culture and test implementation of the partial life-cycle tests in both species. Pre-validation of these SOPs consisted in two ring-tests involving 10 partner labs with different level of expertise in mollusc tests. Effects of cadmium on the individual reproductive outputs were studied over 28-d in *P. antipodarum* and over 56-d in *L. stagnalis*. Pre-validation tests were successful in both species. Indeed, both species could be successfully cultured at the lab. Toxicity tests provided consistent results among laboratories for each species (e.g. homogenous and non-significantly different NOECs, LOECs and EC<sub>50</sub> values for most partner labs). The draft SOPs will be optimized (e.g. statistical tests showed that the duration of the test with *L. stagnalis* can be shortened to from 56-d to 35-d) and further tested for various types of reproductive toxicants including endocrine disruptors. A broad scale ring-test will be conducted in 2014 for both species based upon the consolidated SOPs.

**433 Use of vitellogenin as a biomarker for estrogenic effect: What is the baseline?** J.E. Morthorst, University of Southern Denmark / Department of Biology; K. Mathiesen, University of Southern Denmark / Institute of Biology; H. Holbech, K.L. Pedersen, P. Bjerregaard, University of Southern Denmark / Department of Biology. Vitellogenin is used as a biomarker for estrogenic effect in laboratory-based

regulatory test systems OECD TGs 229, 230 and 234 and vitellogenin levels in the plasma of juvenile or male specimens of various fish species have also been used for monitoring estrogenic impacts in freshwater and estuarine environments, especially in association with discharges from waste water treatment plants. The use of any biomarker requires knowledge about the baseline levels in an uncontaminated environment and the present investigation was initiated to identify baseline vitellogenin levels in juvenile brown trout *Salmo trutta*. Juvenile brown trout were caught by electro-fishing from 15 sites in 12 streams of Funen, Denmark, during late September and early October 2010. The fish were brought to the laboratory, blood samples were taken and plasma vitellogenin concentrations were determined by means of a direct non-competitive sandwich ELISA. Vitellogenin concentrations in the plasma of the 135 male, juvenile brown trout caught during the investigation were all below 52 ng/ml and vitellogenin concentrations in the juvenile, female brown trout were generally one order of magnitude higher. The results indicate that baseline vitellogenin levels in juvenile, male brown trout in an uncontaminated environment are below 100 ng/ml. This also indicates that most of the high values obtained in earlier investigations (2000 to 2004) investigations could be attributed to estrogenic exposure. Six of the streams investigated in 2004 were revisited in the 2010 study and vitellogenin concentrations had decreased. Between 2004 and 2010 some discharges (via septic tanks) from scattered houses in the open land known to discharge estrogenic activity had been removed and this may have reduced the addition of estrogenicity to the streams.

**434 Impact of climate change on a risk assessment for intersex in fish due to steroid estrogens** V. Keller, Centre for Ecology Hydrology; P. Lloyd, Wallingford Hydrosolutions Ltd; R. Williams, Centre for Ecology and Hydrology. The impact of climate on the natural environment has been a reason for concern for many scientists across the globe. Although water quantity and water resources have been the focus of many studies over the past years, water quality raised less attention. In England and Wales, steroid estrogens, namely estrone (E<sub>1</sub>), estradiol (E<sub>2</sub>) and ethinylestradiol (EE<sub>2</sub>) were identified as being the main chemicals causing intersex in male fish. A national risk assessment is already available for intersex in fish arising from these estrogens under current flow conditions. This study, presents to our knowledge, the first set of national catchment-based risk assessments for steroid estrogen under future flow scenarios. A geographically referenced model was used to predict concentrations in surface waters across England and Wales for E<sub>1</sub>, E<sub>2</sub> and EE<sub>2</sub>. The river flows were perturbed using 3 climate change scenarios for the 2050's defined by the 2009 UK Climate Projections (UKCP09). These climate change scenarios were chosen to represent a selection of possible changes: ranging from a relatively dry scenario to the wettest scenario available. The effects of demographic changes on estrogen consumption and population served by sewage treatment works were also included by using population projections for the UK in 2050. These predicted concentrations were then combined into estradiol equivalent (E<sub>eq</sub>) and compared to known biological effect levels to assess the risk of endocrine disruption across England and Wales. This risk was then mapped in order to identify hotspots and quantify how the risk could change in the future compared to the current situation. For the 2050s, depending on the climate scenario selected, between 51 and 54% of the total river length modelled is predicted to be at no risk from endocrine disruption ([E<sub>eq</sub>] < 1 ng/l). A significant proportion of reaches are predicted to be at risk (1 ? [E<sub>eq</sub>] < 10 ng/l): between 43 and 45%, and there are between 3 and 4% of reaches estimated to be at high risk ([E<sub>eq</sub>] ? 10 ng/l). Compared to the present situation (no risk: 61%, at risk: 38, and high risk: 1%), this study indicates the possibility of an increased future risk of endocrine disruption in particular within the high risk category where fish intersex is likely to occur. This study provides a spatial overview of this possible change in risks and may provide regulators and policy makers useful information to prepare for this potential risk.

**435 Formulation additives in the environmental risk assessment of biocidal products** A. Coors, ECT Oekotoxikologie GmbH; F. Sacher,

DVGW-Technologiezentrum Wasser; U. Schoknecht, BAM Federal Institute for Materials Research and Testing; B. Weisbrod, ECT Oekotoxikologie GmbH; A. Kehrer, Federal Environment Agency. Biocidal products often contain a wide variety and considerable number of different formulation additives in addition to one or more active substances. According to the Biocidal Products Directive (BPD) and the Biocidal Product Regulation (BPR), which will replace the current BPD in September 2013, an environmental risk assessment must be performed not only for active substances, but also for any substance of concern contained in a biocidal product. These substances must not be seen isolated, but possible cumulative and synergistic effects shall be taken into account according to the new regulation. In this context, the following question will be addressed in the presentation: Is it sufficient to consider hazardous and dangerous substances together with the active substances in a theoretical mixture toxicity model in order to obtain a toxicity estimate for the product? Theoretical and experimental investigations were conducted here with three wood preservative products and their technical eluates in order to verify if their toxicity can be correctly predicted by the concept of concentration addition (CA). The obtained results demonstrate that formulation additives as well as transformation products of active substances can significantly contribute to or even dominate the toxicity of wood preservatives. For some products and eluates, the consideration of the labelled hazardous components in addition to the active substances was sufficient to predict theoretically by CA the toxicity with a deviation of less than factor 2 from the observed toxicity for a number of typical regulatory endpoints, both acute and long-term. This supports for formulated products the reliability of a theoretical hazard assessment. However, the case of other products and eluates demonstrated that this approach is only reliable if indeed all relevant product ingredients are considered.

**436 The UK's approach to addressing the toxicity of mixed active substance plant protection products** M. Reed, Chemicals Regulation Directorate, HSE; J. O'Leary-Quinn, Chemicals Regulation Directorate. For Plant Protection Products (PPPs) containing more than one active substance (a.s.) the combined effects of simultaneous exposure to all the a.s. and the influence of any co-formulants in a PPP should be considered. The approach varies between areas, partly depending on whether formulation data are usually available. It is important that any approach does not result in increased vertebrate testing, so the approach taken for birds and mammals is different to the approach taken for other groups (the requirement for fish formulation testing is set and is based on the sensitivity of the different aquatic groups). For groups other than birds and mammals the **acute or short-term risk** is usually assessed via the use of formulation studies. As regards **reproductive risk** from PPPs it is considered unlikely that the a.s. and all the co-formulants will remain intact and hence that the long-term risk will primarily come from the a.s.. The **long-term risk to aquatic life** from spray drift of PPPs that contain **more than one** a.s. is assessed using data on the individual a.s.. This is based on the assumption that the formulation will break down into its component a.s. once it enters the water. For **drainflow** the potential additive effects should be considered if the a.s. are likely to co-occur. The fate and behaviour of the individual a.s. will determine the degree of co-occurrence and should be considered in the risk assessment. It should also be considered whether the risk is being driven by one a.s.. If all the a.s. within the formulation pass the risk assessment with a margin of safety then the need for further assessment will be limited. For birds and mammals the first step is to determine if one a.s. in the PPP drives the risk. The size of the toxicity exposure ratio for each a.s. in the PPP can be considered. Where it is necessary to calculate the acute toxicity of the PPP the initial approach is based on the toxicity and the proportions of the each a.s. in the PPP and using an additive toxicity approach as described in EFSA 2009 (based on the 'Finney equation'). CRD currently considers the reproductive risk by firstly looking at the basis of the long term end point for each a.s.. If the NOEC in the reproductive study is based on different parameters then it is considered that the effects are unlikely to be additive and the long term risk from the mixed active PPP can be considered by addressing each active substance independently.

**437 Tiered Mixture Risk Assessment With Modes of Toxic Action: Improving Pesticide Risk Assessment** C. Borgert, Applied Pharmacology Toxicology Inc; L.S. McCarty, LS McCarty Scientific Research Consulting; A. Weyers, Bayer CropScience / BCS-D-EnSa-ETX. The use of simple, highly conservative approaches in risk assessment (RA) can focus effort on mixtures of concern, but additional tiered approaches considering mode of action (MoA) are needed for future mixture regulations and to close knowledge/data gaps. We examined an empirical demonstration offered as justification for applying simple concentration addition (CA) to pesticide mixtures in the aquatic environment irrespective of their toxic MoA. Predicted environmental concentrations (PEC) for 25 herbicides were compared to the observed 24h algal toxicity of 23- and 25- chemical mixtures to an estimate derived by applying CA and IA to the algal EC50s for the individual chemicals, irrespective of their pesticidal MoAs. The analysis concluded: "The toxicity of the tested mixture showed good predictability by CA." and "IA slightly underestimated the actual mixture toxicity." Our analysis indicates that the comparison between CA and IA is flawed as a single MoA (Photosystem II) comprises 80% of the toxicity estimate and the experiment fails to test CA against IA based on the usual understanding of their application to chemicals with similar and dissimilar MoAs, respectively. A tiered approach allows a more detailed RA analysis and better fits the data. In the first tier, both a "de minimus" and the CA toxic unit approach used in the herbicide approach above are employed. As this mixture data fails both Tier 1 assessments, it is necessary to move to a Tier 2 assessment. For Tier 2, the CA potency-weighted sum of the components with the most common mode/mechanism of action strongly suggests that the a single MoA - photosystem II inhibition - dominates the algal toxicity of this mixture of herbicides and also explains why CA approximated the case-specific algae toxicity tests carried out. Also, using a critical body residue (CBR) evaluation it appears unlikely that baseline neutral narcosis is contributing to the herbicide mixture toxicity. Plant protection products (PPP) have specific non-narcotic MoAs and mixture narcosis is unlikely at low exposures. However, this is a useful Tier 2 check, ruling out, in this case, an alternative explanation. MoA is vital for estimating mixture risk assessment and possible for data rich substances such as PPP. A tiered approach to regulatory RA based on concepts that better explain the data is preferred over those assuming CA for all chemicals irrespective of exposure concentrations or MoA.

**438 Predictive mixture toxicity assessment of pesticides in Swedish surface waters** T. Backhaus, M. Gustavsson, University of Gothenburg / Dep. of Biological and Environmental Sciences. Several studies have shown that the combined effect of a pesticide mixture can give rise to higher effects exceeding that of each individual compound. Even if all pesticides are present at levels lower than their no effect concentration (NOEC) the mixture effect might become substantial. We have applied a range of methods based on Concentration Addition to pesticide monitoring data from surface waters in Swedish agricultural areas. A database with data from 6 different locations covering 751 individual weekly samples and a total of roughly 60 000 data provided the input for the study. Ecotoxicological data for each monitored pesticide were extracted from the US EPA database AQUIRE and the EU database eChemPortal. The compiled data comprise results from studies with algae, crustaceans and fish and exposure times of 1-4 days. ECOSAR was used to predict toxicity when experimental data were unavailable. Results indicated that algae are frequently more at risk than crustaceans, which on average are slightly more at risk than fish. There are however individual sampling events where fish and crustaceans have been subjected to a higher mixture toxicity than algae. The average mixture risk quotient is 0.2, ranging from 0.6 to 0.036. Using the typical AF of 1000 would lead in most cases to a mixture risk quotient exceeding 1, which would call for follow-up studies. In more than 60% of the cases the estimated mixture risk exceeded the average risk of an individual pesticide by more than a factor 100. Pesticide risks are expected to fluctuate over seasons, following agricultural activities, with a dip towards the end of the year. However, while in some years almost



no seasonal dependence of pesticide risks was observed (e.g. in 2002 and 2003), an increase in pesticide risk over the year was observed in 2004 and 2008.

#### **439 Environmental Risk Assessment of Pesticide Mixtures – Lessons learned from a project on regulatory implementation** F. Frische,

Federal Environment Agency UBA / Section IV 1.3; R. Altenburger, UFZ Helmholtz Centre for Environmental Research; T. Backhaus, Goteborg University; A. Coors, ECT Oekotoxikologie GmbH; M. Faust, Faust & Backhaus Environmental Consulting GbR; D. Frein, Federal Environment Agency (UBA); D. Zitzkat, UFZ Helmholtz Centre for Environmental Research. A paradigm shift is underway in the environmental risk assessment of chemicals leaving behind the traditional “individual substance approach”. For plant protection products (PPP), the new regulation 1107/2009 thus requires in article 29 that “interaction between the active substance, safeners, synergists and co-formulants shall be taken into account” in the evaluation and authorisation. This explicitly refers to marketed PPP, which are by origin technical mixtures containing one to several active ingredients plus several co-formulants. Moreover, common agricultural practice comprises also the application of two or more PPP simultaneously (tank-mixtures) as well as the repeated application of several PPP during the growing season (serial applications). Despite this well-justified concern for exposure of non-target organisms towards pesticide mixtures in the environment, the associated risks are largely not considered in risk assessment so far. Obviously, today’s lack of sufficiently specific technical guidance is a major obstacle for a widely accepted and consistent implementation of mixture risk assessment under regulation 1107/2009. Against this background, the research project “Ecotoxicological combined effects from chemical mixtures” was commissioned by the German UBA in 2009 addressing the relevance and means to account for mixture toxicity within the environmental risk assessment in the authorization process for PPP (and biocide products). Major outcomes of the project are presented in order to illustrate the lessons learned for the on-going regulatory implementation of mixture risk assessment: (i) The data for performing mixture risk assessment are available (i.e. comparatively data rich situation for PPP). (ii) The methodology for component-based predictive approaches - especially Concentration Addition - is available and seems to provide a reliable tool. (iii) The impact for regulatory decision making appears to be rather modest (i.e. not overly conservative outcomes are to be expected). (iv) Common principles are necessary to define coherent regulatory settings for a mixture risk assessment. (v) Tiered approaches are the way forward to deal with e.g. heterogeneous data (i.e. a three-tiered approach allowing for efficient use of the available data and resources will be discussed) (vi) Some questions remain (i.e. regarding indicators for synergism, higher levels of biological complexity, chronic toxicity, realistic exposure assumptions).

#### **440 Life Cycle Assessment of bio-based ethanol produced from different agricultural feedstock** I. Muñoz, Unilever / Safety and Environmental Assurance Centre SEAC; K. Flury, N. Jungbluth, ESU Services; L. Mila-i-Canals, G. Rigarlfsford, H. King, Unilever.

**Introduction** Bio-based products are often considered sustainable due to their renewable nature. However the environmental performance of products needs to be assessed considering a life cycle perspective to get a complete picture of potential benefits and tradeoffs. In this article we present an attributional life cycle assessment (LCA) of a global commodity, ethanol, produced from different feedstock and geographical origin. The aim is to understand the main drivers for environmental impacts in the production of bio-based ethanol as well as its relative performance compared to a fossil-based alternative. **Methods** Ethanol production is assessed from cradle-to-gate, although end-of-life emissions are also included in order to allow a full comparison of greenhouse-gas (GHG) emissions, assuming degradation of ethanol once emitted to air from household and personal care products. The functional unit is 1 kg ethanol, produced from the following combinations of feedstock and region: maize grain in USA, maize stover in USA, sugarcane in North- East of Brazil, sugarcane in Centre-South of Brazil,

sugar beet in France and wheat in France. As a reference, ethanol produced from ethylene in Western Europe is used. Six impact categories from the ReCiPe assessment method are considered, along with seven novel impact categories on biodiversity and ecosystem services (BES). **Results and discussion** GHG emissions per kg bio-based ethanol range from 0.7 to 1.5 kg CO<sub>2</sub>-eq per kg ethanol, and from 1.3 to 2 kg per kg if emissions at end of life are included. Fossil-based ethanol involves GHG emissions of 1.3 kg CO<sub>2</sub>-eq per kg from cradle-to-gate and 3.7 kg CO<sub>2</sub>-eq per kg if end-of-life is included. Maize stover in USA and sugar beet in France appear as the ones with lowest GHG emissions, although when other impact categories are considered tradeoffs are encountered. BES impact indicators show a clear preference for fossil-based ethanol. The sensitivity analyses performed showed how certain methodological choices (allocation rules, land use change accounting, land biomes), as well as some scenario choices (sugarcane harvest method, maize drying) affect the environmental performance of bio-based ethanol. Also, the uncertainty assessment showed that results for the bio-based alternatives often overlap, making it difficult to tell whether they are significantly different.

#### **441 Integrated assessment of prospective scenarios of energy demand by combining economic equilibrium models and LCA** E. Igos, CRP Henri Tudor / Resource Centre for Environmental Technologies (CRTE); B. Rugani, University of Siena / Resource Centre for Environmental Technologies (CRTE); S. Rege, E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies (CRTE); L. Drouet, D. Zachary, Public Research Centre Henri Tudor / Resource Centre for Environmental Technologies (CRTE); T. Haas, F. Adam, Service Centrale de la Statistique et des Etudes Economique (STATEC). The energy sector at country level is very sensitive both from a political, economical and environmental viewpoint, due to the high dependency from the availability and cost of fossil fuels. Initiated by the Public Research Centre Henri Tudor, the Luxembourgish statistics institute STATEC and the Luxembourgish Ministry of Sustainable Development and Infrastructures, the project LUXEN (*Integrated assessment of future energy scenarios for Luxembourg*) aims at assessing the economic and environmental consequences of possible future scenarios of energy supply consumption in Luxembourg. LUXEN has been carried out in two phases: (1) assessment of future energy supply and demand and their consequences on the Luxembourgish economy, and (2) evaluation of related environmental impacts. The first step has been performed by coupling the Energy Technology Environment Model (ETEM) of Luxembourg with the LUXembourg General Equilibrium Model (LUXGEM). Based on the results of this economic- and energy-based coupling, the environmental impacts related to the entire economy were evaluated. Input-output tables in time-series (from 2005 to 2009) for Luxembourg were extended with environmental satellite accounts both at the domestic production and import levels. Specific EE-IO tables were thus created and further disaggregated within the energy sector to meet the purposes of LUXEN. Indeed, the energy sector (i.e. production and use) within the use table for imports as well as the domestic I/O is replaced by the results of ETEM. The corresponding technologies were adapted from the Ecoinvent database (efficiency ratios and emissions of combustion from ETEM), distinguishing imported or domestically produced inputs and outputs. The results showed that the future energy demand will be mainly covered by additional imports of liquid fuel, gas and electricity, which represent the largest part of the environmental impacts. Transport is the most demanding sector (using liquid fuels), due to the dominance of tertiary activity in Luxembourg and the high number of commuters. These results could steer policy proposals since they reflect forecasts linked to two sustainability pillars at the national scale: economy and environment. The outcomes could be also used to identify future marginal energy supplier to answer to an additional demand (e.g. implementation of electric vehicles) and therefore to assess the environmental impacts using a consequential approach

#### **442 LCA as a decision support tool in the urban water system of Aveiro (Portugal)** A. Dias, D. Lemos, University of Aveiro; X.

Gabarrell, Universitat Autònoma de Barcelona; L. Arroja, University of Aveiro. The objective of this study is to assess the environmental impacts associated with the urban water system of Aveiro municipality (Portugal) using Life Cycle Assessment (LCA), in order to identify the stages and processes with the higher environmental impacts and to propose improvement scenarios. The whole water system was considered, including the following stages: water catchment and treatment, water distribution and sewage, wastewater treatment and water administration. The production of chemicals, fuels and electricity consumed in these stages is also considered, as well as the environmental impacts from deposition both in landfill and agricultural soils of the waste generated in wastewater treatment. The transportation of chemicals and fuel consumption by the vehicle fleet of the administration are also considered, as well as the production of ducts used in water distribution and sewage collection. Data on the inputs and outputs of the water system stages refer to the year 2008 and were mostly obtained directly from the entities responsible for their management. The impact assessment method used is the hierarchist approach of ReCiPe. The results show that the stage of water catchment and treatment was the most relevant for the majority of the impact categories, because most of the electricity consumption happened in this stage. For marine eutrophication and marine ecotoxicity, the stage of wastewater treatment carried alone almost all the burden, due to the release of nitrogen and phosphorus in the sea. Moreover, no stage could be regarded as irrelevant (including water administration). The environmental performance of the system could be improved by adopting some measures, such as installation of units for nitrogen and phosphorus removal in the wastewater treatment stage, reduction of electricity demand by improving the efficiency of pumps and other electrical equipment, decrease of water losses in the water distribution system, reduction of water demand, decrease of water inputs to the sewage collection system, and use of solar or other green energy for the pumping systems and for the other equipment that consume electricity. The proposed improvement measures are a basis for the decision making process regarding future investments by local authorities towards environmental sustainability of the urban water system.

**443 Environmental assessment of electricity scenarios with Life Cycle Assessment** T. LARBI, Mines ParisTech; I. Blanc, Mines ParisTech / Centre for Energy and Processes. The environmental impacts of existing electricity generation systems have already been assessed with Life Cycle Assessment (LCA) studies. However environmental impacts assessment of scenarios is very rarely evaluated through a life cycle perspective partly because of the complex parameters it involves, such as the temporal technology variation and the spatial dependency per country. Moreover some studies only analyze the effect of limited indicators variation. Considering these parameters is however crucial when steering and evaluating policy options with regards to the possible environmental consequences. The main objective of this paper is to present the methodology undertaken to perform an LCA for energy scenarios through the analysis of environmental impacts. Such methodology has been applied to a specific scenario, a "Renewable" scenario and results are analysed for Austria. This scenario has been developed within EnerGEO European project. The methodology relies on the following assumptions and steps: The share of energy pathways for electricity production have been considered in line with the scenario definition. The current country-specific technical parameters (efficiency, lifetime, etc...) of each energy pathway have been modeled. The temporal evolution of future energy performances have been considered. LCA for each energy pathway has been performed for each year based on ecoinvent 2.2 database. We apply this methodology to evaluate the impacts on climate change and human health of our renewable scenario (100% RE) for Austria. In this scenario, electricity generation in Austria increases from 87 TWh in 2000 to 137 TWh in 2050. The renewable energy sources share, mainly wind and biomass, increases from 50.34 % to reach 80 % for the same period. Opposite trends are to be found : a substantial decrease of GHG by 75 % due to the decline of fossil energy sources share and a major increase by tenfold of DALYs due to biomass combustion. These first

results are based accounting for (1) restricted data corresponding to the foreseen future technological performances for this scenario and (2) unknown shares of sub-categories for some energy pathways such as biomass where we had to sort between wood, agricultural crops, biogas...etc.. We therefore propose to customize ecoinvent 2.2 data with additional data from other sources: IEA and NEEDS. With this strategy the unavailability restriction is outstripped.

**444 Database and IT infrastructure for Life Cycle Inventories of Bioenergy in Germany** L. Schebek, Technische Universität Darmstadt / Industrial Material Cycles; W. Poganietz, Karlsruhe Institute of Technology; A. Ciroth, GreendeltaTC GmbH; C. Duepmeier, Karlsruhe Institute of Technology; L. Eltrop, University of Stuttgart; S. Simon, German Aerospace Center; T. Targiel, Wuppertal Institute for Climate, Environment and Energy; H. Wagner, Ruhr-Universität Bochum; T. Zschunke, Hochschule Zittau/Goerlitz. Bioenergy is an important part of Climate Policy in Germany. Within the German Sustainable Biomass Strategy, the assessment of existing and novel technologies on a life cycle approach has been acknowledged as important contribution to achieve policy goals and the "need of an adequate database" has been identified as one focus of the funding program of the Ministry of Environment (BMU). The project BioEnergieDat has been carried out by a consortium of German research organizations from 2010 – 2012 and developed a comprehensive database and IT infrastructure for bioenergy process chains for German framework conditions. The database comprehends data sets for processes and process chains of selected bioenergy technologies and energy carriers for today's state of technology as well as for the projected state of technology of 2020/2030. Based on methodological specifications worked out as part of the project, the unit modules may be combined in a flexible way within different process chains and applications. This concept of a modular data supply was enabled on the technical side by a web-based Open Source IT infrastructure. The methodological specifications are based on the idea of a methodological core that specifies fundamental aspects to be supported by all data sets. Datasets for technologies in 2020/2030 are based on a learning curve approach complemented by an analysis of expected market shares of various technologies. The IT infrastructure includes data storage for unit modules, aggregated processes and complete product models and a calculation tool for LCA modelling. As to data storage, a database application based on Internet technology allows local data storage in the modelling tool, but also shared data storage in the central database. In order to ensure continuous provision, up-dating and support of the database, a host organization is being implemented which takes over the operational responsibilities for the database and will be active in future revision, extension of the database and the IT infrastructure. Summing up, the BioEnergieDat database provides core data sets for bioenergy technologies and energy carriers in Germany to be used in support of climate and technology policy as well as further LCA-based applications. The web-based IT infrastructure enables new possibilities for data exchange between different tools, methods and user environments, connecting the field of bioenergy to surrounding fields of application.

**445 Adaptation of the LCA framework to support land use planning policies** E. Loiseau; P. Roux, Irstea; G. Junqua, Ecole des Mines d'Alès; P. Maurel, V. Bellon-Maurel, Irstea. Since the implementation of the European Directive on strategic environmental assessment (2001), local authorities are in charge of carrying studies on the environmental impacts of land use planning policies. However, they are facing with a lack of standardized approaches to perform such assessment. Methodological developments are therefore needed for the environmental assessment of spatial planning policies adopted on a territory. In order to achieve this, different kinds of tools and methods can be used. Among them, LCA has been identified as a promising tool as it has the ability to avoid burden shifting between environmental impacts as well as between life cycle stages. Yet, no study which performs the environmental assessment of a territory as a whole has been reported. The aim of this presentation is twofold. Firstly, the main

methodological bottlenecks which can partly explain the lack of applications of LCA to territories have been identified and discussed, i.e., (i) functional unit definition, (ii) boundary selection, (iii) data collection and (iv) the refinement of LCIA indicators in order to provide useful information for land planning policies. Secondly, for each bottleneck, proposals have been made to overcome it and to provide a general framework adapted to land planning issues. One of the main adaptations relies on the functional unit definition. The territory (defined by its geographical boundaries) and its related land planning scenario will now be considered as the reference flow. The adapted framework will hence deliver two kinds of indicators (calculated outputs), the environmental impacts and a set of services provided by the territory. This adapted framework offers a new perspective on the issue of the environmental assessment of land planning policies by proposing a global approach which include all the activities located on a territory, avoiding burden shifting between territories, prioritizing environmental issues and identifying the most polluting activities on a territorial context which could need a complementary analysis (e.g., environmental risk analysis).

**446 Practical aspects in water quality monitoring of pesticides in streams; autosamplers and GamTox** M.D. Koster, Amt für Umwelt Thurgau / Gewässerqualitaet. In areas of intensive agricultural land use, monitoring water quality in flowing waterways is often expensive and frustrating. The strategy that the Water Quality Board of Canton Thurgau (Switzerland) has developed for their many small streams involves ecotoxicological testing *in situ* with local Gammarids, pesticide analysis with autosamplers, and benthic fauna indices, besides the usual water chemistry. This presentation aims to show results of small-scale intensive monitoring of a stream in a fruit growing area. It also introduces our passive monitoring device for collective water samples, and our experiences with the caged *Gammarus* GamTox toxicity test. In this case-study, highest pesticide input came from the drainage connected to the farm yard, despite protection measures and Good Agricultural Practice. Key Words: pesticides, Gammarus, stream toxicity test

**447 Calculation of community responses to pesticide exposures from mesocosm data: comparison between SPEAR and PRC** A. Focks, Wageningen UR / Mathematics/Computer Science; R. Bhatta, A. Stegerman, Wageningen University / Aquatic Ecology and Water Quality Management; P.J. van den Brink, Alterra and Wageningen University. Aquatic higher tier testing of pesticides using mesocosm experiments is regularly performed as part of the registration procedure. The increase of the ecological relevance of mesocosm tests takes it toll: often complex response patterns are the result of such experimental approaches. To serve as decision criterion for the registration of pesticides, these complex response patterns need to be aggregated, mostly into a concentration where no community effects of a pesticide could be observed in comparison to controls (NOEC<sub>community</sub>). We compared two techniques for determining NOEC<sub>community</sub> values, the Principal Response Curves (PRC) and the SPEcies At Risk (SPEAR) indicators. Mesocosm data was used for three substances: chlorpyrifos (CPF), fluazinam (FLU) and  $\gamma$ -cyhalothrin (CYH). Numbers of different macroinvertebrate species were counted at the given sampling dates in control mesocosms and cosms exposed to different treatment levels. The PRC analysis was performed using CANOCO (version 4.5). The SPEAR calculation was performed as described in the literature. To adjust the species sensitivities, LC50 values were taken from the ECOTOX database (<http://cfpub.epa.gov/ecotox>) and specific sensitivities were calculated. NOEC<sub>community</sub> calculations were performed for each sampling date by applying the Williams test on the community data set of that particular sampling date by using the two methods. For the CPF data set, the PRC detected most NOEC values at lower concentrations and at more time points than SPEAR. For the FLU data set, PRC detected NOEC values throughout at much lower concentrations (table 2), probably because the sensitivity ranking by SPEAR is done based on data dominated by organophosphates which does not represent

the sensitive towards the fungicide very well. An increase of species at risk over time was indicated by the SPEAR index for this compound (temporal dynamics not shown here). For the CYH data set, the results indicated always lower or equal detection levels for PRC when compared to SPEAR. In our evaluation of the sensitivities of PRC and SPEAR to detect the effects of pesticide exposure from mesocosm data it appeared clearly that PRC detected lower NOEC<sub>community</sub> values in all five evaluated mesocosm experiments. Our results indicate that PRC is applicable with less effort and provide a higher level of detection of effects of pesticides on aquatic communities from the evaluated mesocosm experiments.

**448 Impact of polluted sediment resuspension events on chemical and biological water quality guidelines** E. Prygiel, Université Lille; P. Superville, L. Lesven, D. Dumoulin, B. Ouddane, University Lille1; O. Geffard, A. Francois, Irstea; J. Prygiel, Agence de l'Eau Artois-Picardie and University Lille1; G. Billon, University Lille1. In northern France, numerous rivers have been channeled to allow navigation. During the 19<sup>th</sup> and the 20<sup>th</sup> centuries, the Nord Pas-de-Calais region was an important industrial area where the main activities were coal extraction, heavy industry and textile industry. From these activities, a strong pollution of soils and sediments by metals like Pb, Zn, Cd, Hg, Cr... was inherited and some sites are even considered as among the most polluted in Europe. Although industrial emissions have been dropping drastically these last ten years along with the shutting down of polluting factories (such as that of Metaleurop located near the city of Douai), historical sediment pollution still persist in rivers. In some canals, the navigation has been given up so that the resuspension events are rather limited, but they still occur from time to time during river maintenance, restoration or strong raining occasions. In the largest canals, fluvial transport tend to increase with barges able to contain thousands tons of merchandises. As a consequence, resuspension events of contaminated sediments occur recurrently. The impact of sediment resuspension on the water quality has been studied: (i) at high frequency by voltammetry; (ii) by using integrative chemical sensors (DGT, Diffusive Gradients in Thin films) deployed several days *in situ*; and (iii) biologically with caging experiments. For that purpose, the crustacean *Gammarus fossarum* was selected to act as a bioindicator of the water quality. Three canals were selected for this study: the Deûle River, navigated with highly polluted sediments, the Scarpe River, highly polluted but without navigation and the Sensée River, navigated with relatively low pollution level towards trace metals. Preliminary ecotoxicological data shown that only one of measured life traits (feeding rate) was slightly inhibited in the Deûle River. For the other markers studied (molting, oocyte growth, fecundity and fertility), no significant difference has been observed between the 3 rivers. In the other hand, the trace metal dynamic composition of waters, measured on line by voltammetry, shows relevant daily cycle with higher concentrations daytime in the Deûle River while high labile trace metal fractions were accumulated on DGT. These apparent differences between chemical water quality in the Deûle River and caged organisms responses will be discussed as well as the consequences on the WFD ecological and chemical status assessment.

**449 Behavioural, physiological and biochemical markers in damselfly larvae as a tool for assessing effects of accumulated metal mixtures** N. Van Praet, Antwerp university / Department of Biology; M. De Jonge, University of Antwerp / Biology; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology; R. Stoks, University of Leuven / Department of Biology; L. Bervoets, University of Antwerp / Dept. of Biology, Systemic Physiological and Ecotoxicological Research group. The present study investigated relationships between accumulated metals with behavioural, physiological and biochemical endpoints in the damselfly *Ischnura elegans*. Concentrations of accumulated metals were analyzed in larvae of the damselfly *Ischnura elegans*, captured from seven different Flemish ponds. Furthermore different physiological (energy availability), biochemical (acetylcholinesterase (AChE) and glutathione-S-transferase (GST)) and behavioural endpoints (locomotory

activity and the feeding rate) were measured. Accumulated metal levels significantly differed among ponds, both expressed separately or as metal load. The accumulated metal load influenced some of the measured endpoints on different levels of biological organisation. For all measured levels of organisation pond differences are found but only for GST and energy availability this could be partly predicted by the measured mixed accumulated concentrations, expressed as metal load, whereas non of the behavioural endpoints could be predicted by the accumulated metal load. Moreover the major prediction occurs by GST followed by energy availability. By using biochemical and physiological endpoints the effect of accumulated trace metal mixtures could be investigated under natural field conditions, which can ultimately lead to the construction of more accurate biota standards including various metals mixtures.

**450 Trace element bioaccumulation and effects in indigenous brown trout (*Salmo trutta*) and caged brown trout eggs exposed to shooting range run off** L.S. Heier, UMB / Centre for Environmental

Radioactivity (CERAD CoE); E. Mariussen, Norwegian Defence Research Establishment; H. Teien, Norwegian University of Life Sciences (UMB) / Centre for Environmental Radioactivity (CERAD CoE); B. Salbu, Norwegian University of Life Sciences (UMB) / Department of Plant and Environmental Sciences; B.O. Rosseland, Norwegian University of Life Sciences / Department of Ecology and Natural Resource Management. Concern has been raised regarding release of munitions related contaminants in firing ranges such as metals/metalloids and explosives (e.g trinitrotoluene). In some Norwegian military firing ranges there is a legacy of metal deposits which has led to elevated metal and metalloid concentrations in lakes and streams, mostly lead (Pb), copper (Cu), zinc (Zn) and antimony (Sb). The objective of the present study was to investigate the metal accumulation and potential negative effects in brown trout (*Salmo trutta*) inhabiting a lake affected by run off from ammunition deposits, as well as to investigate the survival and metal accumulation of caged trout eggs in the outlet stream. A lake nearby, not affected by metal contamination from ammunition, was used as a reference. Indigenous brown trout were caught by gillnets. Accumulation of trace elements were determined in gills, blood, liver, kidney, brain, muscle and brain tissue. Determination of delta-aminolevulinic acid dehydratase (ALA-D) were done in red blood cells. Brown trout eggs were dry fertilized and immediately placed in cages in the outlet streams of the lakes. The eggs were checked for mortality and development and some eggs were collected for metal accumulation at 4 different times during the exposure, after approximately 110 day degrees (dd) (eggs), 260 dd (eyed egg), 480 dd (at hatching) and 680 dd (alevins). Water samples for determination of metal concentrations, total organic carbon and major cations and anions were collected at each sampling. The brown trout in the lake had very high bioaccumulation of Pb, and the highest accumulation was observed in the kidney, bones and gills. In addition to high concentrations of Pb there is also an additional stress in periods with low pH and elevated levels of Cu. There was a strong inhibition of ALA-D in the trout confirming exposure to high Pb levels, which may cause negative adverse effects. Trout eggs exposed in the outlet stream accumulated Pb, Cu and Sb, and higher mortality was observed after hatching. The high Pb concentrations along with additional stressors such as Cu and low pH could possibly cause lower reproduction of fish in the lake, explaining the lack of young yearclasses during the test fishing.

**451 POPs in catfish (*Silurus glandis*): bioaccumulation and effects** S. Lacorte, M. Valenzuela, S. Fabregat, M. Faria, IDAEA-CSIC / Environmental Chemistry; C. Barata, CSIC / Environmental Chemistry. Fish have the ability to accumulate Persistent Organic Pollutants (POPs) through the diet and become good indicators of environmental pollution. In areas impacted by historic and continuous releases of POPs, unexpected high levels of POPs may affect fish condition, behavior, reproduction, and may lead to serious consequences for the development of the species, reinforcing the necessity of a better knowledge on the presence of POPs in sensitive and impacted areas which are refuges for

numerous wildlife species. In the small city of Flix, there is a chloro-alkali industrial area which historically has produced organochlorinated compounds such as PCBs and OC pesticides and has resulted in the contamination of river waters and sediments in this area and downstream to the Ebro delta [1,2]. The aim of this study is to study the bioaccumulation and biological effects of organochlorine pesticides and polychlorinated biphenyls in the centran European catfish (*Silurus glandis*) sampled across a pollution gradient of a chlor-alkali superfund site located in the low Ebro River, Spain. Integration of analytical tools and toxicological tools were used to determine the levels and effects of high levels of contaminants in a species which is both invasive and with high gastronomic value.

**452 Ecological risk assessment of atrazine in North American surface waters** K.R. Solomon, University of Guelph / School of Environmental Sciences.

**453 Improving regulatory assessment of the risks from chemicals...probably!** M. Reiley, U.S. Federal Employee.

**454 The Critical Roles for Probabilistic Risk Assessment in Evaluations of Controversial, High Production Volume or Well-Studied Chemicals: An Industry View** S.E. Belanger, The Procter Gamble Company / Central Product Safety Dept.

**455 Panel discussion** E.M. Mihaich, .

**456 Assessing aquatic population and community level risks of pesticides** P.J. van den Brink, Alterra and Wageningen University.

**457 Assessing aquatic population and community level risks of pesticides - a regulatory perspective** M. Reed, Chemicals Regulation Directorate, HSE.

**458 Triumphs, trials and tribulations of using ecological modelling in risk assessments: an industry perspective** P. Thorbek, Syngenta / Environmental Safety.

**459 Panel discussion** E.M. Mihaich, .

**460 A novel bioassay with higher plants for integrative sediment toxicity assessments** A. Geilen, German Federal Institute of Hydrology; S. Buchinger, Federal Institute of Hydrology; R. Klein, Trier University; U. Feiler, Federal Institute of Hydrology. Within the framework of RAISA (Rice Arrays for an Integrative Sediment Assessment), a project of the German Federal Institute of Hydrology, a detailed characterization of cause and effect relationships between sediment-bound pollutants and higher plants is planned, which goes well beyond the methods of traditional sediment assessment by means of geneexpression analysis (DNA-Array und RT-qPCR). As test organism *Oryza sativa* L. ssp. *indica* a semiaquatic macrophyt was chosen. Since *O. sativa* genome is completely sequenced, it is also suitable as testorganism for global toxicogenomic studies. For this purpose a test protocol for a new sediment-contact bioassay with the rice plant was developed. The test design was aligned to the ISO/DIS 16191: 2012 test with *M. aquaticum* as one of the few examples of standardized sediment-contact test with higher plants. Hence, test conditions and parameters were optimized and suitable robust and sensitive test endpoints for the assessment of adverse effects were identified. Elongation of root and shoot proved to be easy to detect and reproducible parameters. In exposure experiments, several sediment relevant inorganic and organic pollutants were tested. From the tested compounds Ni, Cr (III), Cr (VI), were investigated in detail with spiked artificial sediments. Concentrations above and under the effective concentration were determined. According to the data obtained the parameter root growth was more sensitive compared to shoot growth. In general, the model organism *O. sativa* proved to be a suitable test organism for a sediment contact assay due to its emerge growth. The successful development of this assay provides now the basis for subsequent analysis of the

molecular level such as DNA-microarrays and RT-qPCR.

**461 Sulfonylurea reproductive effects on terrestrial plants: Results from greenhouse and field studies** H. Ochoa-Acuna, DuPont Crop Protection / Veterinary Pathobiology; R.A. McKelvey, DuPont Haskell Laboratories. The objective of these studies was to evaluate whether reproductive effects (reduction in number of seeds and/or number of seeds and vigour of resulting seedlings) occurred at exposure rates lower than those used for risk assessment. The effect of a sulfonylurea herbicide applied on soybeans at different development stages under greenhouse and field conditions was determined by measuring the response on biomass and seed production at different stages, including effects on seedling emergence and growth from the resulting seeds. Applications were made at four growth stages in both the greenhouse and field studies: seedling stage, pre-bud initiation, flower bud initiation, and beginning pod set. Parameters measured were: vigour at 21 days after application and at late reproductive stage, number of pods/plant at maturity, total yield at maturity, and weight of seeds. The greenhouse study demonstrated greater effect on seedling vigour, biomass at maturity, and yield at lower rates compared to the field study for most application times. Recovery from initial injury was greater under field conditions. The greenhouse study determined that the most sensitive stage for seed yield was bud initiation, whereas for the field study was beginning pod set. The lowest ER<sub>25</sub> value recorded was for 21-d post-application dry shoot weight for applications at the seedling stage which closely corresponds to the standard vegetative vigour test. No adverse effects were observed in the field study or the greenhouse study on germination, viability, and growth of seedlings harvested from treated soybean plants regardless of the application timing. These results demonstrate that risk assessments based on vegetative vigour endpoints are protective of potential effects on reproduction.

**462 A higher tier approach for non-target terrestrial plant testing and risk refinement** A. Solga, Ecology; M. Dollinger, Bayer CropScience; P. Sowig, Bayer CropScience / Ecotoxicology; D. Jans, Bayer CropScience. The authorization of plant protection products under Regulation 1107/2009 requires an assessment of their potential to affect plants occurring outside the cropped area (non-target plants). Routine testing with non-target terrestrial plants is done in highly standardized greenhouse studies. So far no agreed higher tier options have been established. The method presented here aims to provide a straightforward higher tier approach for non-target terrestrial plant testing. As a case study, plant testing cascade with a sulfonyl-urea product has been selected. Seven sensitive species from a greenhouse vegetative vigour study were tested in a new developed semi-field design. Plants were grown in polyethylene containers under outdoor conditions. Application was conducted simulating normal field application. For two days after application, the containers were kept under a UV permeable roof to ensure full penetration of the product. Afterwards the plants were exposed to real environmental conditions. Assessments of phytotoxicity were made on days 7, 14 and 21 after application. Plant survival and shoot dry weight were determined at test termination. As in the preceding greenhouse trial, the most sensitive parameter under semi-field conditions was shoot dry weight. For most species, dry weight inhibitions were distinctly more pronounced under greenhouse than under semi-field conditions. With regard to phytotoxicity, the recorded symptoms were similar; however, the severity of the symptoms under semi-field conditions was overall lower than in the greenhouse. Since the higher tier study delivered endpoints for seven species, it was possible to calculate a species sensitivity distribution from which an HR5 was obtained. The probabilistic non-target plant risk assessment based on this new endpoint resulted in a distinct reduction of required mitigation measures if compared to the outcome of the standard risk assessment approaches. The method presented here proved to be suitable for higher tier non-target plant testing. While increased realism in ecotoxicological studies is usually linked to larger variation, here a high level of reproducibility could be kept due to the straightforward and standardized test design. This approach may also be suitable for running prolonged studies with

non-target plants or for testing other species.

**463 Uptake, translocation and bioaccumulation of PPCPs in vegetables** J. Gan, University of California, Riverside / Department of Environmental Science; S. Wu, J. Conkle, L. Dodgen, University of California. Treated wastewater is increasingly used to irrigate agricultural crops in different parts of the world. A threshold concern for this reuse, however, is the potential accumulation of trace contaminants such as PPCPs into food produce such as vegetables. It is especially important to identify those PPCPs that have a high potential for plant uptake and translocation. In this study, we grew lettuce, spinach, cucumber and pepper in solutions containing 19 PPCPs at 0.5 or 5 µg/L, and analyzed for their tissue levels in roots and leaves using freeze-drying, sonication for extraction and LC-MS/MS for detection. Triclocarban, fluoxetine, triclosan, and diazepam showed the highest accumulation in roots, and the accumulation was positively related to  $K_{ow}$ . However, fluoxetine, diuron, and carbamazepine exhibited the highest translocation to leaves. Therefore, while hydrophobicity propelled root accumulation, hydrophilicity appeared to control in-plant translocation. Carbamazepine was also detected in vegetables irrigated with treated wastewater under field conditions.

**464 Uptake of nitrosamines and other chemicals of emerging concern in the model plant *Arabidopsis thaliana*** C.E. Mueller, G.H. LeFevre, F.A. Hussain, Stanford University / Civil and Environmental Engineering; E.S. Sattely, Stanford University / Chemical Engineering; R.G. Luthy, Stanford University / Civil and Environmental Engineering. Prevalence of persistent, unregulated trace contaminants in recycled water is of substantial concern when applied to agricultural crops for irrigation. Poly- and perfluoroalkyl substances (PFAS) and benzotriazoles, for example, may not be efficiently removed during wastewater treatment; furthermore, disinfection byproducts such as nitrosamines may be produced during the process. As water re-use for agricultural and landscape irrigation continues to increase, understanding the fate and transport of these contaminants becomes imperative for ecosystem and human health. Nitrosamines are a particularly troublesome class of contaminants for plant uptake due to their high mobility and low sorption affinity. This study seeks to fundamentally understand the vegetative uptake and fate of a range of trace organics with a focus on nitrosamines. In laboratory experiments with the model plant *Arabidopsis thaliana*, we are studying the hydroponic uptake of nitrosamines, PFAS, and benzotriazoles under environmentally relevant concentrations. *Arabidopsis* plants are grown in spiked growth solution for 11, 16, and 21 days, after which plants are harvested. In addition, growth media, plant and wall rinse are collected to close the mass balance. Proof of principle experiments with a range of PFAS (perfluorocarboxylates PFCAs and perfluorosulfonates PFSA) showed a clear temporal trend. At day 11, almost all the PFAS were detected in the media with some sorption to the container walls. On days 16 and 21, a distinct increase of PFAS in plant matter was observed. The high ratio of PFAS in the plant compartment might be due to surface sorption on roots rather than plant uptake, which is reflected by the high fraction of PFAS in the plant rinse as well as the observed chain length dependence. Affinity for the plant tissue increased from C6 to C9 PFCAs and C4 to C8 PFSA, which correlates with higher sorption affinities of longer chain PFAS. However, there was an even greater increase in plant affinity from C6 to C4 PFCAs, which cannot be explained by surface sorption. These short chain PFCAs are very mobile and may therefore be efficiently transported into the plant. Following these preliminary experiments, exposure studies with nitrosamines and benzotriazoles are currently in progress employing the same experimental approach to improve the understanding of temporal trends and partitioning behavior of these important water contaminants.

**465 Effects of chronic exposure to radionuclides in terrestrial plants** S.A. Geraskin, Russian Institute of Agricultural Radiology and Agroecology / Radioecology. One of the major difficulties in the implementation of an ecological risk assessment is a lack of knowledge about the effects from chronic low-level exposures to radioactive

contaminants. To understand effects of real-world contaminant exposure properly we must pay attention to what is actually going on in the field. However, for many wildlife groups and endpoints, there are no, or very few, studies that link accumulation, chronic exposure and biological effects in natural settings. The results of long-term field observations in the 30-km Chernobyl NPP zone, in the vicinity of the radioactive wastes storage facility (Leningrad Region), at radium production industry storage cell territory (the Komi Republic), in the Bryansk Region affected by the Chernobyl accident, and in Semipalatinsk Test Site, Kazakhstan that have been carried out on different species of wild and agricultural plants are discussed. Although radionuclides cause primary damage at the molecular level, there are emergent effects at the level of populations, non-predictable from the knowledge of elementary mechanisms of the pollutants' influence. Plant populations growing in areas with relatively low levels of pollution are characterized by the increased level of both cytogenetic alterations and genetic diversity. Accumulation of cellular alterations may afterward influence biological parameters important for populations such as health and reproduction. Presented data provide evidence that in plant populations inhabiting heavily contaminated territories cytogenetic damage were accompanied by decrease in reproductive ability. In less contaminated sites, because of the scarcity of data available, it is impossible to establish exactly the relationship between cytogenetic effects and reproductive ability. Radioactive contamination of the plants environment activates genetic mechanisms, changing a population's resistance to exposure. However, there are ecological situations in which enhanced resistance has not evolved or has not persisted. Consequently, there are good theoretical and practical reasons for more attention being paid to the mechanisms by which populations become more radioresistant and to those situations where radio-adaptation appears not to be taking place. Since radio-adaptation plays an important role in response of populations on radiation exposure, this process needs to be incorporated into management programmes.

**466 Transcriptomic effects of exposure to 17 $\beta$ -ethynylestradiol during sexual differentiation on genetic male *Xenopus laevis*** M. Hecker, University of Saskatchewan / School of the Environment & Sustainability; A. Tompsett-Higley, Monsanto Company / Regulatory; E.B. Higley, Nuclear Engineering and Radiation Health Physics; J.P. Giesy, University of Saskatchewan; S.B. Wiseman, University of Saskatchewan / Toxicology Centre. Genetic male African clawed frogs (*Xenopus laevis*) display feminized/demasculinized phenotypes after exposure to potent estrogens during the period of sexual determination and differentiation, but little is known about the molecular changes that drive the development of these altered phenotypes. Thus, the transcriptome-level effects of exposure to 17 $\beta$ -ethynylestradiol during this sensitive period were evaluated in *X. laevis* by use of *Illumina* sequencing coupled with RNA-Seq expression analysis. The abundances of a subset of transcripts that were determined to be regulated by RNA-Seq were also measured by use of quantitative polymerase chain reaction (qPCR) to validate the quantitative capacity of RNA-Seq. There was a significant correlation ( $R^2=0.78$ ) between the fold-change values calculated for RNA-Seq and qPCR. Overall, a number of pathways and processes were impacted by exposure to ethynylestradiol, including steroid and xenobiotic signaling and metabolism, steroid biosynthesis, thyroid hormone signaling and metabolism, and testicular development and spermatogenesis. Some of the altered pathways, such as thyroid hormone signaling and metabolism and testicular development, could be linked with observed biological effects on gonadal phenotypes and metamorphosis that were observed in a group of frogs that was exposed to ethynylestradiol throughout larval development.

**467 Protection of amphibians from plant protection products** A. Aldrich, Agroscope ACW. For the registration of plant protection products (PPP), effects on amphibians are currently not studied. It is assumed that the risk assessment of e.g. fish, aquatic invertebrates or mammals covers amphibians. Even if the sensitivity of amphibians was comparable to standard test organisms and the exposure of amphibians in water and on land could be estimated, the protection level may be

different for amphibians, given that in Switzerland 14 of 18 amphibian species (78%) are currently endangered. As a precautionary measure, special risk mitigation measures for amphibians are therefore investigated. In Switzerland, 10% of the spawning areas for amphibians are protected, which are supposed to foster and maintain the amphibian population over the long term. These protected areas, currently roughly 900 in total (13'900 ha), are divided into two zones: zone A contains the water body and zone B contains mainly agricultural land and forests, which function as terrestrial habitat and migration corridors. The application of PPP is prohibited in zone A, but not in zone B. Exposure of amphibians can be by drift deposition or run-off of PPP into zone A, or by direct overspray or contact when crawling in zone B. It is currently unknown if this exposure causes adverse effects on the amphibian populations. If amphibians are at risk by the application of PPP, risk mitigation measures have to be investigated. This poster explores the consequences of two risk mitigation measures. Firstly, buffer strips to zone A and B next to vineyards and orchards. Secondly, no PPP application in area B in spring. As a case study, the proportion of affected agricultural area in canton Thurgau by these measures is estimated by GIS analysis. This way, specific areas can be identified where special application techniques should be applied or physical barriers could be erected to reduce the input of PPP into the spawning areas. In practice, these risk mitigations should be implemented in collaboration with the farmers. The analysis showed that roughly 15% (75 ha) of zone B is comprised of crop and grassland, where PPP are potentially applied. Orchards and vineyards are at least 20m away from zones A and B. Therefore, it is suggested that GIS analysis is a suitable tool to identify areas where PPP application in protected spawning areas might occur and to develop applicable risk mitigation measures to protect amphibians.

**468 Time-dependent uptake of trace metals in larvae of European common frog (*Rana temporaria*)** S. Meland, Norwegian Public Roads Administration Norwegian University of Life Sciences / Environmental Assessment Section; S. Lund Johansen, K. Jensen, B. Rosseland, Norwegian University of Life Sciences; E. Farnen, Norwegian Institute for Water Research (NIVA); L. Sorlie Heier, Norwegian University of Life Sciences. Road traffic is considered as a major source of diffuse pollution in the urban environment. Deposited contaminants originating from the vehicles and the road body are readily washed out during storm events to nearby recipients. Thus, aquatic organisms inhabiting small and sensitive recipients may be negatively harmed. As a consequence, wet sedimentation ponds are implemented along roads to protect the aquatic environment from chemical perturbations. These artificial ponds are very similar to ponds existing naturally in the landscape and recent findings suggest that these ponds may be an important habitat for several aquatic organisms, such as insect and amphibians. These organisms are therefore likely to be exposed to e.g. elevated metal concentrations during storm episodes. The common European frog (*Rana temporaria*) is a widely distributed vertebrate all over Scandinavia and is the most abundant frog in Norway. In addition, this species seem to be present in many wet sedimentation ponds along Norwegian roads. Amphibians worldwide are declining in both abundance and species diversity due to many anthropogenic factors. However, *R. temporaria* is currently not considered threatened but local declines are registered. Nevertheless, research on how chemicals interact with amphibians in the environment is warranted. The present study aimed to document the spatial accumulation of metals in larvae of *R. temporaria* inhabiting two wet sedimentation ponds (Skullerud and Vassum) and one naturally occurring pond (Prinsdal). In addition to metal concentrations, the concentration of metallothionein (MT) was determined as it is a well-known biomarker for metal exposure and oxidative stress. The results showed that the frog larvae of *R. temporaria* accumulate metals during their early developmental stages. This was evident for the majority of the measured metals. Increased metal-uptake in larvae from Prinsdal and Skullerud were coherent with increased levels of MT. This was, however, not apparent in larvae from Vassum although they appeared to have the highest metal body burdens.

**469 Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data** L. Weltje, BASF SE / Agricultural Centre; P. Simpson, WCA Environment Ltd; M. Gross, WCA Environment; C. Merrington, WCA Environment Ltd; J.R. Wheeler, Syngenta Ltd. Amphibian toxicity testing is not specifically required for the authorisation of plant protection products or other chemicals in Europe. However, in recent years there has been increasing interest in developing a specific amphibian risk assessment approach due to a perception that the aquatic life stages of amphibians are not protected by current risk assessment schemes. This presentation describes the results of an analysis of acute and chronic amphibian and fish toxicity data performed to determine whether sensitivity differs systematically between these two groups. Acute and Chronic toxicity data for amphibians and fish were principally obtained from the US EPA ECOTOX database (February 2012 search). There were sufficient acute data for comparisons of amphibian and fish sensitivity for 55 chemicals. Amphibians were more sensitive than fish in only 16 out of 55 cases, despite the data selection criteria favouring a higher sensitivity for amphibians. In 14 of these cases the sensitivity of amphibians was less than 100-fold more than fish, which would be accounted for by standard assessment factors. Two comparisons suggested greater than 100-fold sensitivity for amphibians: *p*-nonylphenol and dimethoate. However, both of these comparisons were subsequently determined to be unreliable and, therefore, the weight of evidence suggests that fish and amphibians are similarly sensitive after acute exposure. There were sufficient chronic data for comparisons of amphibian and fish sensitivity for 52 chemicals. Amphibians were more than 10-fold more sensitive than fish on only three occasions (dexamethasone, carbaryl and sodium perchlorate). The relative sensitivity of amphibians to carbaryl and sodium perchlorate was considered to be an artefact of the experimental designs of the studies involved, rather than evidence for greater amphibian sensitivity. This analyses, in common with most other comparative studies, demonstrates that in the majority of cases fish are more sensitive than amphibians to chemical exposure. The exceptions identified appear to be artefacts of either study design or data selection. Only substances that specifically interfere with amphibian metamorphosis may not be detected when using fish as surrogates (e.g. dexamethasone). These data support the notion that additional aquatic amphibian testing is not necessary during aquatic risk assessment.

**470 Advances in reptile ecological risk assessment: measuring and estimating exposure and effects in fence lizards** C.J. Salice, Texas Tech University / Environmental Toxicology; S.M. Weir, The Institute of environmental and human health / Environmental Toxicology. There is a long-standing and well recognized need to incorporate reptiles and amphibians into ecotoxicology and ecological risk assessment. While considerable research has been conducted on the effects of a wide range of chemicals on larval amphibians, by comparison, there are hardly any data on reptiles. However, ecotoxicological data on Western fence lizards and lacertid lizards has indicated that these species may be suitable models for ecotoxicity studies for reptiles. Protocols exist to conduct toxicity studies on reptiles but particularly lacking are studies and models that attempt to better understand and estimate contaminant exposure to reptiles. The objective of our overall research program is to better understand and develop approaches for incorporating reptiles into the ecological risk assessment process. We report on studies aimed at understanding non-dietary routes of exposure. Specifically, we evaluated the nature and extent of dermal exposure to phthalates in Western fence lizards as well as the inhalation toxicity of hydrogen sulfide gas. We also have developed models for estimating dermal exposure. Our model for dermal exposure in reptiles was similar to our experimental exposure results in that dermal exposure was generally less than dietary but nonetheless represented a significant source. One important insight is that chemical with moderate Log *k*<sub>ow</sub> values (approximately 4-5) may result in the highest dermal exposure levels. Taken as a whole, our results suggest that dermal exposure is likely important for reptiles and generally may be more relatively important to total exposure for reptiles compared to surrogate avian species. Our experiments to evaluate the

toxicity of hydrogen sulfide indicated that this noxious gas common to petroleum production areas did not result in any observable toxicity, even in behavioral assays. Our results show that inhalation exposure and toxicity data are obtainable for reptiles but that they may be particularly resistant to gas phase toxicants. This may be the result of a generally lower metabolic rate and, hence, lower respiration rate. We acknowledge that there are very few cases where reptiles are likely to be exposed to gaseous toxicants but nonetheless, our study provides a methodological approach for assessing inhalation toxicity in reptiles. Future efforts in reptile ecotoxicology should be pursued with an eye towards applicability in risk assessment.

**471 Impact assessment of a rodenticide containing Strychnine on wild Gophersnakes (*Pituophis catenifer deserticola*)** C.A. Bishop, Environment Canada; K. WILLIAMS, Environmental Consultant; D.A. Kirk, Aquila Conservation & Environment Consulting; P. Nantel, Parks Canada; J.E. Elliott, Environment Canada. Vineyards and orchards currently represent a significant proportion of the land cover in the Okanagan valley BC. Rodents, such as pocket gophers (*Thomomys talpoides*), eat young roots and chew bark which can girdle the tree or vine. Strychnine, the active ingredient in the product Gopher Getter, is put down holes and tunnels to kill the pocket gophers and bring their populations back under control. Great Basingophersnakes (*Pituophis catenifer deserticola*) are known to eat pocket gophers across their range. We created a model to estimate the exposure of gophersnakes to strychnine, incorporating for example, feeding frequency, prey composition and amount, body mass, and lethal doses of strychnine for pocket gophers and gophersnakes. We evaluated dose at 2 levels, indicating the lowest and highest probable numbers of gophersnakes impacted by strychnine in the Okanagan Valley. We obtained various GIS layers to visualize the extent of the various factors involved, including TEM (terrestrial ecosystem mapping) maps of the south Okanagan and partial TEM maps of the central and north okanagan, Ministry of Agriculture land use coverage of orchards and vineyards in the Okanagan south of the Peachland area, and a TEM gophersnake habitat suitability model. Data was obtained from the pesticide division of Ministry of environment in Penticton, BC, from vendors local to the south Okanagan. We obtained records for two years from 2005 – 2006 and averaged yearly data to obtain our yearly volume sold (1712 kg). Using population viability analysis, we estimated that only 15% of the population killed by rodenticide poisoning (~30 individuals) per year would change annual growth in the population ( $\lambda$ ) from 1.0 to 0.93 and hence within 25 years only 17% of the population would remain.

**472 The Stockholm Convention – an Industry perspective** D. van Wijk, S. Presow, Euro Chlor. The Stockholm Convention on Persistent Organic Pollutants is an international treaty to eliminate and/or control the production and use of environmentally problematic substances, in particular those showing persistence, bioaccumulation and toxicity, as well as long range transport. A risk profile is developed that must demonstrate that the substance “is likely, as a result of its long-range environmental transport, to lead to significant adverse human health and/or environmental effects, such that global action is warranted.” From an industry perspective, the Convention’s success has been limited. One particular concern is the lack of interest of the POPs Review Committee to apply recent developments in the scientific peer-reviewed literature in the discussion of POP substances and risk. The conclusions of a SETAC Pellston workshop on PBT and POP identification were published in a special series in IEAM in 2009. Indeed, one particular publication from this workshop examined and critiqued several risk profiles developed for the POPRC to consider. Unfortunately, there is little evidence that the conclusions of this state-of-the-art workshop have been incorporated into the working of POPRC. In this presentation we discuss this, with particular reference to the persistence, bioaccumulation and risk assessment of hexachlorobutadiene. Given there have now been 8 annual POPRC meetings, it is still unclear if the application of scientific risk assessment approaches will lead to appropriate discrimination of the substances nominated to the Convention that truly warrant concerted global action, from those that may have intrinsic properties of concern



but do not deserve such action.

**473 Analytical and economic assessments for demonstrating progress in implementation of the Convention** H. Fiedler, UNEPDTIE Chemicals Branch. The Stockholm Convention on Persistent Organic Pollutants (POPs) entered into force in 2004 and presently has 179 parties. In a number of provisions, quantitative information needs to be generated such as release inventories for unintentional POPs (article 5) or concentrations of POPs in the environment and in humans (article 16). This paper presents such measured or estimated data generated under the Convention and assesses them on a national, regional and global basis. In addition, for PCDD/PCDF, a first economic assessment is undertaken by linking these informations to economic status of the countries. Quantitative data have been generated and reported using the Standardized Toolkit for developing national release inventories of PCDD/PCDF. The data on concentrations of POPs in mothers' milk have been generated by the UNEP/WHO reference laboratory at the Chemisches Untersuchungsamt Freiburg, Germany. Data for Gross Domestic Product is provided by the World Bank. Food consumption is analysed based on data from the Food and Agriculture Organization. In 2011, 68 release inventories have been available, which showed total releases of 58,700 g TEQ per year. The main vector of release was air corresponding to 45% of total releases, second highest was releases in residues (34%), whereas releases to water only played minor role (2%). Depending on the assessment, such as  $\text{per source group per capita, per square kilometer or per unit of gross national product}$ , the ranking of countries with high or low emissions changed considerably. For mothers' milk, time has evolved as the main discriminating factor. Although results generated under the Stockholm Convention are on a national basis and highly aggregated, socio-economic assessments can be used for determining priority actions under the Convention.

**474 Influence of global climate change on chemical fate: temporal trends of persistent organic pollutants in the atmosphere** D. Kong, I.T. Cousins, Stockholm University / Applied Environmental Science (ITM). Research on temporal trends of persistent organic pollutants (POPs) in the environment has previously been undertaken to provide valuable information on the effectiveness of emission reductions resulting from voluntary actions by industry or as a consequence of legislation, the varied roles of primary and secondary emission sources and even the influence of global climate change (GCC). Recently, a nonparametric Mann-Kendall (MK) test with an additional process to remove the effect of serial correlation has been developed and applied to several time series of monitored atmospheric POPs. This method is more robust in examining the statistical significance of temporal trends in time series despite the limited duration and large variability of most time series. We use this MK test method and aim to detect the temporal trends in all available time series of monitored atmospheric concentrations of POPs from all available monitoring stations. We surveyed one of the well-known online databases, i.e., the EBAS online database hosted by the Norwegian Institute for Air Research. 656 time series were identified to have a time span equal or longer than at least 10 years. They were collected at 20 monitoring sites from Canada, Czech Republic, Iceland, Norway, Sweden, and the United States. At all monitoring sites, 576 of 656 analysed, a downward trend could be observed in time series of monitored atmospheric concentrations. This supports similar previous findings, i.e., the atmospheric levels of most POPs are declining in North America, Europe and the Arctic. This declining trend has been mainly attributed to the actions taken globally over the last two decades to reduce or eliminate production, use and emissions of POPs. Inclines in the detrended time series could be observed, especially in detrended time series of those POPs listed in the Stockholm Convention such as PCBs and HCHs. These inclines have been suggested to be due to remobilization through volatilization of legacy POPs from surface compartments to air increased from the late 1990s to the present day. A study further argued that these inclines are induced by recent temperature increases resulting from GCC. We do not

agree, however, that GCC is the only possible cause for these observed inclines. We support the views that the observed inclines could also be caused by e.g. surface-to-air fluxes driven by a strong surface:air fugacity gradients and buffering emission reductions (with or without climate change).

**475 POPs: Have we achieved our objectives and where do we go from here?** K.R. Solomon, University of Guelph / School of Environmental Sciences. Our knowledge of the behavior of the persistent organic pollutants (POPs) has grown considerably since the establishment of the Stockholm Convention. The Stockholm Convention was grounded in the understanding to the classical POPs at that time (1995), DDT, dioxins, furans, and other halogenated pesticides. These compounds were identified empirically on the basis of observed persistence, bioaccumulation, and toxicity (PBT). The Stockholm Convention has continued to identify more POPs and some, such as endosulfan, are on the cusp in terms of properties such as trophic magnification and their classification has been debated and many levels. The criteria for identification of POPs have not changed and have not addressed new knowledge about these compounds. More recent programs have expanded to address a larger range to chemicals in general via REACH and EC1107 in the EU and similar legal instruments in other jurisdictions. In this process we have learned that POPs do not have to share all the properties of the dirty dozen to present a risk to the environment. Experience with the perfluorinated compounds in the environment shows that while persistence is still key, incorporation into the food chain does not require partitioning into lipids associated with compounds of high  $K_{ow}$ . Even compounds with high  $K_{ow}$  may not be problematic if other properties such as  $K_{aw}$  result in partitioning into the atmosphere where rapid degradation occurs. With the increasing focus on POPs and compounds with PBT properties will place burdens on evaluators and lack of consistency across and within jurisdictions will complicate the identification of POPs and PBTs and may result in false positives and negatives.

**476 Panel discussion** D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division. The goal is to address issues raised during the previous platform presentations, and to focus on Stockholm convention effectiveness and where it should go in the future. \n

**477 Current and future developments concerning the testing and regulation of endocrine disrupters - an overview** P. Matthiessen, Independent Consultant. This paper provides an overview of the conclusions of a SETAC-Europe Special Science Symposium (SESSS) on Environmental Endocrine Disrupter Testing and Evaluation which was held in Brussels on 24-25 October 2012. The purpose of the meeting was to consider the various pieces of European, American and Japanese regulatory legislation concerning endocrine disrupting chemicals (EDCs), to evaluate experiences to date with new testing methods for EDCs, to assess how these tests can be effectively deployed in chemical assessment schemes, and to scan the horizon for developments in testing approaches and methodology. It was explained that new legislation in Europe and the USA which seeks to regulate EDCs requires the application of a suite of relatively new chemical testing techniques. Some testing methods and guidance documents for substances with estrogen/ androgen/ thyroid/ steroidogenic (EATS) modalities are now available, but there remain many gaps, both in terms of the taxa protected and the modes of endocrine action covered. This is partly due to poor understanding of the types of endocrine disruption which are actually occurring in the field (although the impacts which are known about fully justify generic regulatory action on chemicals), and partly due to a lack of knowledge about the endocrinology of many invertebrates. Additional new testing techniques and assessment procedures will be needed to address these gaps. It will also be necessary to develop environmental risk assessment methods which recognise the special properties of some EDCs such as non-monotonic dose-responses and delayed effects of brief early-lifestage exposures. Finally, there remains a need to continue monitoring the environment for

the impacts of EDCs in order to focus regulatory efforts on the most relevant chemical groups and modes of action. The paper will conclude by describing the major new research directions which are needed to underpin regulatory programmes in this field.

#### **478 The Law of Unintended Consequences: The Endocrine**

**Regulations** L.S. Ortego, Bayer CropScience / Environmental Toxicology and Risk Assessment; E.M. Mihaich, . In 1996, in response to public concern that some chemicals may interact with the endocrine system in humans, regulations were adopted in the US directing the US EPA to establish a screening program using "validated test systems" to investigate the potential for adverse health effects to be induced through endocrine pathways. While the charge sounded simple enough, it neglected to consider the complex system being targeted for evaluation. Policies and procedures had to be put in place, screens and tests had to be developed and validated, and significant resources had to be earmarked in order to comply with the program. The US Endocrine Disruptor Screening Program (EDSP) has taken more than 10 years and millions of dollars to reach the point of initiating test orders for the performance of the screening battery for the first 67 chemicals in the program. Now, US EPA is evaluating the screening data from this first list of chemicals and making decisions on what, if any, additional testing is required to determine if adverse effects are occurring as a result of exposure to these chemicals. In this talk, we look at the challenges faced in this complex regulatory program. There were costs and impacts, expected and unexpected, in the development of the lists of chemicals to be tested, the performance of the screens and in the collection and reporting of the screening-level data. Going forward, it is important to consider the progress made and any lessons learned as the program evolves to consider high-throughput screens and adverse outcome pathways. It is also relevant to consider the impact testing results may have outside of the US as various regions around the globe work to implement their own endocrine programs.

#### **479 Predicting Reproductive Toxicity by Available Non-testing Tools – Are These Applicable for Prioritization in REACH?**

Rybacka, Chemistry Department; C. Ruden, Stockholm University / Department of Applied Environmental Sciences; P.L. Andersson, Umea University / Chemistry Department. According to Article X in REACH, compounds produced in quantities  $\geq 1$  ton/year and identified with carcinogenic, mutagenic, or toxic to reproduction (CMR) properties or as persistent, bioaccumulative, and toxic (PBT) or very persistent and very bioaccumulative (vPvB) should be prioritized for further testing. Of particular interest is R classification appointing chemicals that cause effects on different developmental stages and mechanisms related to reproduction. These compounds known as endocrine disruptors (EDCs) can alter normal hormone levels in the organisms and hence affect functions that these hormones control. Identification and assessment problems of numerous endocrine disruptors could be solved by using non-testing methods, e.g., expert systems. In this study we focused on 94 chemically diverse organic chemicals selected from the priority lists assigned by the Commission according to the Existing Substances Regulation and evaluated a number of *in silico* tools including (Q)SAR Toolbox, LAZAR, Toxtree, DEREK, VEGA and TEST on their use to predict various R properties. Data on models statistics, i.e., sensitivity, precision, and balanced F-measure were calculated and compared. Furthermore, the chemicals were studied on a chemical map including European high and low production volume chemicals. This map, described in detail elsewhere<sup>1</sup>, was constructed using PCA and a range of chemical descriptors and revealed that the studied chemicals are representative for currently used industrial chemicals. The study showed the highest F-measure for estrogen receptor binding; however, for this endpoint 88% of chemicals were classified as out of domain. Rest of models performed substantially worse (mean and the lowest F-measure: 0.34 and 0.09) leading to many false negatives. These mispredictions result from models' errors but also due to misleading experimental data, e.g., assays that lack premetabolised products that would result in the toxic answer. We are currently investigating compounds commonly predicted as false negatives and analyzing chemical maps of compounds

based on other types of descriptors, e.g., atom-centred fragments, to see if grouping of chemicals can improve the poor performance of the models for R. [1] Rännar S and Andersson PL. 2010. A Novel Approach Using Hierarchical Clustering to Select Industrial Chemicals for Environmental Impact Assessment. J Chem Inf Model 50(1): 30-36

#### **480 Hazard, risk and the need for a scientific approach to the identification of endocrine disrupting properties**

J.R. Wheeler, Syngenta Ltd. In recent years, endocrine disruption has become a topic of increasing public and regulatory concern. This presentation will outline some of the current approaches being taken by European regulatory agencies, US-EPA and industry working groups in order to define suitable criteria for the identification and management of endocrine disrupting chemicals. Plant protection products are likely to be impacted by recent changes which will create hazard based cut-off (ineligibility for registration) criteria for endocrine disrupting properties and so are used here to illustrate the approaches under development. A key component of all the EU member state and NGO proposed schemes is the establishment of weight of evidence methodologies. These aim to provide an independent assessment of the data's reliability/repeatability and relevance to inform the question of significance. Then an overall weight of evidence, incorporating all available information, can be used to conclude whether a substance has sufficient evidence to be judged against specified criteria or if further testing is required.

**481 Assuring transparency and objectivity: Application of an hypothesis-based weight of evidence framework to ESB data**E.M. Mihaich; C. Borgert, Applied Pharmacology Toxicology Inc; L.S. Ortego, Bayer CropScience / Environmental Toxicology and Risk Assessment; S. Marty, Dow Chemical Company; J.M. Brausch, F. Hess, BASF Corporation. A comprehensive, hypothesis-based weight of evidence (HB-WoE) framework was developed to be applicable to any determination relying on experimental data, and a specific formulation was proposed for evaluating results of the U.S. EPA's Tier 1 Endocrine Screening Battery (ESB) (Borgert et al., 2011). The key elements of this HB-WoE process are development of *a priori* specific hypotheses, a systematic review of relevant literature including an evaluation of data quality and reliability (including primary validity of the measurements, reliability of data reporting, and probative capability of the study design to evaluate causation), and an assessment of consistency, specificity and reproducibility of effects. The framework requires that before any WoE determinations are considered, each experimental endpoint be weighted according to its relevance ( $W_{REL}$ ) for deciding each of 8 hypothesis addressed by the ESB. The purpose of these requirements, especially *a priori* hypothesis formulation and  $W_{REL}$  development, is to ensure a level of transparency and objectivity not possible from WoE processes claiming a basis in professional judgment alone. To make WoE determinations for a particular substance, the framework requires combining  $W_{REL}$  values/rankings for each hypothesis with response weightings ( $W_{RES}$ ) derived from the ESB data. Data for the test chemicals are evaluated for each hypothesis, beginning with Rank 1 and continuing through Rank 3 endpoints. The response to Rank 1 endpoints guides the evaluation and interpretation of information from lower-ranked endpoints. Consistency is evaluated within and between rankings to ensure a comprehensive and reproducible process. This presentation demonstrates application of the HB-WoE process using two example chemicals. Data for chemical A was recently produced in response to EPA test orders and has not yet been publicly released. Data for chemical B was gleaned from the scientific literature. The evaluation for chemical A illustrates how the HB-WoE process streamlines refutation of the ESB hypotheses, and chemical B illustrates how potential ambiguities are handled. The identities of the chemicals will be revealed and the overall WoE determinations discussed in detail.

**482 Human susceptibility risk assessment to bisphenol A from multiple exposure pathways** C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering; K. Fu, C. Chio, National Taiwan University. The purpose of this study was to investigate the age-specific human susceptibility risk for environmental

bisphenol A (BPA) exposure based on a probabilistic multiple-exposure-pathway approach. Five health responses were taken into account including (i) immune response, (ii) uterotrophic assay, (iii) cardiovascular disease, (iv) diabetes, and (v) behavior change. A Hill model was used to best fit the dose-response profiles adopted from animal and epidemiological data. A physiologically-based pharmacokinetic (PBPK) model was used to estimate urinary BPA concentration from external exposure. Here we showed that daily average exposure concentrations of BPA were consistent with those of the published literature. Our results also indicated that the urinary BPA estimates are close to published data associated with the Chinese populations. We found that BPA were unlikely to pose significant risk for infant (0 – 1 year) and adults (male and female > 20 years) with less than  $10^6$  fold increase for uterus weight and immune response outcomes, respectively. Moreover, results showed that approximately  $10^4$  –  $10^2$  fold increase were estimated for cardiovascular disease, diabetes, and behavior change outcomes with and without skin adsorptions at exceedance risk of 50%. Our findings suggest that environmental BPA concentrations are unlikely to pose significant threat on human health among specific subgroups. However, the integrated impacts of BPA on human populations are needed to be further investigated.

**483 Environmental risk assessment of GM crops: what is the regulatory process and how does it compare with plant protection products?** F. Tencalla, T. Petry, Tox minds BVBA M. Garcia-Alonso, Estel Consult Ltd. In the European Union (EU), distinct legislation exists covering the placing on the market of plant protection products (PPPs) and genetically modified organisms (GMOs). Plant protection product legislation has been in place for several decades and was reformed in 2009. GMO legislation dates from the early 1990's and was updated at the beginning of the years 2000. For both, new guidance documents are regularly released, following the development of science and knowledge in the respective areas. Overall, the human and environmental risk assessment approaches taken for PPPs and for genetically modified (GM) crops, a category of GMOs, are quite different. In part, this is due to the fact that chemicals and plants cannot be evaluated in the same way, although there are sometimes overlaps (for example crops that act as pesticides, for example Bt plants which produces one or more proteins specifically toxic to certain types of ravaging pests, and crops which work together with pesticides, for example herbicide-tolerant plants which may grow in the presence of a specific weed-removing chemical). Also, historically, methodologies and expertise evolved partly in parallel with sometimes only limited cross-fostering. This presentation proposes to look in detail at the regulatory requirements in the EU for the environmental risk assessment (ERA) of PPPs and GM plants. The focus is on chemicals and crops, and not microorganisms. The goal is to highlight the main similarities and differences. This represents one aspect of a broader discussion within the proposed SETAC session on 'ERA of GM crops. Similarities and differences with the ERA methods used for plant protection products'.

**484 Evaluation of potential adverse effects of GM plants on non-target organisms - an EFSA GMO Panel perspective** Y. Devos, GMO Unit. In November 2010, the Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) issued guidelines for the environmental risk assessment of GM plants, which includes the evaluation of their potential adverse effects of GM plants on non-target organisms (NTOs). Issues to which special attention was paid in these guidelines and that will be presented in this talk are the consideration of functional biodiversity as protection goal, criteria for the selection of NTOs for risk assessment purposes, and approaches to assess the consequences of intended and unintended changes in GM plants on NTOs. During the development of the above mentioned guidelines, EFSA has consulted EU Member States and all relevant stakeholders via public consultations. Some of the scientific comments received from EU Member States and stakeholders will be presented.

**485 Describing the receiving environment: a database on European**

**arthropods inhabiting arable crops** M. Meissle, Agroscope ART / Biosafety. Like plant protection products, genetically engineered (GE) plants are subject to risk analysis before they can be placed on the market. This includes an environmental risk assessment, covering several areas of environmental concern, one of which is the possible risk to valued non-target (NT) organisms including beneficial and protected arthropods. Knowledge of the receiving environment of a new GE plant is valuable in order to generate appropriate risk hypotheses and consequently relevant data to support the environmental risk assessment. The presented database, administered by EFSA, makes knowledge on the composition of the arthropod fauna in different arable crops available. Information on species attributes and collection records has been stored in a SQL-queryable database. In a systematic literature search, suitable publications on arthropods counted or collected in European maize, oilseed rape, potato, beet, soybean, cotton, and rice fields were identified. The database contains more than 3000 species and 14'750 records that were extracted from more than 1000 publications. Records from 31 European countries were available. The functional groups of predators and herbivores are represented with the highest number of records, followed by parasitoids, decomposers, and pollinators. Within the group of predators, beetles (carabids, ladybirds and rove beetles) and spiders were clearly the taxa collected with highest intensity. While most information is available for ground- and plant-dwelling species, only little data on species level has been published for soil-living arthropods. Maize is the crop for which most records and species have been identified. Rice is unique because it supports a large number of aquatic species. One limitation of the database is the fact that the total number of records per species represents collection effort of researchers rather than total abundance of a species. Nevertheless, the presented database provides a detailed picture of the arthropod fauna of European arable crops. For the environmental risk assessment for GE crops, it can help to identify species to be assessed and support the selection of species for laboratory studies, for higher tier studies, and for case-specific monitoring. For the environmental risk assessment of plant protection products, the database could be used in a similar way.

**486 Selecting test species for early-tier risk assessment studies of insect-resistant transgenic plants** J. Romeis, Agroscope ART / Biosafety; A. Raybould, Syngenta; F. Bigler, Agroscope ART; M.P. Candolfi, Innovative Environmental Services (IES) Ltd; R.L. Hellmich, USDA-ARS & Iowa State University; J.E. Huesing, Purdue University; A.M. Shelton, Cornell University/NYSAES. Arthropods form a major part of the biodiversity in agricultural landscapes. Many species are valued because they provide ecosystem services, including biological control, pollination and decomposition, or because they are of conservation value. Some arthropods reduce crop yield and quality, and conventional chemical pesticides, biological control agents and genetically engineered (GE) crops are used to control them. A common concern addressed in the ecological risk assessment (ERA) that precedes regulatory approval of these pest control methods is their potential to adversely affect valued non-target arthropods (NTAs). A key concept of ERA is early-tier testing using worst-case exposure conditions in the laboratory and surrogate test species that are most likely to reveal an adverse effect. If no adverse effects are observed in those species at high exposures, confidence of negligible ecological risk from the use of the pest control method is increased. From experience with chemical pesticides and biological control agents, an approach is proposed for selecting test species for early-tier ERA of GE arthropod-resistant crops. Surrogate species should be selected that most closely meet three criteria: (i) *Potential sensitivity*: species should be the most likely to be sensitive to the arthropod-active compound based on the known spectrum of activity of the active ingredient, its mode of action, and the phylogenetic relatedness of the test and target species; (ii) *Relevance*: species should be representative of valued taxa or functional groups that are most likely to be exposed to the arthropod-active compound in the field. (iii) *Availability and reliability*: suitable life-stages of the test species must be obtainable in sufficient quantity and quality, and validated test protocols must be available that allow consistent detection of adverse effects on ecologically relevant parameters. Our proposed

approach ensures that the most suitable species are selected for testing and that the resulting data provide the most rigorous test of the risk hypothesis of no adverse effect in order to increase the quality and efficiency of ERAs for cultivation of GE crops.

**487 Considerations for adapting ecotoxicology studies designed for crop protection products for assessing risk of genetically modified crops** A. Nelson, Syngenta Crop Protection. Crystal proteins derived from *Bacillus thuringiensis* have been introduced into crops to provide control of certain pest species. Laboratory studies are conducted with invertebrate nontarget organisms (NTOs) to determine if there are adverse effects of oral exposure to the Bt toxins. NTO test methods exist for chemical crop protection products, however many of these involve spray application of the test material on to an inert substrate. Therefore, certain considerations must be taken into account when adapting methodology traditionally used for crop protection effects testing for use in assessing the effects of genetically modified crops. Firstly, a palatable diet is needed in which the transgenic protein remains fairly stable throughout the duration of the test. Proteins must be handled such that the integrity of the protein is maintained. Additionally, diet components should be treated to limit potential protease activity when combined with the protein. The diet must be prepared so that the transgenic protein is homogeneously integrated into the diet. Finally, in order to confirm exposure of the NTO to the transgenic protein, the diet is analyzed to measure the concentration of the transgenic protein and verify that the protein has maintained its biological activity. Confirmation of biological activity is conducted by incorporating the NTO diet into the diet of a sensitive pest species. Therefore, when selecting test levels in an NTO study the concentration must be greater than the estimated environmental concentration and also high enough to elicit a response in the sensitive pest species. In conclusion, the guidelines written for testing effects of chemical crop protection products provide an excellent basis with regards to replication, validity criteria and endpoints. With a few modifications highlighted above, it is possible to alter these test designs to examine for effects of oral exposure of Bt toxins.

**488 Approaches and Challenges to Assessing Potential Interactions of Bt Proteins in Support of Safety Assessments for Genetically Modified Crops** S.L. Levine, Monsanto Company / Regulatory Sciences; G. von Merwy, Monsanto / Regulatory Affairs. Over the past decade a number of genetically modified (GM) crops have been developed that produce more than one *Bacillus thuringiensis* (Bt) proteins that provide protection from feeding damage caused by specific pest species. Consequently, an assessment of potential interactions (antagonism, synergism, potentiation) among Bt proteins is often provided as part of the safety assessment for genetically engineered crops expressing two or more Bt proteins. Typically, these studies are performed in 7-day laboratory diet-incorporation bioassays using an insect species of that is susceptible to one or more of the Bt proteins. This presentation will provide an overview of first principles in assessing mixture toxicity drawn from the chemical toxicological literature and draw linkages to how these approaches are used to support Bt producing GM crops. Additionally, this presentation will provide examples of experimental designs, approaches for data analysis and how the results from these studies are used to support environmental safety assessment for GM crops expressing multiple Bt proteins.

**489 Complementary company initiatives to reduce environmental exposure to pesticides: Product stewardship example bentazone** V. Laabs, B. Jene, J. Leyendecker, BASF SE / Global Product Safety and Registration. The process for registration of crop protection products (pesticides) in the EU is one of the strictest worldwide. As a result, the number of registered pesticides has decreased by more than 50% and maintaining an adequate toolbox of crop protection products in the EU to manage resistance of weeds and pests is now becoming increasingly important. Although in principle the exposure of the environment to pesticides is reduced to an acceptable level by the EU regulatory system, unaccountable exposure pathways exist (e.g. point sources, extreme environmental conditions) that can lead to higher than

expected exposure at times. As these latter exposure scenarios cannot be adequately addressed by regulatory measures, industry-driven product stewardship programs may be a solution to manage such risks during product use on farm. Bentazone product stewardship is presented as example for such a program, aiming at a further reduction of the exposure of water resources. For bentazone, which is a well established herbicide with essential resistance management functions (e.g. in legumes, rice) and comparatively favorable (eco)toxicological properties, the only area of concern is its mobility in soil. This has led in some EU countries over the last decades to regular, but local and transient, exceedances of the generic EU limit value of 0.1 µg/L in groundwater, triggering a company decision to safeguard this a.i. in the long term via a product stewardship program. Based on an in-depth analysis of the history of exceedances of bentazone, three main pollution pathways were identified: (i) point sources, (ii) diffuse leaching in vulnerable areas, and (iii) stream bank infiltration from e.g. ditches. On this basis, a set of seven measures were identified to reduce the frequency of exceedances in groundwater (among these: clarification of and follow-up on exceedances, restriction of seasonal use, use restrictions in defined vulnerable areas). This set of measures was put into a management-approved product stewardship guidance, which is rolled out by BASF units at country level using detailed country-specific implementation plans. Implementation progressed in nine EU countries in 2011/2012 and will start in further countries in 2013. The implementation process is monitored via key performance indicators and first experience has shown that regular information campaigns/refreshers are needed to maintain outreach to and awareness among farmers.

**490 User-engagement is key to implementing low-drift nozzles. Generic learnings from year 1 of UK Chlorpyrifos Say-No-to-Drift campaign.** S. Norman, Ridgeway Eco. The insecticide chlorpyrifos is highly toxic to aquatic arthropods. Hence, it is imperative to prevent spray drift reaching ponds & streams. To protect aquatic ecosystems while maintaining availability of this substance to UK farmers, the 'Say No to Drift' campaign was launched in autumn '11 by Dow AgroSciences, Makheshim Agan, & Headland, supported by ADAS & Pinstone. Experiences from year-1 are relevant for implementing low-drift nozzles (LDN) in general, this active ingredient being used in all major sectors (arable, grassland, vegetables, soft fruit & orchards). Drift-reduction is also fundamental to Directive on Sustainable Use of Pesticides, & National Action Plans (NAP) which enact it (e.g. UK NAP 2012). Aim was to have LDN used for all chlorpyrifos sprays in UK, plus extended buffer zones. The principle of LDN is inclusion of air in the liquid stream, which reduces fine driftable droplets by 95%. Over the last 10 years, use of LDN has increased in UK arable sector (due to LERAP scheme), now accounting for around 80% of applications. However, in orchards (the use which creates most drift), uptake of LDN in UK was minimal. Possible reason is that no orchard LDN are currently rated for LERAP & because LDN require major shift in mind-set to put into practice. The 'Say No to Drift' philosophy is clear-communication, direct-engagement, active-listening & team-working. Realised via: events & articles - widely reported by press & online; a website [www.saynotodrift.co.uk](http://www.saynotodrift.co.uk); listening & talking to growers, agronomists, distributors, & associations - empowering & encouraging to 'spread the word'; & on-farm training events. Dialogue with UK Chemicals Regulation Directorate has also been essential in building faith & gaining permission for Pesticides Usage Survey Group (PUSG) to make independent survey of uptake. For 2012, PUSG postal survey obtained scores of 9 out of 10 for agronomic importance of this insecticide in all sectors, with high awareness of campaign (arable: 97%, orchards: 99%). For orchards, reported uptake of LDN increased from 6% in 2011 to 88% in 2012. A face-to-face survey by PUSG is ongoing; with the campaign continuing in 2013. The experiences are proving valuable for pesticide use in general and for other EU countries. For example, a similar program is starting in Italy. LDN give potential for win-win of agricultural production & environmental protection.

**491 Risk mitigation measures to protect non-target arthropods off-crop** D. Ruf, E. Kohlschmid, O. Daniel, Agroscope Changins-

Wädenswil. Non-target arthropods in off-crop areas are exposed to plant protection products mainly due to spray drift. Off-crop areas can vary considerably in their purpose and in their habitat quality for non-target arthropods (ranging from roads to nature conservation areas). Thus, they can differ in required as well as in reasonable levels of protection. Defining protection goals for off-crop areas has to take this into account and has to balance the contrasting interests of biodiversity preservation and agriculture. It is beyond dispute that the protection goal for nature conservation areas is to preserve biodiversity and thus only limited, short lived effects on NTA should be tolerated at the edge. In order to reach this goal adequate risk mitigation measures, such as buffer zones and/or the obligatory use of drift reducing nozzles (75-90% reduction) are suggested. Regarding other off-crop areas, the situation can be more complex. In Switzerland, farmers are financially rewarded for the establishment of ecological compensation areas (e.g. rotational fallow- and wildflower strips, conservation headlands, hedgerows, extensively farmed meadows), aiming to support the preservation of biodiversity. Buffer zones to protect such areas can lead to a substantial reduction of arable land for farmers and consequently to a loss of interest in establishing important ecological compensation areas. To prevent such an unfavourable situation, buffer zones must be substituted with other risk mitigation measures. Here, we show a concept for the definition of protection goals for different off-crop areas and corresponding risk mitigation measures.

**492 The TOPPS-prowadis project: classifying and mitigating runoff risks from agricultural land to surface water.** Dyson, Syngenta International AG / Sustainable Agriculture and Stewardship. Runoff is a fast route to surface water, reducing the residence time of precipitation and irrigation in fields. This means that in drier landscapes where efficient water harvesting for crop production is important, runoff can result in yield losses for farmers. And in all landscapes during high intensity rainfall, when runoff travels more rapidly over fields, soil erosion levels can become unsustainable, degrading land and its value. Runoff and erosion also transfer fertilizers, micro-organisms, pesticides and sediments to surface water, making it both a land use and water management issue for society that must be tackled in an integrated way. The European Crop Protection Association (ECPA) takes pesticide stewardship seriously, particularly to show how to implement the Sustainable Use Directive for pesticides. Therefore, a 3-year project (TOPPS-prowadis) was started in 2011, engaging with external partners (from BE, DE, DK, ES, FR, IT, PL) to develop and disseminate Best Management Practice (BMP) for pesticide runoff mitigation (Note: spray drift mitigation is part of the project, but not this paper). Two partners - Arvalis and Irstea (formerly Cemagref) from France - have extensive practical experience in mitigating runoff. ECPA and its partners have worked on delivering similar results to those required to pass risk assessments for pesticide water protection, by taking a practical risk management approach. That is, by dealing with runoff holistically to meet stakeholder needs, particularly farmers having to implement the approach and meet crop productivity demands. The objective of this paper is to describe this approach to BMP and what it can achieve. The TOPPS-prowadis project provides an effective entry point for managing all the risks associated with runoff, not just pesticides. Using generally agreed advisory tools will help farmers manage water protection while meeting crop productivity challenges. Agri-environment support will help farmers to implement advice.

**493 MagPIE summary: Mitigating the risk of Plant Protection Products In the Environment** A. Alix, Dow Agrosciences / Risk Management.

**494 It's a long Way to a Harmonised Risk Mitigation System in Europe - How to transfer the existing national system and also consider new requirements** B. Smith, BVL; S. Matezki; C. Kula, Fed Office for Consumer Protection Food Safety; J. Wogram, German Federal Environmental Agency / IV 2.4 - Ecotoxicological Assessm.; M. Streloke, BVL / Plant Protection Products. Considerable progress has been made in harmonising regulatory risk assessment schemes for the

authorisation of plant protection products within the EU over the last 10-15 years. Setting of risk mitigation measures - according to Article 31 (4a) of Regulation (EC) 1107/2009 - when authorising products has become an effective tool to manage risks posed to the environment by Plant-Protection-Products. A process of harmonisation has started with the FOCUS-Landscape Group (SANCO/10422/2005, version 2, vol.1+2), later on single EU-member states like the Netherlands took the lead for specific areas like spray drift. ("Workshop Harmonisation of drift ...", Wageningen 2010). A SETAC workshop on risk mitigation measures will be organised in 2012 together with the EU-Commission. Here the current state in Germany is summarised. Harmonised phrases are laid down in regulation (EU) 547/2011 and are ready for direct use when it comes to zonal authorisations. Unfortunately this list contains only a limited set of measures and the wording needs to be improved to ease enforcement operations. Cross-references to other legislation like the sustainable use directive 2009/128/EC are to be considered. A considerable number of national safety precautions concerning the protection of the environment have been developed in Germany over the last 20 years in close cooperation with regulatory bodies responsible for enforcement of these legally binding restrictions. However, this system of risk mitigation measures has to be adjusted to fulfil the requirements of regulation (EU) 547/2011. By developing a new system Germany aims to give more advice on critical (legal) issues to the farmers. This poster presentation is focused on measures to protect non-target terrestrial life to demonstrate the difficulties of the transposition in SPE-phrases. This covers key issues like the need to define spatial boundaries that need protection as recently discussed in the EFSA panel groups working on the revision of the terrestrial guidance document (EFSA Journal 2009; 7(11):1375). The new protection goal biodiversity (Art. 4 (1) (e) (iii) of regulation (EC) 1107/2009) needs also to be considered. For Germany, specific and implemented management tools are shortly introduced, inter alia a GIS-based system which considers semi-natural structures aggregated on an administrative unit (communities) to consider spatial aspects of the environmental risk assessment on a landscape level.

**495 Consumption based footprint of a city** S. Worbe, VEOLIA Environnement Recherche et Innovation / Health and Environment; A. Gallice, A. Flesch, F. Tarrisse-Vicard, S. Mehier, VEOLIA Environnement Recherche et Innovation. Since a few years, there is a growing interest for consumption based indicators reflecting the environmental impacts generated by citizen final demand. Considering the complexity and the variety of environmental and intermediate flows in an urban territory, constructing life cycle inventory with classical bottom up approaches for data collection is not a pragmatic option for LCA practitioners. This study focuses on a consistent combination of local emission and activity data with Environmentally Extended Input Output Analysis (EEIOA), into a hybrid EEIO-LCA to assess the environmental impacts generated by the final demand of a city. A hybrid EEIO-LCA has been carried out to capture the footprint generated by a French city. To integrate the city specificities, regional input-output table is estimated from French input output table, using location quotients derived from local employment data. The obtained Leontief matrix is coupled with national environmental extensions and foreign trade data. This approach provides a comprehensive supplement to local sparse environmental data, mainly available for energy use and road transport. The priority was given to local available data and special care was taken to avoid double counting. This inventory is then aggregated into a combined footprint approach (carbon, water, biodiversity and resources) to reflect the environmental impacts generated by citizen's consumption. As expected for a high density population territory, where consumed goods and services are broadly imported, indirect impacts represent a major contribution to the footprint of the city. The results suggest environmental footprint is highly sensitive to consumer choices and expenses allocations. The approach provides a promising solution to couple top down information with local available data, in order to get a full picture of the environmental pressures generated by a large city. Regionalizing economic tables enable to capture the specificities of local domestic businesses. A natural continuation would be to regionalize final demand with local expenses allocation features. The approach

could be used as a screening assessment tool for decision makers, to target potential hotspots of improvement, in a sustainable perspective. The study also illustrates some lack of data availability to comprehensively account for the city impacts and could guide data collection, both from a local and a national level.

**496 GHG emissions and MDL feasibility of representative wastewater treatment technologies in Latin-America and the Caribbean** C. Carius, UNAM; L.P. Guereca, UNAM / Environmental Engineering; J.M. Morgan, UNAM; A. Noyola, UNAM / Environmental Engineering. Abstract. The objective of this paper is to determine the feasibility to apply Clean Development Mechanism (CDM) to Wastewater Treatment Technologies (WWTT), in order to reduce emissions of Greenhouse Gas (GHG), which would contribute to sustainable water management and the reduction of GHG. There is an area of opportunity in the field of WWTT for CDM projects, proposing improvements in technologies such as biogas capture and utilization for electricity or heat generation, besides environmental benefits by decreasing GHG, avoiding the energy consumption of the network reflected in financial savings. The present document analyses the three more used WWTT of Latin America and the Caribbean (LAC), from a baseline, that is the constructed an implemented Wastewater Treatment Plant (WWTP) to an Project scenario that is the progressive reduction of GHG in our project. This project was made using the guidelines of Intergovernmental Panel on Climate Change/United Nation Framework Convention on Climate Change for apply the CDM in each one. Results shows that the stabilization ponds are the technology that produces more methane, due to the anaerobic digestion of organic matter, this anaerobic system do not use oxygen so it's electricity consumption is low, nevertheless they produce more CO<sub>2</sub> than the aerobic digester or UASB. Also, the UASB and the aerobic digester can be used to burn the gas and produce electricity, becoming more sustainable. Further, the economic factor was also analyzed, after quantifying GHG emissions of the three scenarios in LAC, we make an economic feasibility analysis that allowed to establish a set of parameters that relate the potential of the treatment with the capacity of electricity generation, capital costs and annual operation. After gather all the necessary information and making financial calculations the results showed that scenario with coupled activated sludge gravity thickening, anaerobic digestion and centrifugation is the more feasible in the economic matter because because it has a high internal rate of return without any financial or federal support.

**497 Methodological issues of LCA application to building sector: challenges, risks and opportunities** B. Rivea, Technical University of Madrid / Construction and Technology in Architecture; J. Neila, Technical University of Madrid / Construction and Technology in Architecture. Sustainability criteria application must be understood as the essential procedure for the necessary restructuring of the construction sector, which mobilizes 10% of the world economy, accounting for more than one third of the consumption of the world's resources, around 30 - 40% of energy consumption and emissions of greenhouse gases, 30-40% of waste generation and 12% of all the fresh water use in the world. In a previous work, more than 80 case studies have been reviewed in order to describe and classify the results of the LCA studies regarding their goal and scope and their practical methodology implications, by discussing the variability associated to main hypotheses such as the functional equivalent selected and the choice of impact categories. This review allowed to establish conclusions about the degree of consistency with the future regulatory environment and to identify two priority needs for action: the need for harmonization, given the strong methodological inconsistencies detected that prevent the comparison of results obtained in assessment works; and the need for simplification, given the inherent complexity of the assessment. The thread of the present research sets out from the need to establish a description of the basic features and limitations of the methodology of LCA applied to building sector. As a next step, the research focuses on the analysis of the work of CEN/TC 350 and Joint Research Centre - Institute for Environment and Sustainability (JRC-

IES). A critical review of the works that have been developed in recent years is conducted, proceeding to deepen the state of the art of LCA applied to the building sector. It can be stated that the application of LCA is fundamental to sustainability and improvement in building and construction. Methodological issues must integrate general principles of LCA methodology with the protocol established in the European standard, also considering the regulatory standards to construction practices in the different contexts in order to make its implementation possible. Adapting "real life" to the new normative environment is a challenge we are facing nowadays. By systematically directing environmental issues towards standardization, the environmental impacts of products and services could therefore be reduced. There is no need for development of new indicators or methodologies: there is a strong need for coordination and join efforts for better synergies.

**498 Open-Loop Recycling: Market-Based Modeling and its Policy Implications** T. Ekvall, IVL Swedish Environmental Research Institute; P. Soderholm, Luleå University of Technology. For many important materials (paper, steel, aluminium, etc.) recycled material competes with virgin material on an international or even global market. The supply to this common market is provided through the extraction of natural resources and through the separation and recycling of waste materials. In the long run, the supply of virgin materials is much more price elastic than the supply of recycled materials. For this reason, it is mainly the supply of virgin materials that will be affected by an increased use of material in a life cycle or country, with little regard to whether this increase will be in the use of virgin or recycled material. For the same reason, the material collected for recycling in a country, or at the end of a product life cycle, will mainly displace virgin material. The implications for LCA modelling is that most credits for recycling should go to the life cycle that supplies recycled material, and that the use of recycled material should be considered nearly equivalent to using virgin material. The implications for policy is that global recycling is more affected by national instruments that increase collection for recycling, such as the extended producer responsibility, than by instruments that increase the national use of recycled materials, such as a national system for recycling certificates.

**499 Electrical and Electronic Equipment Environmental Product Declarations: Towards a Harmonized System?** A. Lanfranco, M. Jacquot, Bureau Veritas CODDE; J. Orgelet, Bureau Veritas CODDE / EcoDesign. Due to new regulations such as ETS directive (2009/29/EC) in Europe, Grenelle laws including environmental labelling or RT2012 in France, the increasing demand on performance proofs from Public bodies, Construction leaders to end customers motivates manufacturers to communicate on the global environmental performance of their products. From a European point of view, FDES and IBU formats are examples of type III declarations for building products. Swedish EPDs are environmental declarations for all kinds of products supported by the Swedish Environmental Management Council. Finally, PEP Ecopassport® programme is managing the scheme for EEE environmental declarations, mostly developed in France. All these programs will drag towards a common approach in compliance with the EN 15804:2012 standard. It sets PCR for EPDs related to building products (building materials, EEE and climatic engineering products). Its implementation aims at harmonizing the different methodologies developed in Europe for communicating on environmental performances of building products, taking into account new notions such as Reference Service Life, Declared Units and the principle of modularity. Nevertheless, it has concretely appeared that this PCR does not fit enough the EEE perspective. There are indeed cross-sectorial problems impeding a good implementation of this standard for EEE. That's why the creation of a specific complementary "PCR" for EEE appears to be the solution to these problems. It is indeed aiming at giving a framework as regards as an easier implementation of EN 15804 standard. It shall be the link between the simple still in practice PEP Ecopassport® and the more complex EN15804 PCRs. It requires a revision of the data collection system, to simplify it as much as possible, in particular concerning transport steps and process losses. As a better understanding

of the product chain is necessary for the implementation of this specific PCR, a collaborative work between all the participants of the product chain is demanded.

#### **500 The development of Product Environmental Footprint (PEF)**

**Category Rules (PEFCR)** *E.M. Schau*, European Commission DG Joint Research Centre Sustainability Assessment / Sustainability Assessment; K. Allacker, C. De Camillis, European Commission, Joint Research Centre / Sustainability Assessment; R. Pant, European Commission. The European Commission's "Roadmap to a Resource Efficient Europe" proposes ways to increase resource productivity and to decouple economic growth from both resource use and environmental impacts, taking a life-cycle perspective. One of its objectives is to: "Establish a common methodological approach to enable Member States and the private sector to assess, display and benchmark the environmental performance of products, services and companies based on a comprehensive assessment of environmental impacts over the life-cycle ('environmental footprint')". The European Council invited the Commission to develop supporting methodologies. The Environmental Footprint (EF), launched by the European Commission's Joint Research Centre in close cooperation with Directorate-General for the Environment, gives specific guidance for comprehensive, robust and consistent environmental assessment of products and organisations. This is an important step forward to ensure robust decision support for business and policy. However, for to be more relevant to the situation and problems of specific product categories more specific guidance on how to conduct the EF study is required. The guides on Product EF (PEF) and Organisation EF (OEF) provide more specific requirements that need to be defined in so called Product Environmental Footprint Category Rules (PEFCRs). These PEFCRs are seen as crucial for EF studies aiming at business-to-business (B2B) and business-to-consumer (B2C) communication intended to be used for comparisons and comparative assertions. The role of PEFCRs are to increase the reproducibility, consistency, comparability and relevance of EF studies, but also to increase the efficiency (reduce time, efforts and costs) of EF studies by directing the focus on the most important processes and impact categories. First steps are taken by the European Commission to develop PEFCRs through several pilot studies. These will build on: The recent development of guidelines on the development of Product Category Rules (PCRs) by the United States Environmental Protection Agency Existing PCRs and specific sector guidance such as the European Food Sustainable Consumption and Production (SCP) Round Table, PAS2050, GEDNets PCRs for Environmental Product Declaration (EPD) and the Repository of good practice in France (related to BP X 30-323) Knowledge and experience from a broad spectre of stakeholders For the development of PEFCR there are at least three major challenges that will be discussed in the presentation: definition of the Product Category/Sector in a way that allows for meaningful comparison of products fulfilling an equivalent function, definition and modelling of an average product(s) and identifying and focussing on what matters most. This contribution gives an overview of what PEFCRs are and highlights some issues relating to their development. Keywords: Environmental Footprint (EF), Product Category Rules (PCR), Product Environmental Footprint Category Rules (PEFCRs)

#### **501 Used of fish immune parameters in environmental risk**

**assessment** *A. Bado-Nilles*; S. Jolly, URCA/Ineris; J. Porcher, O. Palluel, INERIS; A. Geffard, URCA; B. Gagnaire, IRSN; C. Blanchard, C. Le Rohic, ONEMA; S. Betouille, URCA; W. Sanchez, INERIS. Currently, for environmental risk assessment, multi-biomarker approach based on complementary parameters in term of substances, measured effect and biological integration levels were developed with some biochemical biomarkers, such as ethoxresorufine-o-deethylase (EROD), glutathione-S-transferase (GST), glutathione peroxidase (GPx), lipid peroxidation (TBARS) and acetylcholinesterase (AChE). This approach allows integration of each environmental factor by restitution of qualitative and quantitative exposure variation. Nevertheless, use of these biochemical parameters was not enough to

obtain clear information about fish and ecosystem health. Now, components of the fish immune system are considered as an attractive non-specific marker for environmental biomonitoring which integrate measure of exposure over time and may reflect the combined results of simultaneous contamination to several chemicals. Moreover, immune parameters were attractive due to their ecological importance by direct implications in individual fitness and population growth. In this way, the present work proposed the use of fish immunomarkers (i.e. apoptosis, necrosis and respiratory burst activity) together with more current biochemical biomarkers (i.e. EROD, GST, GPx, TBARS and AChE) to better evaluate fish health. The determination of a relationship between fish immune capacities and Fish Based Index (FBI), a useful indicator of ecosystem health by monitoring fish population, were also search. This work demonstrated that selected immune and biochemical biomarkers were able to discriminate sites in function of contaminant effect since sampling conditions were optimized to reduce biomarker variability and increase parameters robustness. In the multiple discriminant analysis, the sites were highly correlated with apoptosis, respiratory burst, GST and EROD activity. Moreover, the use together of biochemical and immune markers increase the percentage of fish correctly classed in each site (from 45 % with only biochemical data to 68 % with immune/biochemical values) and enhanced site separation (increase of dotted line from 5.51 to 11.71 after agglomerative hierarchical clustering). The relationship between immune capacities and FBI must further be demonstrated. So, this study argues for attractive utilization of immunomarkers for determination of environmental risk assessment in addition with a set of biochemical biomarkers currently used.

#### **502 Using exclusively endobenthic organisms to derive sediment quality guidelines (SQGs)**

*M. Brinke*, Federal Institute of Hydrology / Biochemistry and Ecotoxicology; E. Claus, Federal Institute of Hydrology (BfG); G. Reifferscheid; S. Höss, Ecossa; W. Traunsperger, Bielefeld University / Department Animal Ecology; P. Heininger, Federal Institute of Hydrology (BfG). Sediment quality guidelines (SQGs) have shown to be predictors of the toxic potential of sediments. Several approaches are available to derive those guidelines, such as the spiked-sediment bioassay approach, the equilibrium partitioning approach or co-occurrence approaches (i.e. simultaneous presence of pollutants and biological effects). One example of the latter is the screening level concentration approach (SLCA) that uses information of whole benthic communities found in polluted sediment samples. In this presentation, SQGs are shown that were calculated with the SLCA based on chemical and nematode community analysis of freshwater sediment samples from several river basins across Germany (e.g., Elbe, Rhine, Danube). Additionally, the logistic regression modelling approach (LRMA) was applied for SQG derivation, however, using a new community index, the NemaSPEAR (Nematode SPecies At Risk), as matching toxicity data for calculation of the proportion of toxic samples. Nematodes were chosen because they are frequently the most abundant and diverse taxonomic group in sediments and they fulfil key positions in benthic food webs due to their various feeding types. In addition, as endobenthic organisms, they are subjected via various pathways to any noxious substances in sediments throughout their whole life-cycle. Moreover, nematodes are often more dominant than macroinvertebrates in fine sediments, which are of particular interest in terms of water quality due to their high potential to accumulate pollutants. The results show that SQGs can be derived on the basis of nematode communities and that threshold effect concentrations (TECs, i.e. lower limits) are for many substances similar to existing ones based on macrobenthic organisms. However, calculated probable effect concentrations (PECs, i.e. upper limits) were for several substances higher compared to existing ones, indicating a high tolerance range within nematode communities. In addition, the results from the LRMA provide further evidence that the NemaSPEAR is a feasible indicator of sediment toxicity. On the one hand, this study demonstrates that the protection of ecologically important meiobenthic organisms is at least to some extent already covered by existing SQGs, but that vice versa nematode-based SQGs might also protect sediment-dwelling macroinvertebrates. On the other hand, this study additionally underlines the value and applicability



of nematodes for sediment quality assessment in general.

**503 Pollution-induced community tolerance to anti-inflammatory drugs in fluvial biofilm communities** N. Corcoll, Resources and Ecosystems; V. Acuña; D. Barcelo, IQABCSIC; M. Casellas, ICRA; H. Guasch, University of Girona (UdG); B. Huerta Buitrago, Catalan Institute for Water Research ICRA / Department of Water Quality; L. Ponsati, Water Institute for Water Research (ICRA); S. Rodriguez-Mozaz, Institute for Water Research ICRA; s. sabater, ICRA. Effluents from wastewater treatment plants (WWTP) are considered an important and continuous source of pharmaceuticals into aquatic ecosystems. In this study the pollution-induced community tolerance (PCT) approach was used as an indicator of anti-inflammatory drugs (Ibuprofen, Diclofenac and their binary mixture) effects on fluvial biofilms. To assess biofilm tolerance to these anti-inflammatory drugs, an experiment was performed in the Segre River (N Catalonia, Spain), which has a gradient of anti-inflammatory drugs pollution after the entrance of the WWTP. The sensitivity of different microorganisms composing biofilm (algae and bacteria) to anti-inflammatory drugs was also addressed. Biofilms were developed for six weeks on artificial substrata in a non-polluted site (before the WWTP) and in several sites downstream, presenting decreasing levels of anti-inflammatory drugs pollution (after a WWTP). PCT to Ibuprofen, Diclofenac and their binary mixture was determined using photosynthetic efficiency and  $\beta$ -glucosidase activity bioassays. From each sampling site water was physicochemical characterized. Biofilm communities from polluted and non-polluted sites were compared in terms of algal biomass (chl-a), abundances of main algal groups (Phyto-PAM), live and dead bacteria (Live/Dead® Bacteria Viability kit) and the microbial community level physiological profiles (CLPP) based on Biolog® EcoPlate assay. Short-term bioassay toxicities were low for Ibuprofen and Diclofenac, with EC<sub>50</sub> in the range from 100 to 1000 mg/L, Diclofenac being the most toxic compound. Autotrophic and heterotrophic organisms composing biofilms from the highest polluted sites induced tolerance to these anti-inflammatory compounds in the highest polluted sites. The results show that considerable effects can occur on aquatic biota even though anti-inflammatory drugs occur at very low concentrations in fluvial ecosystems. *Keywords: anti-inflammatory drugs, biofilms, rivers, PICT concept* *Acknowledgements* This study was financed by Scarce Consolidar- Ingenio 2010 (Csd2009-00065). "Assessing and Predicting Effects On Water Quantity and Quality In Iberian Rivers Caused By Global Change (2009-2014)" and Spanish Ministry of Economy and Competitiveness.

**504 Sensitivity and recovery of zooplankton and macroinvertebrate communities exposed to a fungicide or a hydrocarbon mixture in outdoor pond mesocosms** Y. Bayona, INRAAgrocampus Ouest / UMR 985; A. Roucaute, INRA; M. Roucaute, INRA / UMR ESE; K. Cailleaud, Total Petrochemicals France / PERL; A. Basseres, TotalFinaElf; L.L. Lagadic, INRA / UMR INRAAgrocampus Ouest Ecology and Ecosystem Health; T. Caquet, INRA / UMR ESE 0985. Mesocosms give the opportunity to assess the effects of toxic substances on various biological compartments providing an overview of direct and indirect impacts of exposures. Using these systems, it is also possible to compare the responses of organisms that differ in terms of size, of life-cycle and of recovery potential (e.g., presence of resistance forms such as diapausing stages or resting eggs). For example, it is anticipated that zooplankton would show a faster response to toxicants and a faster recovery as compared to macroinvertebrates. In order to test this hypothesis, a several month-long experiment was implemented to assess the effects of two chemicals on zooplankton and macroinvertebrates in pond mesocosms. After a 3 month-long maturation period, outdoor pond mesocosms (7.5 m<sup>3</sup>, water depth ca. 0.9 m) were exposed with either thiram, a dithiocarbamate fungicide (2 nominal concentrations in duplicates: 35 and 170 µg/L) or a hydrocarbon mixture (4 nominal concentrations in duplicates using Water Accommodated Fractions for application: 0.01, 0.4, 2 and 20 mg/L). Three other systems were kept as controls. The mesocosms were treated weekly for 4 weeks. The treatment period was followed by a 10 month-long recovery phase.

Zooplankton was sampled weekly using water column samplers whereas macroinvertebrates were collected every 3 weeks using artificial substrates. Planktonic and benthic invertebrates were then identified and enumerated in order to characterize the structure of their respective communities in control and treated mesocosms using taxa abundances and various indices (e.g., diversity and dominance indices). A significant impact of exposure on the structure of the macroinvertebrate community was shown for the hydrocarbon mixture, without recovery (for 20 mg/L), whereas thiram had no effect. Zooplankton community structure was affected by the exposure to both chemicals during the treatment period but complete recovery was observed before the end of the experiment in all treated ponds. Our results show that some zooplankton taxa were impacted by both chemicals and may therefore be considered as broadly sensitive taxa, whereas others seemed to be more specifically sensitive to one chemical. Overall, zooplankton taxa were more sensitive during dosing than macroinvertebrates. Combined use of zooplankton and macroinvertebrate communities therefore provides assessment during exposure and post-exposure period on the effect of chemicals.

**505 The influence of forest patches on pesticide effects in agricultural streams** P. Khrycheva, UFZ / Bioenergy; R. Muenze, UFZ; M.A. Beketov, UFZ - Helmholtz Centre for Environmental Research / Department of System Ecotoxicology; M. Liess, UFZ Center for Environmental Research / Department of System Ecotoxicology. It is well-known that the response of macroinvertebrate communities to pesticide exposure in agricultural streams can vary due to non-chemical factors, such as the riparian land use upstream. Previous studies have shown that the presence of upstream riparian forest patches reduces the impacts of pesticides on the macroinvertebrate community composition up to 5 km downstream. We investigated the factors, which determine this effect, such as the size and the relative location of the riparian forest patches and the concentrations of pesticides in water. Our goal was to determine and define the most relevant parameters of the forest patches for macroinvertebrate communities at the time of pesticide application. In order to do so, we analysed the pesticide data and macroinvertebrate assemblages from three field studies conducted in June 2000, 2010 and 2011 in central Germany. The parameters of the forest patches, such as the surface area, the distance to the sampling site, and the length of the riparian forest were measured in ArcGIS 10 using the ATKIS land use data. The results of this analysis showed that the area of the upstream forest patch and the length of the riparian forest had the strongest and significant impact (p-values < 0.05) on the macroinvertebrate community in contaminated sites. The SPEARpesticides indicator for pesticide effects successfully responded to pesticide contamination, as shown by a significant negative correlation (p-value < 0.0001) between the percentage of the pesticide-sensitive species and the toxic units *D. magna* (TU). The SPEARpesticide value was significantly affected by several forest patch parameters (p-values < 0.05), except for the sites with high concentrations of pesticides, where only TU had a significant (p-value < 0.05) influence (TU < -3.5). Our results confirm that the riparian forests upstream of the sites contaminated with pesticides play a role for macroinvertebrate community composition even in June, at the time of intensive pesticide application. They are also in agreement with other studies, which show that at high concentrations of pesticides, non-chemical factors lose their importance. The efficacy of upstream riparian forest patches on macroinvertebrate communities should be taken into account, when assessing pesticide risks and planning risk reduction measures. The most relevant factors to consider are outlined in our study.

**506 An integrated study of metals behaviour in stream ecosystems.** N. Roig, Universitat Rovira i Virgili; J. Sierra, Universitat Rovira i Virgili / Laboratori d'edafologia; J. Ortiz, G. Merseburger, Working group on stream ecosystems. Association for the conservation of natural ecosystems; M. Nadal, University Rovira i Virgili; M. Schuhmacher, Rovira i Virgili University; J. Domingo, Universitat Rovira i Virgili. The aims of the present study are to: (1) assess the distribution, bioavailability and ecotoxicity of some potentially toxic elements (PTEs) in the Francolí stream basin (NE Catalonia, Spain), and (2)

evaluate the relationship between stream metal concentrations and the macroinvertebrate community. For this purpose, the concentration of some heavy metals was analyzed in stream water, sediments, and Baetidae mayfly nymphs collected in 4 sites within the basin, during spring 2011. Three sampling reaches were selected according to the presence of different human pressure agents (agriculture, urban, and industrial discharges), while the fourth reach, was selected as a reference site. In each sampling reach, one sample of water, sediment, and Baetidae nymphs were collected for metal analysis. Benthic macroinvertebrates were sampled with a Surber sampler to estimate density, richness, Shannon diversity, and the EPT index at family level. In addition, diffusive gradient in thin-films (DGTs), were disposed for 7 days in order to assess the potential bioavailable fraction. In sediments, metal bioavailability was calculated by a sequential extraction according to the Community Bureau of Reference (BCR) method. Moreover, bioaccumulation factors (BAF) for each metal have been calculated for water and sediment. The ecotoxicity of sediment samples was evaluated in *Vibrio fischeri* by Microtox Acute Test. The results revealed that metals present different behaviour depending on its chemical form. Anions tend to predominate in solution and are not sorbed by DGT devices, contrary of cations that can be sampled by DGTs. Zn and Cu have been the most bioavailable elements in water and sediments, and also presented the highest concentrations in both environmental matrices. Respect the metal concentration in Baetidae mayfly nymphs, Zn, followed by Cu have been the mostly accumulated elements, in agreement with DGTs and sediment BCR prediction. Moreover, Zn has been the element with the highest BAF in these macroinvertebrates. Urban and industrial areas presented the highest concentrations of metals and showed the highest toxicity and the lowest score for all biological indices measured. This study demonstrates that the integration of metal analysis (total metals and potential bioavailable fraction), ecotoxicity assays and biological indices could be crucial to understand the hazard of metals in aquatic ecosystems.

**507 Introduction** J. Van Wensem, TCB.

**508 The Environmental Chemistry of Single Malt: From *Hordeum vulgare* to the turbinates** K.R. Solomon, University of Guelph / School of Environmental Sciences. It is now recognized that the art of the production of the great Scottish libation, single malt whisky (SM), is dependent on a large number of environmental factors and interactions between these, the chemical properties of the distillate, and the receptor organism. Factors influence the chemistry of single malt from early in the production process to the final consumption and are responsible for the unique character of the malt. The choice of energy source for the drying of the malt is the primary driver of the distinctive character of smoke in some SMs and results from of condensation and absorption of phenolics and smoke flavors in the drying malt. These flavours carried through the distillation process and provide a range of flavors that depend on the sources of the SM. Lightly peated malts contain phenolics in the range of 1-5 mg/L while, at the other end of the distribution are the Isla malts such as Ardbeg (30 mg/L) and Smokehead (50 ?g/L). The more subtle flavors of the SM come from several sources, some from the malt, some from the nature of the distillation process, but most importantly from the wood used to make the barrels in which the spirit is aged. These barrels have invariably been used by others (a life-cycle benefit) and therefore impart different nuances of flavour that depend on the source of the oak, the previous contents, the contact time, the environmental temperature, and the chemical reactions that occur in storage. Last, but not least, is the tasting. How this is conducted has important effects the receptor organisms that may change the response to the exposure. This talk will offer an overview of the topic with a flavor of environmental chemistry and toxicology.

**509 Overview of Ecosystem Services** L. Maltby, The University of Sheffield / Dpt of Animal Plant Sciences; J. Van Wensem, TCB. Ecosystems are essential to human health and well-being and underpin our cultural, social and economic development. They provide us with clean air and water, fresh food and medicines, energy and the raw

materials required to construct our homes and to produce the myriad of consumer products that we purchase every day. They control our climate, protect us from floods, control pests and diseases and clean up our waste. We are totally dependent on these ecosystem goods and services, yet about a third of the ecosystem services provided by UK habitats are in decline, and worldwide, 60% of ecosystem services are degraded or being used unsustainably. Habitat destruction and habitat degradation are primary drivers of this decline. To counteract degradation and unsustainable use of ecosystems, the EU member states are working, with assistance of the European Commission, on mapping and assessing the state of ecosystems and the ecosystem services they provide. And early example of such an exercise is the UK National Ecosystem Assessment. This action should lead to assessment of the (economic) value of ecosystem services, and thereby facilitate integration of these values in accounting and reporting systems. One of the ultimate goals of these activities is to achieve better informed environmental management, that can help optimise the delivery of a suite of services at a landscape scale. With 'local' examples we will illustrate the major characteristics of an ecosystem services approach and provide the wider context for the following presentation, which focuses more directly on whisky production.

**510 Whisky, Ecosystem Services and the Ecosystem Approach** M. Everard, UK Environment Agency; J. Van Wensem, TCB. Whisky, its supply and value chains and cultural context, confers multiple values to Scottish and UK society. Ecosystem services are defined as the benefits that ecosystems provide to humanity, and many are evident in the production and enjoyment of Whisky recognising a broad range of value systems from the economic to the spiritual and cultural. The ecosystem services framework is a central element, albeit a vital one, of the broader 'ecosystem approach', which also addresses broader dimensions of economics, ecological carrying capacity, spatial and temporal impacts, equity and participatory decision-making. Considering the 'life cycle' of Whisky in these broader contexts sheds light on its importance and further opportunities for sustainable development.

**511 Economics of Whisky in Scotland** R. Marsh, 4-Consulting. The environmental, social and economic impact of Scotch Whisky will outline the economic and social benefits arising from Scotch Whisky production alongside the environmental impact including energy, consumption and recycling of raw materials.

**512 Colloquy reflecting on Raw Spirits and 99 Drams** R.J. McCormick, Bureau of Land Management; L. Kapustka, LK Consultancy. There is a rich history associated with whisky that wends through politics, technology, and ecology. This history is captured in different ways by two intriguing authors, Iain Banks and Kate Hopkins. Both authors used the literary tool of a travelogue to present their encounters with 'the water of life.' We will draw from these two entertaining works to explore evolving cultural norms and political follies surrounding the production and use of whiskies. We will highlight how flows of ecosystem services play out locally and connect globally in the challenge to sustain the whisky industry.

**513 Panel Q&A with Audience** L. Kapustka, LK Consultancy.

**514 QSARs and nanoparticles: a challenging relationship** E. Papa, QSAR Res. Unit Environ. Chem/Dep. Structural Functional Biology; A. Rizza, University of Insubria / Department of Theoretical and Applied Sciences; L. van der Wal, Joint Research Centre / Department of Theoretical and Applied Sciences; J. Ponti, European Commission, Joint Research Centre, Institute for Health and Consumer Protection; P. Gramatica, University of Insubria / QSAR Res. Unit Environ. Chem. Ecotox./Dep. Structural & Functional Biology. The current use of nanoparticles (NPs) for industrial and pharmacological applications has become a reason of increasing concern due to their possible toxicological activity in humans and in the environment. The investigation and prediction of toxicological and physico-chemical properties of NPs, as well as the interaction of organics with NPs

surface, are challenging topics. The main problems associated to the application of QSAR type studies in this field are related to the lack of experimental information available to describe the structural properties of these new materials, and a lack of in vivo or in vitro measured activities for large sets of NPs. However, different approaches based on quantitative structure (property)-activity relationships (QS(P)AR) can be applied to investigate the potential activity of NPs. In this presentation we want to show some examples of models to study: a) the adsorption of small organics on the surface of multiwalled carbon nanotubes (MWCNTs), b) the effects of changes in the organic coating of coated NPs on cellular uptake phenomena, c) the in vitro toxicity of heterogeneous NPs. Three data sets were used to develop QSAR models for different endpoints, related to the above. The first two datasets were structurally described by conventional molecular descriptors, which were calculated using commercial and freely available online software (DRAGON 5.5, PADEL 2.13). The final dataset was structurally characterized by experimentally measured properties such as size, surface charge and NPs behaviour in selected biological media. The QSAR models were developed according to the OECD principles, thus, particular effort was made to produce, externally validated, predictive models, with verified structural applicability domains. Multiple linear regression models were developed by Ordinary Least Squares (OLS) method, and the best combinations of descriptors were selected through the application of the All Subset and, if necessary, the Genetic Algorithm Variable Subset Selection procedures. Final aim of this presentation is to highlight the strengths and weaknesses of the performed approaches, thereby informing potential users on which issues represent limitations and possibilities for the future improvement and application of QSAR-based approaches in NPs-related scientific and regulatory fields.

**515 An evaluation of studies on nano TiO<sub>2</sub> fate and ecotoxicity for risk assessment – experiences from the OECD Sponsorship Programme** K. Hund-Rinke, Fraunhofer IME / Oekotoxikologie; D. Hennecke, Fraunhofer Institute for Molecular Biology and Applied Ecology. The quality of the available test results and reports determines the accuracy of the risk assessment of nanomaterials. Experiments are often performed using standardised ecotoxicological test guidelines that include various optional experimental settings, e.g. test media, which may affect the results. In addition, individual modifications of the test guidelines or individual test procedures are applied in order to achieve higher effects or include further endpoints. Based on the TiO<sub>2</sub> materials selected for the OECD Sponsorship Programme and the requirements according to REACH, the aim of the study was to (i) describe a data and report assessment procedure, (ii) evaluate the reports and published papers, (iii) recommend criteria for the assessment of additional endpoints regarding their suitability for risk assessment, and (iv) draw general conclusions on the scope of testing, the sensitivity of test organisms and endpoints, and on the robustness of the test procedures. Our evaluation showed that the Klimisch-criteria and their adaptation by ECETOC are suitable for the assessment of TiO<sub>2</sub> test reports. Soil populations and soil functions must be protected by the risk assessment. Therefore, suitable selection criteria for additional endpoint inclusion depend on whether respective information is provided. Endpoints that do not fulfil the selection criteria can only be used for supporting information. All additional endpoints currently applied for TiO<sub>2</sub> are also applicable for the assessment of conventional chemicals as there are none specific to TiO<sub>2</sub> nanomaterials. The sensitivity of standardised endpoints is equal for TiO<sub>2</sub> and conventional chemical risk assessments. The available information is not sufficient to comment on the robustness of the test guidelines except to say that round robin tests between laboratories are recommended. Agglomeration behaviour and zeta-potential of the nanomaterials are dependent on the test conditions indicating a need to clearly specify the test medium in order to reduce the variability of test results. A comprehensive risk assessment should consider both environments, so long as the overall information remains limited. A selection of tests that are based upon the annual production volume of TiO<sub>2</sub> is considered less suitable, especially as accumulation in the terrestrial environment cannot be excluded.

**516 Assessing aquatic hazards of nanomaterials – first experiences with REACH registrations** N.B. Hartmann, European Commission Joint Research Centre / Nanobiosciences Unit Institute for Health and Consumer Protection; J. Fabrega, European Chemicals Agency; F.M. Christensen, K. Aschberger, European Commission - Joint Research Centre / Nanobiosciences Unit - Institute for Health and Consumer Protection. By 1 December 2010, substances manufactured or imported in the EU > 1000 t had to be registered under REACH. The Joint Research Centre (JRC), in close cooperation with the European Chemicals Agency (ECHA), carried out an assessment of information on nanomaterials in the submitted registration dossiers (Nanosupport Project). From approx. 26000 submitted registration dossiers covering ca. 4700 substances, 25 dossiers covering 19 substances could be identified to most likely address nanomaterials or nanoforms. On this basis, the adequacy of REACH requirements for nanomaterials was examined and endpoint-specific recommendations were made for adaptation of REACH. Here we evaluate how environmental safety of the registered nanomaterials was ensured by examining environmental fate and hazard endpoint information with particular emphasis on aquatic hazards. A general ambiguity was identified in the 'scope of the registered substance' and the assessment highlighted a need for addressing nanoforms in a specific and transparent manner throughout the dossiers and within endpoint sections. Data on short-term aquatic toxicity was provided for the majority of registrations and otherwise addressed by read-across, weight of evidence approaches, QSAR, or waving. The long-term toxicity endpoint was generally waived but in a few cases testing data or read-across data was provided. In some dossiers, the experimental data included tests on nanomaterials/nanoforms of a substance. In a few dossiers a distinction was made between the nano- and bulk form of the substance or the nanoform was addressed as a 'worst case'. However, in general discussion was found to be lacking regarding potential differences in toxic effects between different forms/sizes of the registered substance. Other identified issues relate to: test waiving based insolubility, testing of supernatants from saturated solution of the test material and use of read-across from other substances (usually a metal oxide or metal salt) – all of which can be considered not taking into account possible particle-related effects. On this basis a number of general and endpoint-specific recommendations were made for adaptation of REACH in order to best address the identified issues. Step 2 of the Nanosupport project is currently ongoing with the purpose of assessing the consequences for industry, consumers, human health and the environment of the implementation of such adaptations.

**517 State of knowledge on nano-pesticides and implications for environmental exposure assessment in the EU** M. Kah, University of Vienna / Department of Environmental Geosciences; S. Beulke, S. Monteiro, K. Tiede, Food and Environment Research Agency; T. Hofmann, University of Vienna. Deliberate application of nano-particles as plant protection products within agricultural practices could result in one of the rare intentionally diffuse inputs of engineered nano-particles into the environment. The anticipated new or enhanced activity of nano-pesticides will inevitably result in both new risks and new benefits to human and environmental health. It is unclear whether the current regulatory framework is adequate for the evaluation of these new products. First, a literature review [1] was carried out with the objectives (i) to explore potential applications of nano-technology within the pesticide formulation sector, (ii) to identify possible impacts on environmental fate, and (iii) to analyse the suitability of current exposure assessment procedures to account for their novel properties within the EU regulatory context. A variety of sources were extensively searched and relevant information was combined from published literature, company websites, patent databases, reports from governmental and non-governmental institutions. Second, experimental work was performed on a series of nano-pesticides. The suitability of standard regulatory protocols to determine fate parameters will be evaluated and discussed in the context of pesticide regulatory assessment in the EU. Discrepancies in fate between free and nano-

formulated active ingredient will also be analysed by combining more realistic experimental set up to extensive characterization of the nano-formulation under a range of conditions likely to occur in the aquatic environment. A more robust risk assessments framework for nano-pesticides is urgently needed. In this context, priority for research are to (i) identify the assumptions currently applied that are not valid in the case of nano-pesticides, (ii) evaluate the points or situations in which differences may impact significantly on the exposure assessment outcomes, and (iii) refine or adapt current assessment protocols as required. [1] Kah M, Tiede K, Beulke S, Hofmann T 2012. Nano-pesticides: state of knowledge, environmental fate and exposure modeling. *Critical Reviews of Environmental Science and Technology*. DOI:10.1080/10643389.2012.671750

**518 An approach to determine appropriate dose metrics for nanomaterials** W.J. Peijnenburg, RIVM / Laboratory for Ecological Risk Assessment. Risk assessors derive safe exposure limits based on information on the toxic potential of substances. For soluble substances, a unique measure of dose is the total mass of the substance or, equivalently, the total number of molecules administered, and exposure limits are generally based on mass concentrations. With nanotechnology facilitating the creation of complex structures of different sizes, a whole new array of often insoluble chemicals made its appearance. The characteristics of these nanomaterials (e.g. size, shape, polymorph form) all may determine their toxic potential. For example, X mg of nanomaterial (NM) with particle size d may be more toxic than X mg of NM with particle size d<sub>2</sub>, despite consisting of the same chemical substance Y. Thus, information on the administered mass of the chemical substance alone may not be a sufficient description of the dose that determines the response of NMs. As a result, risk assessors are faced with the question of what dose description to use when setting exposure limits for NMs. It has been speculated that limits based on particle numbers may be more appropriate, while toxicity of NMs may also be determined by the surface area. An adequate dose metric for NMs should describe all relevant characteristics that are necessary to explain differences between responses in experiments. Hence, the dose metric should be able to discriminate doses with different responses. In its most complete form, the dose of a NM can be described by a (distribution) function PN that specifies the number of particles in the ensemble with specific characteristics. For example PN(d, ?, ..., ...) may give the number of particles N with diameter d, surface potential ? and crystal structure ?. Ideally, a dose metric should be as concise as possible, i.e. should describe the dose with as few dimensions as possible. A reduced dose metric, (for example requiring only information on administered total number of particles, total or volume or total surface area of a NM consisting of chemical substance Y), would be most pragmatic, since only one exposure limit would have to be derived for various different NMs consisting of chemical substance Y. The existence of a reduced dose metric should be established experimentally. In this contribution, we present a method to determine whether a reduced dose metric for NMs exists. As an illustration, the method is applied to analyse results from experiments with various NMs.

**519 Evaluation of Critical Body Residue Data for Acute Narcosis in Aquatic Organisms** L.S. McCarty, LS McCarty Scientific Research Consulting; J.A. Arnot, ARC Arnot Research Consulting / Department of Physical Environmental Science; D. Mackay, Trent University. The Environmental Residue Effects Database (ERED) was evaluated to identify critical body residues (CBRs) of organic chemicals causing acute baseline neutral narcosis in aquatic organisms. More than 15,000 records for over 400 chemicals were evaluated, giving a putative narcosis CBR data set of 161 records for 29 chemicals. Mean molar CBRs were within ranges previously published; however, overall variance was ~3 orders of magnitude. CBRs varied within and between chemicals and species and whole body lipid normalization did not consistently decrease variability. Rainbow trout and *Hyalella azteca* exhibited lower CBRs with neutral organics. All 29 chemicals in the selected subset are believed to be baseline neutral narcotics, but they and/or their metabolites may be biochemically reactive non-narcotics in

some circumstances. Biotransformation of test chemicals can be a significant source of variability, particularly for some polycyclic aromatic hydrocarbons and missing metabolite measurements complicates data interpretation. Other confounding issues such as poor or missing information on lipid content and composition and steady-state partitioning status reflect inadequacies in aquatic toxicity test design, implementation, and reporting. These limitations impede the precise, accurate CBR determination necessary for sound toxicological comparisons. Four organism-based metrics of toxicity are discussed: CBR on a wet weight and lipid weight basis, percent volume fraction in organism lipid phase, and chemical activity. All have specific advantages that can be useful when assessing toxicity of organic substances to aquatic organisms, but none were free of influences found to confound test interpretation. Thus, both exposure-based (e.g. LC50) and whole-body dose surrogate metrics can introduce substantial variability/uncertainty in estimates of relative toxicity. Changes to aquatic toxicity testing designs and methods are required to improve data collection and interpretation and facilitate scientifically-sound risk assessment uses.

**520 Screening for Low Aquatic Bioaccumulation: Weight of Evidence with the BCF Waiving Scheme** M. Nendza, Analytisches Laboratorium; R. Kuehne, Helmholtz Centre for Environmental Research – UFZ; T. Aldenberg, RIVM; G. Schuurmann, Helmholtz Centre for Environmental Research UFZ / Department of Ecological Chemistry. Aquatic bioconcentration factors (BCF) are critical in PBT and risk assessment of chemicals under REACH. High costs and consumption of more than 100 experimental animals per standard BCF study (OECD 305) call for filters based on quantitative structure-activity relationships (QSARs). The BCF waiving scheme allows to identify substances with low aquatic bioaccumulation (BCF < 2000) that cannot classify as PBT. Their biotesting with guideline studies may be waived because of low concern with regard to the B criterion. The BCF waiving scheme is based on physico-chemical properties related to media-specific exposures and bioavailability [1]. Pragmatic cut-off criteria in lipophilicity (log Kow10), solubility and volatility (log Henry constant5% dissociation at pH 7 and log D < 3) reliably discriminate nonB chemicals from possibly bioaccumulating compounds. The decision tree performs with 100% sensitivity (no false negatives) on industrial and new chemicals, pesticides as well as known bioaccumulative (B/vB) compounds and allows to securely deprioritise more than 50% of the nonB chemicals. Prediction confidence of the BCF waiving scheme relies on applicability domains defined by structural features (chemical classes, atom centred fragments (ACF)) and ranges and characteristic combinations of physico-chemical properties. Weight-of-Evidence (WoE) for low aquatic bioaccumulation is supported by similarity to substances correctly predicted, number of physico-chemical property criteria triggered by a query compound, distance of predictions from thresholds and consistency with other available information including estimates from independent QSARs. Bayesian statistics provide quantitative measures of the WoE and inform users about the reliability of the classifications as either nonB (BCF definitively < 2000) or "unknown" (BCF may be >2000). *Acknowledgement* - This work was supported by the EU 6th Framework Integrated Project OSIRIS (contract no. GOCE-ET-2007-037017). [1] Nendza M, Herbst T. 2011. Screening for low aquatic bioaccumulation (2): Physico-chemical constraints. *SAR QSAR Environ. Res.* 22, 351-364.

**521 Statistical handling of reproduction data for concentration-response modelling** M. Delignette-Muller, VetAgro Sup; C. Forfait, Université de Lyon; C. Lopes, Université Lyon UMR CNRS; S. Charles, University Lyon / Laboratory of Biometry and Evolutionary Biology. During the last decades, many scientists advocated the ban of the NOEC and its replacement by the EC. Everybody seems to agree that EC has advantages over NOEC and should be considered as an appropriate approach as far as data are sufficient and properly analysed to fit a concentration-response curve. Nevertheless, other scientists proposed an opposed view, essentially based on the criticism of the way

regression methods are sometimes misused (from a statistical point of view) to calculate an EC<sub>50</sub> value. These criticisms are well founded and should not be neglected by the users of concentration-response modelling. Within this context, we are personally convinced that new methods should be proposed to improve statistical handling of different types of data for calculating EC<sub>50</sub> values. In this present work, we focus on the analysis of reproduction data that are commonly used to estimate EC<sub>50</sub> values from chronic toxicity tests. reproduction data are generally count data, corresponding to a cumulated number of offspring at the end of the test. Ordinary nonlinear regression is often performed to fit a concentration-response curve to reproduction data, such as the log-logistic model, ignoring the nature of the data. A Poisson model, best suited for count data, is sometimes proposed for reproduction data but its drawback is that it ignores the replicate nature of the experiments. Mortality is another problem often encountered in the analysis of reproduction data. When parent mortality occurs, OECD preconises that offspring of that parents be excluded from the calculations and to work with the total number of offspring produced per parent animal still alive at the end of the test. In some cases, we may unfortunately lose valuable data following this recommendation. In fact, parents may have reproduced before dying, and we should not drop valuable information provided by the corresponding data. By doing this, we could even bias the results by dropping such reproduction data which certainly correspond to the response of the most sensitive organisms. In this present work, we propose a new way to use all reproduction data by calculating the period each parent is staying alive during the experiment, and to fit them with an extension of the Poisson model that takes into account the inter-replicate variability often observed in such data.

## 522 Statistical refinements for data analysis of mollusc reproduction tests: an example with *Lymnaea stagnalis*

**S. Charles**, University Lyon / Laboratory of Biometry and Evolutionary Biology; **C. Forfait**, Université de Lyon; **V. Ducrot**, INRA / Ecotoxicology and Quality of Aquatic Ecosystems; **H. Holbech**, University of Southern Denmark / Department of Biology; **T. Hutchinson**, School of Biological Sciences Plymouth University; **L.L. Lagadic**, INRA / UMR INRAAgrocampus Ouest Ecology and Ecosystem Health; **J. Oehlmann**, Johann Wolfgang Goethe Universität Frankfurt am / Aquatic Ecotoxicology; **L. Weltje**, BASF SE / Agricultural Centre; **M. Delignette-Muller**, VetAgro Sup. Since 2012, European experts work towards the development and validation of an OECD test guideline for mollusc reproductive toxicity with the freshwater gastropod *Lymnaea stagnalis*. A ring-test involving six laboratories allowed studying reproducibility of results, based on survival and reproduction data of snails monitored over 56 days exposure to cadmium. A classical statistical analysis of data was initially conducted by hypothesis tests and fit of parametric concentration-response models. However, as mortality occurred in exposed snails, these analyses require to be refined, particularly in avoiding bias that exists when the number of clutches/eggs is analysed without accounting for mortality, or when replicates where mortality occurred are excluded; in the latter case, a number of organisms are discarded and valuable data can be lost. In this context, the purpose of our statistical study was twofold. First, we refined the statistical analyses of reproduction data accounting for mortality all along the test period. The variable "number of clutches/eggs produced per individual-day" was used for EC<sub>50</sub> modelling, as classically done in epidemiology in order to account for the time-contribution of each individual to the measured response. Furthermore, the combination of a Gamma-Poisson stochastic part with a Weibull concentration-response model allowed accounting for the inter-replicate variability. Second, we checked for the possibility of optimizing the initial experimental design through the reduction of exposure duration and/or number of replicates. Based on the six datasets, we show that using the 'per individual-day' unit in ecotoxicology avoids the exclusion of data (as a consequence the EC<sub>50</sub> could be not estimated from remaining data) and ensures an unbiased reproduction data analysis when mortality occurs in exposed animals. We also show that the experimental design may be optimized, depending on what should be prioritized. Even if further studies would be necessary with other kinds of compounds, we illustrate the fact that, in the case of

cadmium, and if 6 replicates are kept, a 35-day exposure duration would be sufficient to characterize the toxicity. In the same way, 3-4 replicates appear sufficient if the exposure duration stays at 56 days. However, before the reproduction test with *L. stagnalis* can be standardized, other works are necessary to further refine the experimental design, e.g., by regarding exposure duration and replication simultaneously.

**523 The freshwater amphipod *Hyalella azteca* as alternative test organism for bioaccumulation studies** **C. Schlechtriem**, Fraunhofer IME / Oekotoxikologie; **H. Bruckert**, **H. Juerling**, **I. Goeritz**, **V. Kosfeld**, **C. Schaefers**, Fraunhofer IME. The ultimate decisive bioaccumulation-criterion as part of the REACH regulation (Annex XIII) is the bioconcentration factor (BCF) reflecting the uptake of a test substance from the contaminated surrounding medium. Bioconcentration factors (BCF) for regulatory purposes are usually determined by fish flow-through tests according to TGD OECD 305. Alternatively, biomagnification factors (BMF) can be determined in dietary exposure tests for such groups of substances where this is considered more suitable than an aqueous exposure test. Fish bioaccumulation studies are time consuming, expensive, and use many animals. Alternative methods that replace the use of fish for BCF (BMF) testing would therefore be of value. The aim of this study was to investigate whether the freshwater amphipod *H. azteca* can be used as alternative test organism for bioaccumulation studies. In a first approach, a diet suitable to grow the freshwater amphipod was identified. The selected diet was enriched with the highly lipophilic test items hexachlorobenzene (HCB) and ortho-terphenyl (o-TP) as well as the perfluorinated acid perfluorooctane sulfonate (PFOS) and was then applied in dietary exposure tests with *H. azteca*. In a further approach the uptake and accumulation of the same substances and of a low lipophilic pesticide from water was investigated in flow-through tests. Column generated concentrations were applied to avoid the use of solvents. The bioaccumulation of test items in male and female animals was compared. Animals collected during the bioaccumulation studies were analysed for their tissue concentrations, which were normalized to a lipid content of 5%. Based on the kinetic study design the depuration and uptake rates for the test items were determined which were further used to calculate species specific BCF or BMF estimates. The results were compared with BCF and BMF values obtained from fish bioaccumulation studies in rainbow trout (*Oncorhynchus mykiss*). The results show that *H. azteca* provides the opportunity to investigate bioaccumulation from water (bioconcentration) and food (biomagnification) separately. BCF and BMF estimates from bioaccumulation studies with *H. azteca* are similar to those obtained from fish tests. Bioaccumulation studies with *H. azteca* support animal welfare considerations using a non-vertebrate species, improve efficiency and reduce costs for BCF/BMF-testing and thus have a high potential to be used as alternative method for bioaccumulation studies.

**524 Are acute avian toxicity tests with formulated plant protection products necessary?** **S.K. Maynard**, Syngenta / Environmental Safety; **P.J. Edwards**, Syngenta Ltd; **M. Douglas**, DOW Agro Sciences Ltd; **R. Green**, 3National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs); **J.R. Wheeler**, Syngenta Ltd. All national plant protection product (PPP) registration schemes require information on the acute avian toxicity of the end-use product. In Europe this can be assessed using toxicity data for constituent active substance(s) (a.s.) rather than the formulated PPP itself. In some countries this is not permitted as there is a requirement to perform specific acute toxicity tests on each individual formulated PPP. The objective of this study was to determine if the conduct of additional studies on formulated PPPs added any useful information beyond that which can be obtained by testing the constituent a.s. This was achieved by compiling a database of LD<sub>50</sub> values for formulated PPPs and their constituent a.s. Data for 199 PPPs were compiled from acute toxicity studies conducted with northern bobwhite or Japanese quail (Syngenta and Dow Agro Sciences unpublished regulatory literature). Of the 199 PPPs studied 77 contained only a.s. with undefined LD<sub>50</sub> values (i.e. LD<sub>50</sub> >

**525 Passive sampling as a monitoring tool for organic substances in coastal and marine waters - strength & limitations of an emerging monitoring technique** U.R. Kraus; R. Gunold, A. Paschke, Helmholtz Centre for Environmental Research (UFZ); N. Theobald, German Federal Maritime and Hydrographic Agency (BSH). The continuous anthropogenic impact on coastal and marine ecosystems results in a rising number of European frameworks and legislative requirements both already in effect as the Water Framework Directive or under development as the Marine Strategy Framework Directive and HELCOM CORESET. They all demand the close monitoring of a wide range of both priority and new substances of concern. However, in coastal and marine environments, traditional sampling methods based on grab sampling are inefficient due to high logistical costs, low contaminant concentrations and the incapacity to detect episodic changes in contaminant concentrations by spot sampling. Offering an alternate technique, passive sampling devices (PSDs) combine the provision of time-weighted average concentrations with low detection limits and technical robustness independent of environmental conditions. Therefore, PSDs are considered as complimentary monitoring tools under legislative regulations but there is a demand of further research and validation. Within the presented study the applicability of two types of PSDs – silicone rubber sheets and LDPE membranes – for the monitoring of 73 organic pollutants (PAHs, PCBs, halogenated and polar pesticides, PFASs, fire retardants, pharmaceuticals) in coastal and marine environments was tested under real-world conditions. This included logistical issues as well as the development of PSD-related extraction procedures and analytical methods for the large number of both polar and non-polar compounds. By the use of performance reference compounds (PRCs), the calculation of time-weighted average concentrations ( $c_{TWA}$ ) was carried out and results from passive samplers were related to those of conventional sampling methods.

**526 Applicability of passive samplers in automated sampling stations for the time-integrated monitoring of hydrophobic pollutants in limnic ecosystems** R. Gunold, Department of Ecological Chemistry; U.R. Kraus; A. Paschke, G. Schuurmann, Helmholtz Centre for Environmental Research UFZ / Department of Ecological Chemistry. Recent studies have shown the suitability of passive samplers as an alternative tool to traditional sampling techniques for the chemical analysis of surface waters [1]. However, the majority of aquatic monitoring carried out by state and federal authorities involves automated sampling stations, where composite water and sediment samples are collected through a bypass containing pumped river water. The latter sample treatment and analysis is usually done by the associated state or federal laboratory. Operating those stations as well as transport and processing of samples is connected with cost- and labour-intensive expenses, interfering with limited budgets of environmental authorities. During a research project funded by the Federal Environment Agency of Germany (UBA), five different passive sampling devices (PSD) were deployed in automated sampling stations in Saxony-Anhalt. They were exposed in self-constructed flow-through vessels made from stainless steel, being connected to the bypass of the stations in Magdeburg (Elbe) and Dessau (Mulde). The investigated PSDs have been LDPE strips [2], silicone sheets [3], MESCO [4,5] and bare silicone rod [5]. Next to 7-day composite water samples, grab samples were collected at the bypass and investigated. In addition the analytical results of composite sediment samples, taken monthly by the State Office for Flood Protection and Water Management of Saxony-Anhalt (LHW), could be used for comparison. This lecture will provide an overview of the study and discusses the most important outcomes.  
**References:** [1] Allan IJ, Booij K, Paschke A, Vrana B, Mills GA, Greenwood R. 2009. Environ Sci Tech 43:5383

**527 Passive sampling of perfluorinated chemicals in water: in situ correction of flow effects on chemical sampling rates** S. Kaserzon, University of Queensland / National Research Centre for Environmental Toxicology; D. Hawker, Griffith University / School of Environment; K.

Booij, NIOZ Royal Netherlands Institute for Sea Research; D. O'Brien; K.E. Kennedy, University of Queensland; E. Vermeirssen, Eawag / Dept. of Environmental Toxicology; J. Mueller, Entox / National Research Centre for Environmental Toxicology (Entox). Perfluorinated chemicals (PFCs) are a family of anthropogenic pollutants that are the subject of increasing scrutiny and concern due to their wide-spread environmental distribution and the persistence, bioaccumulation and toxic properties of some members. Consequently regulation and monitoring of some PFCs has begun, and the requirement for monitoring of these chemicals is expected to increase. A recently developed modified polar organic chemical integrative sampler (POCIS) provides a means for quantifying PFCs in water. However, changes in external flow rates may alter POCIS sampling behaviour and consequently affect estimated aqueous concentrations of PFC analytes. Therefore it is important that water flow rate should be accounted for during field deployments. In this study sampling behaviour of the modified POCIS was calibrated using a previously developed (O'Brien et al. 2011) passive flow monitor (PFM) to account for *in-situ* water flow conditions. Results from this study showed daily mass loss rate of the PFM device ( $r_{PFM}$  g d<sup>-1</sup>) corresponded well with that from a previous study (O'Brien et al. 2011) ( $R^2=0.95$ ). In addition the predicted water flow rate derived from the PFM was consistent with results from a hand held flow monitor, within a flow rate range of 6 – 15 cm s<sup>-1</sup>. A plot of sampling rate ( $R$  L d<sup>-1</sup>) for the uptake of PFCs into the POCIS versus  $r_{PFM}$  (PFM loss rate, g d<sup>-1</sup>) showed  $R$  to be a linear function of  $r_{PFM}$  (within the range of 1-4 g d<sup>-1</sup>). The use of the PFM can therefore be used to allow direct estimation of  $R$  for the PFCs in this study, within this range, which corresponds to a water flow rate of 6–15 cm s<sup>-1</sup>. This study represents the first calibration of the modified POCIS using a co-deployed PFM device for selected PFCs. It suggests the PFM can be an effective *in-situ* calibration tool to improve the estimation of POCIS derived water concentrations. The range applicable for effective PFM deployment falls within the range of water velocities likely encountered under field conditions. At higher  $R$  where the PFM is less effective, a plateau of  $R$  for some chemicals has been observed. This could suggest further investigation into non-linear relationships would be required at highly turbulent flow rate. Under typical field deployment conditions however, such flow rates are rare. Given appropriate co-calibration the PFM could have a wider application for deployment with a range of POCIS devices and analytes.

**528 Kinetic limitations in measuring concentrations of labile metals by diffusive gradients in thin films** C. Gueguen, Trent University / Chemistry; O. Clarisse, Université de Moncton; A. Perroud, Trent University. Dissolved metals may exist in natural waters as hydrated ions and as complexes with inorganic (such as carbonates, chlorides or hydroxides) or organic (such as DOM) compounds. From a geochemical and ecotoxicological point of view, the **master species** are the free and small labile complexes as they are able to cross cell membranes, leading to an increase in bioavailability. In light of these considerations, the determination of free/labile species has become an important field of research in aquatic sciences and biogeochemistry. In speciation studies, sampling and storage of samples can irreversibly change the speciation of metals, resulting in erroneous information about the system. The application of *in situ* techniques using passive samplers can considerably reduce this problem. There is a limited number of *in situ* techniques with the required sensitivity and selectivity. One of the most promising is the diffusive gradient in thin-film gels or DGT that involves the diffusion of metal species from solution through diffusion gel to binding resin. The amount of metals that accumulates on the resin depends on concentration and speciation of metal in solution, time of deployment, properties of diffusion gel, temperature-dependent diffusion coefficients of metal species and whether dissolved metal is labile. This work provides new information on the dissociation kinetics of metal organic ligand complexes in natural waters. Diffusive gradients in thin films devices (DGT) were deployed at 49 sites located in arctic and boreal river watersheds. The apparent diffusive boundary layer (ADBL) thickness at the gel/solution interface showed the highest values for the trivalent metal Fe and the lowest for Cd at most sites. Significant

differences in ADBL may be attributable to several factors including the metal: DOM ratios and the quality of the organic ligands.

**529 In situ Measurement of Solution Concentrations and Fluxes of Antibiotics in Soil Using o-DGT** C. chen, H. Zhang, Lancaster University / Lancaster Environment Centre; G. Ying, Chinese Academy of Sciences Guangzhou Institute of Geochemistry / State Key Laboratory of Organic Geochemistry; K.C. Jones, Lancaster University / Lancaster Environment Centre. Adverse effects of antibiotics, particularly their promotion of antibiotic resistance, has raised concerns in environmental science and ecology. The availability, rather than total concentration in the soil determines the toxicity of antibiotics in soil. Techniques which either minimally disturb the soil or perturb the solution in a controlled way are most likely to provide relevant information. Herein, we report the first use o-DGT (Diffusive Gradients in Thin-film for organics) in soil systems to gain insight to the mobility and lability of four antibiotics - sulfamethoxazole (SMX), sulfamethazine (SMZ), and sulfadimethoxine (SDM), trimethoprim (TMP) in soil. Directly measured concentrations ( $C_{\text{free}}$ ) of these antibiotics in soil solution were in the order:  $\text{SMX} > \text{SMZ} > \text{SDM} > \text{TMP}$ . The  $R$  values (ratio of o-DGT measured concentration -  $C_{\text{free}}$  to  $C_{\text{free}}$ ) for TMP, SMZ, SMX and SDM were 0.55, 0.40, 0.28 and 0.40, respectively, indicating that the removal of these antibiotics from the solution can be to some extent rapidly (minutes) resupplied by release from the solid phase. The nonlinearity of the relationship between o-DGT fluxes and the reciprocal of diffusive layer thickness ( $1/g$ ) also suggested that concentration in the soil solution were only partially sustained by release of these antibiotics from the solid phase. The initial solution concentrations derived from the measurements of o-DGT with thicker diffusive layers were 29, 152, 207 and 83 ppb for TMP, SMZ, SMX and SDM, respectively, which were comparable to the directly measured values. The potential fluxes of these antibiotics in this soil were 1.2, 3.6, 5.4 and 2.4  $\text{pg}/\text{cm}^2/\text{s}$  for TMP, SMZ, SMX and SDM, respectively. O-DGT is a promising tool for understanding the fate and behaviours of polar organic chemicals in soil, and it provides a potential *in situ* approach for assessing their bioavailability.

**530 Solid phase micro extraction as a tool to study ecological effects of PAHs in Baltic Sea sediments** S. Lang, Hamburg University of Applied Sciences; D. Schulz-Bull, Leibniz Institute of Baltic Sea Research / Marine Chemistry; G. Witt, HAW Hamburg / Faculty of Life Sciences, Environmental Technology. Polycyclic aromatic hydrocarbons (PAHs) belong to the category of "Priority Pollutants" in today's marine environmental risk assessment. Whether complex environmental matrices like sediments serve as sink or source for PAHs in the water column, and other ecologically relevant processes such as diffusion, bioavailability, bioconcentration in sediment organisms and baseline toxicity, are mostly controlled by the chemical activity of a substance rather than by its total sediment concentration. This study provides the first comprehensive dataset on freely dissolved sediment porewater concentrations and pollutant chemical activities in Baltic Sea sediments. To determine freely dissolved concentrations ( $C_{\text{free}}$ ) and chemical activity of PAHs in sediment pore water samples from sediment cores of the Baltic Sea, and hence to study ecological effects, we used solid phase microextraction (SPME) as a passive sampling tool for our investigations. Gradients in  $C_{\text{free}}$  and chemical activity were used to maintain a spatial characterisation of PAH exposure and to determine the direction of diffusion within the sediment and at the sediment water interface. The contribution of PAHs to the baseline toxic potential was assessed on the basis of the chemical activities. Finally total sediment concentrations and  $C_{\text{free}}$  were used to calculate site specific sediment distribution ratios of the PAHs. The results indicated different concentration ranges of  $C_{\text{free}}$  among the sampling stations. The comparison of the composition of  $C_{\text{free}}$  and chemical activity reveals that lower molecular weight PAHs predominate  $C_{\text{free}}$ , while higher molecular weight PAHs predominate chemical activity. This clearly shows that the contribution to the baseline toxic potential is not depending solely on the amount of PAHs in general, but rather than on its composition, e.g., BbF, BkF and BaP build the lowest fraction of

C but the effect they cause is highest compared to all other PAHs. The investigation shows that  $C_{\text{free}}$  and chemical activity are important exposure parameters for the prediction of bioavailability and baseline toxicity of Baltic Sea sediments and their measurement should be therefore included in monitoring programmes, risk-assessment and pollution-management strategies.

**531 How should we define a Planetary Boundary for Chemical Pollution?** M.L. Diamond, University of Toronto / Department of Earth Sciences. The centre piece of the call for Planetary Boundaries (PB) is the need for global governance to manage human manipulations of biophysical processes. We live in an era where financial and industrial sectors are highly and effectively globalized, but such globalized governance and stewardship of our biophysical system are sorely lacking. The planetary boundaries concept speaks to moving what Garrett Harding described in 1968 as "The Tragedy of the Commons", beyond the local to a global scale. For the chemical pollution, the boundary acknowledges the global distribution of persistent compounds such as POPs. As well, the PB should recognize the increasing prevalence of "pseudo-persistent" chemicals emitted at levels beyond the assimilative capacity of the receiving environment. The implications of, and driving force for a chemical PB is the mounting evidence of widespread and sometimes serious adverse health impacts to biota and humans. Leaving aside, for the moment, the rate limiting and vexing question of a lack of global governance to implement a chemical PB, the boundary for chemical pollution is scientifically challenging to define because of the myriad chemicals in commerce and observed adverse effects. As a first step, I suggest reducing the universe of chemicals under consideration to synthetic and metal-containing substances, and exclude construction materials that constitute by far the greatest mass of substances in commerce. We can further constraint the chemicals under consideration to the limited number of high production volume chemicals. Although a chemical PB ought to be effects-based, this may be unknowable for many compounds. I suggest we debate the merits of using production rates of high production volume chemicals (excluding construction materials) as a proxy for emissions and the potential for adverse effects. This argument is based on four points. Based on these points, I suggest defining a planetary boundary for chemical pollution for POPs and high production volume chemicals (excluding construction materials) based on production levels, following the logic of the boundary being a "fixed cap" on emissions. This boundary acknowledges the global scale of chemical production and release, the limited assimilative capacity of the receiving environment, and our unsustainable rate of resource use.

**533 Mechanistic Modelling in Search of the Human Most Exposed to Polychlorinated Biphenyls** T.N. Brown, Helmholtz Centre for Environmental Research UFZ / Department of Analytical Environmental Chemistry; E. Undeman, Stockholm University / Dept. of Applied Environmental Sc.; E. Wania, University of Toronto at Scarborough / Department of Physical & Environmental Sciences; M.S. McLachlan, Stockholm University. Recently, the concept of a "safe operating space for humanity" has been proposed, and a challenge issued to identify the "planetary boundaries" with respect to nine global environmental issues, beyond which humanity cannot venture without disastrous consequences (Rockström et al. Nature 461, 472-475). The authors concluded that there is presently no scientific framework for estimating this biophysical threshold for chemical pollution. We will argue that this boundary has to protect those humans most likely to suffer detrimental effects from globally distributed contaminants. Because the susceptibility of different humans to suffer toxic effects from a certain dose of a contaminant varies within a relatively small range, this vulnerability will largely be determined by exposure susceptibility, i.e. the likelihood to accumulate high levels of that contaminant in the body, which will vary by many orders of magnitude. As a proof of concept, we identify for a single contaminant, polychlorinated biphenyl (PCB) congener 153, the human population worldwide that is likely to accumulate the highest body residues. This is accomplished by feeding the geographically explicit output of the global



fate and transport model BETR-Global into the human food chain bioaccumulation model ACC-Human, parameterized for a range of human sub-population living in different climates and eating different, locally grown diets. Both models are dynamic and thus also identify the human generation in history that was most exposed to PCB-153 during a century that saw increasing and decreasing emissions of PCBs. Those eating high in the food chain AND living close to sources suffered the highest human PCB exposure worldwide.

#### **534 Area directed Groundwater control: a combination of tools**

M.H. Wagelmans, M. Henssen, M. Luitwieler, S. Lieten, Bioclear. Due to industrialization, groundwater in urban areas has become polluted. Often similar industries were located in the same area of a city so the groundwater became polluted with similar compounds. In the Netherlands there is a change in remediation procedures from site specific remediation to area specific remediation in which all of these similar polluted sites are managed (figure 1a). Area directed groundwater control can be a valuable tool in managing polluted groundwater provided that the risks of this approach can be controlled. Possible risks that can be identified are: Seepage in water streams or natural areas Interference with groundwater extraction/drinking water production Uncontrolled spreading of pollution Interference with aquifer thermal energy storage Insight in these risks can be gained by using a combination of tools: pollution transport modeling, molecular tools, biodegradation, ecological risk assessment, geohydrological calculations. This presentation will give an overview of some projects in the Netherlands, the tools that were used for making area directed plans and the results of these plans. In times of changing legislation and remediation procedures, a consideration can be made between site directed remediation of all individual sites versus the area directed approach of combining the sites and manage the combined pollution. However, it is even more important to have insight in groundwater distribution, biological processes, possible interferences with groundwater extraction and other groundwater uses and goals in the area. This presentation will show that different tools are available and can be used to make a sound based consideration that will be accepted by the authorities and other stakeholders.

#### **535 A sedimentary approach to determine the extent of spatial and temporal Hg contamination in a large, subalpine lake (Lago Maggiore, Italy)**

D.A. Vignati, CNRS / LIEC UMR; G. Piero, CNR-ISE; R. Bettinetti, B. Ponti, Università dell'Insubria; G. Tartari, CNR-IRSA / UOS Brugherio. Lago Maggiore is a large subalpine lake lying along the border between Italy and Switzerland. The lake consists of a main basin elongated in the north to south direction and a large secondary basin, the Pallanza Basin, located on its western part. This basin receives the waters of the River Toce (the second most important tributary of the lake) that, in turn, has carried the effluents of a chlor-alkali plant for most of the 20<sup>th</sup> century. Legacy Hg pollution is therefore an important issue in Lago Maggiore and priority areas for long-term monitoring and, possibly, remediation have been identified. These areas include stretches of River Toce, the Pallanza Basin and the central portion of the main basin, with exclusion of its northernmost and southernmost parts. Recent studies on the circulation of water masses in Lago Maggiore showed that the waters of River Toce tend to be transported southwards, meaning that Hg inputs could tend to accumulate precisely in zones currently not considered as priority areas for monitoring and risk management. Total Hg levels in recent sediments (2000–2011) were determined in nine cores collected at different locations and confirmed that the highest Hg levels occurred in the southern part of the lake. Three additional cores, one from the Pallanza Basin (i.e., closer to potential Hg inputs from River Toce) and two from the southern part of the lake, were analysed to reconstruct the geochronology of Hg contamination. These cores were sliced in sections of 1 cm and dated using biological markers (i.e., changes in diatom species composition) and radiometric methods. The three cores had very similar Hg profiles, providing strong evidence of a common polluting source that, following historical knowledge, can be reasonably traced to a chlor-alkali plant in the River Toce basin. These findings show that a

properly implemented sedimentary study of Hg contamination can bring invaluable information for the regulatory follow-up of ecosystems with a legacy of Hg pollution.

#### **536 Interactions between Hg in the aquatic environment and macrophytes**

C. Cosio, Geneva University; N. Regier, University of Geneva. The UNEP Global Mercury Partnership aims to protect human health and the environment from the release of mercury (Hg) by minimizing or eliminating anthropogenic mercury releases to air, water and land. Notably Hg impact, dispersion and biomagnification in the aquatic environment are still of great concern because of the amount of Hg circulating in the environment. Accumulation of Hg in primary producers is the first step of Hg uptake in food chain and result in the highest biomagnification step. In shallow waters the highly toxic methyl-Hg (MeHg) is formed and macrophytes – aquatic photosynthetic organisms that can be seen by naked eyes – are the predominant primary producers playing a key role in the structure and functioning of the ecosystem. Bioaccumulation of Hg in macrophytes under these conditions has been regularly reported. A high Hg and/or MeHg production or proportion in this environment may constitute a major pathway of Hg uptake into aquatic food webs. Moreover macrophytes can reflect site specific bioavailability and notably Hg concentrations found at the base of the food web. Macrophytes that are exposed to sediments and water column are therefore particularly interesting in the context of the Hg biogeochemical cycle. The present work describes the role of macrophytes in Hg fate that have been identified until now including Hg cycles in sediments, methylation, bioaccumulation, and biomagnification in the food chain. Data will be illustrated with our findings regarding *Elodea nuttallii* interactions with Hg, namely a high Hg and MeHg accumulation and tolerance in the field and the laboratory, a change of microbial communities associated with rhizospheric sediments, an increase of MeHg proportion in rhizospheric sediments, a mobilization of MeHg from the sediments to the shoots, a carrier mediated internalization of both inorganic Hg and MeHg and evidences of trophic transfer of Hg from these plants in the field. Results highlight the need for an increased understanding and consideration of the role of macrophytes in the functioning of Hg biogeochemical cycle in aquatic environment and in risk assessment of this highly toxic metal as shallow water represent a significant surface of our planet. Based on the current work concerning interaction of Hg with macrophytes, we believe that the definition of a global Hg policy must take in consideration the shallow water ecosystems in the UNEP monitoring and assessment mercury programs.

#### **537 Revisiting the framework for Life Cycle Sustainability**

**Assessment** A. Zamagni, P. Buttol, O. Amerighi, ENEA; B. Burchi, Pisa University / Economics Department; P. Masoni, ENEA / Protezione e Sviluppo dell'Ambiente e del Territorio. Alessandra Zamagni<sup>1</sup>, Patrizia Buttoli<sup>1</sup>, Oscar Amerighi<sup>1</sup>, Barbara Burchi<sup>2</sup>, and Paolo Masoni<sup>1</sup>  
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The Life Cycle Sustainability Assessment (LCSA) framework is considered suitable for evaluating the sustainability of products/systems/services, by means of a combined evaluation of environmental, economic and social impacts through Life Cycle Assessment (LCA), Life Cycle Costing (eLCC) and Social Life Cycle Assessment (SLCA), respectively. The framework works under the hypothesis of carrying out the three assessments keeping consistent (ideally equivalent) system boundary and the same functional unit. The results of the three assessments are thus evaluated together, without any compensation and/or substitution among the three results but adopting a strong sustainability perspective. First applications of the framework do not discuss the validity of the approach but they focus on two main aspects: data availability and interpretation of results. On the contrary, in this paper we intend to draw the attention to the LCSA methodological framework and to discuss the two main aspects that guide its application: the problem at hand and the decision context. These two aspects contribute to define whether a producer or a public policy maker perspective should be adopted for carrying out the study.

In this paper, we provide a revisitation of the LCSA framework able to take into account the diversity of applications.

### **538 Beyond compliance: Social impact assessment driving upgrading and innovation in the product/service life cycle**

**Wangel**, DTU Management Engineering. Social life cycle assessment has emerged within the LCA community to supplement the environmental and economic dimensions, as an integration of the three pillars is sought to build a methodology for the full life cycle sustainability assessment. However, the contributions to a SLCA adopting similar methodological principles as LCA have shown to be problematic in several areas, and also social acceptance of some solutions suggested seems doubtful. The paper will detail the challenges to SLCA on its own account by reviewing recent literature and discussions. Then, in comparison, alternative approaches to capture social impacts in product chains to support decision making are discussed to characterise donor-driven as well as private sector interventions, which goes beyond compliance with existing standards and adopts a much broader agenda of social and economic development taking its point of departure in the dynamics of a given product chain. To strengthen efforts to alleviate poverty in Africa, donors are giving priority to private sector support for upgrading of agricultural value chains focusing on improving the livelihood of primary producers and workers. This approach adopts a chain perspective to strategize optimal action points for upgrading. Social impacts are captured under the headings of poverty, gender and environment. In recent years, mainly larger companies, who are lead firms in global value chains, have embarked upon efforts to extend and improve social impacts of their chain, often in partnership with non-governmental organisations, and build new, corresponding business models. Given the limitations of Social LCA, there is a need to seek the foundations for an approach to capture social impacts in full throughout all life cycle stages, which do respond to the concern of the Northern consumer observing his or her enhanced well-being as connected to the living conditions of those who produced that product or service, while at the same time pointing to chain innovation in products and business models for improved and additional social impacts. Returning to the basic issues further implies a questioning of the three pillar sustainability concept, whereby social impacts are not regarded as discrete, fragmented events but as integral to a social system interacting with ecological systems.

### **539 Addressing the sustainability assessment of complex systems with Resilience, Emergy and LCA**

**B. Rugani**, University of Siena / Resource Centre for Environmental Technologies (CRTE); **A. Marvuglia**, CRP Henri Tudor / Cork Constraint Computation Centre; **D. Arbault**, CRP Henri Tudor; **E. Benetto**, CRP Henri Tudor (CRPHT) / CRTE. In ecology, resilience and efficiency are related to the levels of diversity and connectivity of ecosystem networks, but in opposite directions: these indicators are essentially complementary because the streamlining that increases efficiency automatically reduces resilience, where greater efficiency means less resilience, and, conversely, greater resilience means less efficiency. As a consequence, the long-term maintenance of vitality (i.e. sustainability) appears to be intrinsically related to efficiency and resilience, and systems that lie between these two extremes are likely to endure (to be sustainable). The aim of this contribution is to trace and discuss feasible research paths to enhance the characterization of sustainability in LCA by using the resilience and efficiency principles in combination with the Emergy concept. Indeed, the Emergy method is usually reputed being able to measure sustainability through the use of several intensive measures of system's performance. The Unit Emergy Value (UEV, i.e. an estimate of the solar energy required to generate one unit of a product), in particular, is considered an indicator of quality and environmental efficiency when comparing alternative products. However, the extent to which we can accept the UEV meaning as such is not yet proved, and literature is actually contrasting on this issue. Recent analyses suggest that UEVs can be consistently calculated in complex LCI networks through the application of a specific search algorithm. We have advanced this calculation on two representative large and complex LCI matrices and

then observed how the Emergy accumulated over the visited nodes of the network. A strong accumulation of Emergy over one or very few nodes may mean that the output is strongly constrained by the availability of that or those few processes. Despite this can be regarded as a way of increasing the system efficiency (i.e. a low number of sources/nodes is used to obtain the output), at the same time it can be considered an index of low flexibility and thus low resilience (i.e. low capability of using other nodes/processes whether the main ones are disrupted). Discussions and reflections are provided here on this finding. Current research is also addressed to identify correlation patterns between Emergy, resilience and efficiency in those highly complex LCI systems.

### **540 'Comeback of mills?' - Sustainability Assessment of small hydro power plants in Germany in a historical perspective**

**F. Becker**, TU Darmstadt / Industrial Material Cycles; **L. Schebek**, Technische Universität Darmstadt / Industrial Material Cycles; **C. Zumbraegel**, TU Darmstadt / Department of the History of Technology. The interdisciplinary project 'Comeback of mills?' investigates small hydro power plants in Germany by collaborative research of historic and engineering science based on a Life Cycle approach. In Germany, due to challenging climate and energy policy, renewable energies are to be extended considerably in the next years, e. g. hydropower. However, the implementation of renewable energy technologies often results in conflicts on local scale (e.g. fish habitats, ecosystem, cultural landscape heritage). Sustainability assessment therefore has to cover all these effects and to envisage both local and global perspective. So, a comprehensive sustainability assessment of small hydro power plants was done. Therein technological efficiency and nature conservation as well as cultural and social aspects were taken into account. Furthermore the past (historical use of mills) was searched for traces and clues to find out how to cope with the challenges of our present and future towards local energy supply. Based on a typology of small scale hydro power, technological key indicators and a first Life Cycle Assessment (LCA) characterize performance of generic types of small scale hydro power technologies. From these findings, regional sites (based on historical investigation - 'Sauerland' and 'Alpen-Vorland') are selected for in-depth local and on-site investigation. For these sites, the first LCA on generic technology types integrates information on local conditions in the inventory, but notably in the impact assessment. Impacts on local ecosystems are assessed resulting a second, now regionalized LCA. As to criteria for sustainability assessment, two levels are discerned: on the macro-economic level, energetic performance, material demand and general impacts on economy are main aspects. On the local level, a list of criteria has been developed for environment and social effects which have been derived from current framework documents of natural protection (e.g. Council Directive 92/43/EEC) but notably also the study of historical background in these regions. The project is designed to provide insights specifically for societal decisions on small hydro power plant. Taking into account that collaborative research with historical science is novel within the scope of Sustainability Assessment, it is above that also designed to explore chances from this kind of interdisciplinary research for sustainability assessment of technology in a more general way.

### **541 A Novel Methodology for the Sustainability Assessment of New Technologies**

**B. van Haaster**, Utrecht University / Copernicus institute department energy and resources. The introduction of new technologies can have profound impact on society and nature. Therefore, there is a need for a method that can provide a balanced assessment of new technologies on all its different impacts. Within the European Prosuite project, the aim is to develop a methodology for the sustainability assessment of existing and emerging technologies. In this article, existing approaches of sustainability assessment are reviewed. Using concepts developed initially for environmental life-cycle assessment, we propose an approach consisting of the following major impact categories: 1) impact on human health; 2) impact on social well-being; 3) impact on prosperity; 4) impact on natural environment; 5) impact on exhaustible resources. All primary impacts can be grouped

under these categories. For several of these categories still quite some analysis of mechanisms in the cause-effect chain is necessary and there will be substantial remaining uncertainties for the others. For a complete assessment, the five major impact categories need to be integrated. Several approaches are available for this purpose, such as multicriteria analysis with or without weighted aggregation.

#### **542 Viewpoints to social life cycle assessment of biodiesel production**

C. Macombe, Irstea; P. Leskinen, University of Turku; P. Feschet, CIRAD Montpellier; R. Antikainen, Finnish Environment Institute. Assessment of social impacts of different production systems and services has gained increasing interest in the society. Life cycle assessment (LCA) is a key tool developed to estimate the impacts from cradle to grave. However, traditionally LCA has focused on environmental impacts, but recently approaches for social life cycle assessment (SLCA) have been developed as well. The aim of this presentation is to analyse possibilities and future development needs in evaluation of social impacts in LCA. We analyse the field in general and take a closer look at the case of biodiesel production, which is a timely topic in view of global climate change mitigation objectives. The analysis is carried out at three different levels – company, regional, and state level – since different levels of decision-making require different types of information. Despite active recent development in the field of SLCA, it is concluded that in many cases carrying out comprehensive SLCA is not possible yet, even though important information based on SLCA can be produced for decision-makers. Finally, we outline topics of future research that would further improve the methodological and empirical basis of SLCA at various levels of decision-making.

#### **543 Potential use of a population model of the wood mouse in pesticide risk assessment of sublethal effects**

C. Liu, Syngenta / Environmental Safety; M. Ebeling, Environmental Safety; W. Schmitt, Bayer CropScience AG / Environmental Modelling; R. Luttik; R. Sibly, University of Reading; V. Grimm, Helmholtz Centre for Environmental Research UFZ / Department of Ecological Modeling; P. Thorbek, Syngenta / Environmental Safety. In pesticide risk assessment of small mammals, the assessment of sublethal effects can be challenging, due to unrealistically ubiquitous assumptions of dietary dose. The population-level consequence is difficult to define and evaluate because most reproductive studies are conducted under static lab conditions. Population modelling can be a useful refinement tool as it can simulate animal spatial behaviour more realistically. It can also extrapolate individual-level endpoints to population-level effects, such as changes in population size and the ability to recover. However, the robustness of a model needs to be justified and the representative scenarios, such as appropriate landscape settings, need to be clarified before a model is used in risk assessment. In a recently held SETAC workshop, MODELINK, such topics were explored and available models were used in case studies for stakeholders to discuss how such models can be used in risk assessment. Here we present the results of a wood mouse model used as one of the examples in the “small mammal” working group. First, we justified the robustness of the model using the “pattern-oriented validation” approach. We then used the model to test the population-level effects of different reproductive endpoints (i.e. reduced litter size, delayed first reproduction and lower infant survival) and how they are affected by variant landscape settings. Results showed that with realistic settings of the individual endpoints, the effects on population size were negligible. When the individual endpoints were decreased by 10 times in order to show an effect, the population-level consequences varied with the field size and proportion of off-crop habitats, with the more optimal habitats, the larger the population size, meanwhile a larger proportion of the population being affected by pesticide exposure. Among the different reproductive effects, changes in litter size caused most reduction in population size while delay in first reproduction had minor effect. Despite the difference, population recovered to pre-treated level in all cases. With such insights, we discussed how protection goals can be better interpreted and how acceptable level of effects can be more profoundly suggested.

#### **544 Linking variable spatial exposures of chemicals to individual and population level effects in soil invertebrates: a modelling approach for current RA**

M. Meli, Roskilde University / Department of Environmental, Social and Spatial Change; A.S. Johnston, University of Reading / Biological Sciences; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; R.A. Pastorok, Integral Consulting Inc; F. Voss, Dr Knoell Consult GmbH / Environmental FateModellingGIS; S. Chelinho, IMAR CMA / Department of Zoology; H. Sundberg, Swedish Chemicals Agency; T. Alvarez, EcoRisk Solutions Ltd / Dept. of Ecological Sciences; P. van Vliet; M. Reed, Chemicals Regulation Directorate, HSE. Current risk assessment methods for measuring the toxicity of plant protection products (PPPs) on soil invertebrates use standardised laboratory conditions to determine acute effects on mortality and sublethal effects on reproduction. If an unacceptable risk is identified at the lower tier, population level effects are assessed using semi-field and field trials at a higher tier. Yet, field trials are expensive, time-consuming and cannot be applied to variable landscape scenarios, whilst there is currently no way of extrapolating available lower tier information to population effects. Mechanistic modelling of chemical effects on individual responses to PPPs shows great potential in fulfilling such a need, aiding ecologically informed extrapolations from this basic information. Here, we introduce and evaluate the potential of two models using ubiquitous soil invertebrates (collembolans and earthworms) as refinement options in current risk assessment. Both are spatially explicit agent-based models (ABMs), incorporating both individual and landscape variability. These models were presented to interested stakeholders from academia, industry and regulatory authorities at the first MODELINK workshop. Initial discussions defined the necessary modelling criteria for these models to be considered in risk assessment. Stakeholders outlined a need for clear model documentation following the available guidance documents and thorough validation of individual life cycle and population level effects; as to show the model incorporates all necessary detail. For assessing chemical effects, stakeholders also pointed to the need for spatio-temporal exposure modelling to be incorporated within these effects models and a desire for flexible model outputs, highlighting the importance of changes in population density and biomass as indicators of population effects. As a workable problem formulation for the modellers to apply to their ABMs, a relevant case study scenario was developed for applying and testing these modelling refinements. Results are to be presented at the second MODELINK workshop, and comparisons with homogeneous applications will highlight the usefulness of these additional tools.

#### **545 Population-level effects and recovery of aquatic invertebrates – a case study of the SETAC workshop MODELINK**

T.G. Preuss, RWTH Aachen University / Institute for Environmental Research; A. Basseres, TotalFinaElf; F. De Laender, Université de Namur ASBL / Lab of Env.Tox&Appl.Ecol.; N. Galic, AlterraWageningen UR / School of Biological Sciences; M. Hamer, Syngenta; B. Martin, Helmholtz Center for Environmental Research / Department of Ecological Modelling; T. Strauss, RWTH Aachen University / Research Institute Gaiac; P. van den Brink, AlterraWageningen UR / (a) Aquatic Ecology and Water Quality Management Group; (b) Alterra; L. Wendt-Rasch, Swedish Chemical Agency-Kemi; P. Dohmen, BASF SE / Landw. Versuchsstation, APD/RO. Recently EFSA has published a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides. Within this framework ecological modelling (e.g. individual level models like TK/TD models, population models, and ecosystem models) is identified as a promising tool to link the results of ecotoxicological studies to such specific protection goals. Specifically, ecological modelling enables extrapolation from laboratory conditions and test species to population level or community level effects under field conditions. However, even when ecological models are available, which are well documented and tested, there are currently no recommendations for how to apply them to risk assessments. The general aim of the SETAC Europe technical workshop MODELINK is therefore to provide guidance for when and how to apply ecological models to regulatory risk assessments.

Therefore 6 case studies are conducted during the workshop, from which the outcome of the case study for aquatic invertebrates will be presented here. This case study explores how to use mechanistic effect models to conduct an aquatic risk assessment of a rapidly-dissipating compound, which may be applied several times. For this compound a classical risk assessment was conducted and a model strategy to answer the open question was developed. It was decided to use a combination of TK/TD models with population models for the most sensitive species *Gammarus pulex* and *Chaoborus obscuripes*. The need of defined ecological scenarios for this models were indicated and the models will be used to identify important features of these scenarios.

**546 Testing toxicokinetic and toxicodynamic macrophyte models in herbicide risk assessment** S. Heine, RWTH Aachen University / Institute for Environmental Research; G. Arts, Alterra Wageningen University and Research Centre / Environmental Risk Assessment; T. Brock, Wageningen UR; S. Duquesne, UBA Federal Environment agency; P. Manson, Cheminova A/S; G. Meregalli, Dow Agrosciences / Regulatory Scies & Government Aff.; H. Ochoa-Acuna, DuPont Crop Protection / Veterinary Pathobiology; W. Schmitt, Bayer CropScience AG / Environmental Modelling; P. van Vliet; U. Hommen, Fraunhofer IME. Ecological models have the potential to deliver information for risk assessments which cannot be obtained by standard laboratory assays. However, ecological models are rarely used in risk assessments because of missing guidance on how to use them and unknown model properties. The SETAC Europe technical workshop MODELINK was initiated to provide guidance on how to use ecological models and to identify possible applications of ecological models in risk assessments. The work presented here is the outcome of one group of the MODELINK workshop focusing on macrophytes. In this group, two toxicokinetic and toxicodynamic macrophyte growth models have been used in a new risk assessment approach and were compared with results based on classical risk assessments which are accepted by regulators. One of the models used simulates growth of *Lemna spp.*, the other one growth of *Myriophyllum spicatum*. The toxicokinetic and toxicodynamic properties of the models enable the consideration of effects caused by time variable exposure. Both risk assessments (classical & model based) evaluated the same case study describing varying exposure of a sulfonyl urea herbicide caused by a single spray application in spring. Advantages and disadvantages of the different approaches are compared and discussed.

**547 Population level vs. standard risk assessment: A comparative approach** M. Wang, WSC Scientific GmbH / Dept. Efaté & Modelling. In recent years population models are increasingly being considered as a useful method for chemical risk assessment, especially for addressing protection goals within the ecosystem services concept. However, it is frequently debated how population models shall be used for risk assessment and whether their output can be considered realistic or not. Population models are necessarily more complex than standard methods used in pesticide risk assessment. Due to the higher complexity, risk assessors sometimes assume that these models include a considerable level of uncertainty. However, in standard risk assessment uncertainties are not routinely addressed systematically. Instead assessment factors or worst-case assumptions are combined. To evaluate the level of realism in both standard (and higher tier) risk assessments and population level risk assessments based on a mammal population model, we compare both approaches. This comparison is based on the recent guidance on birds and mammals. We show that population models do offer advantages, especially addressing uncertainty and variability, compared to standard risk assessments.

**548 Prediction of the environmental fate and ecological impact of PAHs and pesticides in two streams in Luxembourg using AQUATOX model** r. carafa, TUODOR / CRTE; S. Massarin, M. Frelat, V. Huck, T. Galle, CRP Henri Tudor / CRTE. One of the main current challenges in the implementation of the WFD is the identification of the pressures that cause the deterioration of ecological status of water bodies and the capability to predict the ecosystem

evolution upon mitigating these pressures. Different modelling approaches, linked to monitoring strategies, have been recently proposed to support stakeholders, but these models are often not integrative and/or not predictive and extremely data intensive. In this study the model AQUATOX, an integrative ecological and ecotoxicological model developed by R. Park and J. Clough and released by EPA has been tested in two case study catchments in Luxembourg, Wark and Mamer, which are subject respectively to strong agricultural and urban pressure. The direct, indirect and synergistic effects of nutrients and micro-pollutants (pesticides and polycyclic aromatic hydrocarbons, PAHs) have been analysed to support river basin management and to predict the efficiency of Programs of Measures. The models are fed with data on food webs composition provided by public authorities and data on nutrients and organic loading and contaminants dynamics as well as sediment and periphyton budgets provided by 2-year field campaigns. The calibration has been performed fitting simulated and measured data on integrative measurements of river metabolism: gross primary productivity (GPP) and ecosystem respiration (ER). The model validation is based on diel oxygen profiles. Simplified food webs corresponding to lead macrobenthic species of the saprobic index classification and matching the corresponding water quality classes have been built within AQUATOX, calibrating nutrients and contaminants tolerance and species traits based on literature data. This approach allows to predict ecological status in water bodies as it is measured in WFD and to distinguish between nutrients/organic and toxicants effects as well as between direct and indirect effects. The model predictions suggest that effects of real exposure patterns of pesticides among the modelled trophic network strongly depend on indirect effects and boundary conditions, whereas real exposure patterns of PAHs mixtures shows significant direct toxic effects.

**549 Metallophytes: the quest for plants with 'mettle' and their exploitation in developing phytotechnologies** A. Baker, The University of Melbourne, Faculty of Science, School of Botany. Metallophytes – plants that have evolved on metal-enriched soils – have key 'values' that must drive research on their unique properties, and ultimately their conservation. The ability of metallophytes to tolerate extreme metal concentrations commends them as the optimal choice for phytostabilization and ecological restoration of mineral wastes and metal-contaminated soils. Metallophytes, and in particular metal-hyperaccumulating plants, have also spawned several novel phytotechnologies, including phytoremediation, phytoextraction and phytomining. Other new potentials for exploiting their unique properties are emerging. The last decade has seen an ever-increasing interest in metal-tolerant and metal-accumulating plants both from an academic standpoint and in developing their potentials in phytotechnologies. Few studies have highlighted the need to conserve these species. This presentation identifies future research needs for the conservation and utilization of the global metallophyte biodiversity.

**550 Dynamic speciation and phytoavailability of trace metals in contaminated soils: Why bother with rhizosphere chemistry?** M. Bravin, CIRAD. The conceptual, analytical and modelling advances achieved in the past few years on the chemical processes induced by root activities in the rhizosphere brought new insights on the biogeochemistry of trace elements in the soil-plant system and their consequences on the phytoavailability and phytotoxicity of trace elements. These advances should contribute substantially to drive the implementation of phytoremediation strategy in contaminated soils. However, in comparison with e.g. the engineering of plant internal physiology as related to (hyper)accumulation, tolerance or exclusion of trace elements by plants, the potential interest of managing rhizosphere chemistry is still poorly taken into account in the operational implementation of phytoremediation. This is partly explained by the fact that the investigations of rhizosphere chemistry are usually performed under controlled conditions and are slow to be connected with field-scale validation as it is a technical and time-consuming challenge to study rhizosphere processes in situ. Considering this issue, the present lecture firstly aimed at giving additional and updated insights on the

governing chemical processes (e.g. pH, binding capacity of dissolved organic matters) and the related dynamic speciation of trace elements in the rhizosphere. In a second part, a case study on a wine-making area in the southern France will be presented to illustrate the interest of an in-depth understanding of rhizosphere chemistry for an efficient and sustainable phytomanagement of these copper-contaminated former vineyard soils. Finally, this lecture should give further evidence of how powerful rhizosphere drivers are for the phytoremediation of contaminated soils.

**551 The fate of arsenic in soil-plant systems: a focus on As behaviour in native flora** E. Moreno, Universidad Autónoma de Madrid; E. Esteban, J. Peñalosa, Universidad Autónoma de Madrid / Department of Agricultural Chemistry. Arsenic is a trace element naturally found in soils, with pyritic mining are amongst the main contributing sources. Arsenic is frequently found as inorganic species and its availability in soils is low and driven by multiple soil properties. Most plant species exclude this metalloid but once inside root cells, As(V) is quickly reduced to As(III), and complexed in many plant species. Plants cope with As using detoxification mechanisms (complexation, compartmentalization, etc), but phytotoxicity symptoms appear once those mechanisms are saturated. Phytotoxic effects include growth inhibition, chlorophyll degradation, nutrient depletion or oxidative stress. Phosphorus nutrition will also influence As(V) uptake and toxicity in plants. Plant screening and phytoremediation experiments were carried out in As-polluted mine soils at the Centre and South of Spain. Strategies for how to apply our understanding of As in soil-plant systems will be discussed, especially as native plants and metallophytes are useful in the reclamation of As-polluted sites.

**552 Database about heavy metal rich habitats, their geology, ecology and biodiversity** L. Nebel, University of Vienna, Center for Earth Sciences; I. Lichtscheidl, Cell Imaging and Ultrastructure Research University of Vienna.

**553 Climate change driven plant-metal-microbe interactions** H. Freitas, University of Coimbra.

**555 Progress and Challenges for applying spICPMS to the detection and quantification of nano particles in biological samples** J.E. Ranville, Colorado School of Mines / Chemistry and Geochemistry.

**556 Application of Carbon-14 Labeled Nanotubes for Quantification in Complex Environmental Matrices** E.J. Petersen, National Institute of Standards Technology.

**557 Solubility of ZnO nanoparticles in various media from AGNES determinations of the free Zn concentration** J. Galceran, C. David, C. Rey-Castro, Universitat de Lleida / Department of Chemistry; E. Companys, Química; J. Salvador, J. Puy, Universitat de Lleida / Department of Chemistry. The toxicity of nanoparticles (NPs) on a given organism depends on a number of factors ranging from size and shape to exposure concentration. In the case of dissolving nanoparticles (such as ZnO or CuO), the delivery of metal ions to the medium has raised the debate on whether the toxicity is due to the nanosized characteristics or to a high concentration of released cations. In this context, the development of analytical techniques which can contribute information on the scale of the dissolution process is key for the correct elucidation of the relationship between chemical species in the solution and toxic effects to the organism. The most relevant chemical species, according to the Free Ion Activity Model and to the Biotic Ligand Model, is the free ion. So, the free metal ion concentration is a very relevant analytical target. The electroanalytical technique AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) has proved useful for the measurement of free metal ion concentrations in many systems: river water, seawater, humic acids, soil extracts, etc. In the field of nanomaterials, AGNES has been applied to follow the dissolution of Cd quantum dots and of ZnO nanoparticles. AGNES provides the free Zn concentration,  $[Zn^{2+}]$ , in solutions and dispersions

without the need of any extraction procedure. The specific application of AGNES to NP is straightforward, except in the case of large amount of capping agents which adsorb onto the electrode. AGNES not only provides thermodynamic information on NP dispersions, but also kinetic information on the dissolution processes. The solubility of ZnO is extremely dependent on pH, so pH along toxicity experiments should be carefully monitored or controlled. In order to facilitate the interpretation of nanoparticle effects on organisms, it is convenient to check that the exposure concentration is above the solubility threshold of the medium. As expected from thermodynamics,  $[Zn^{2+}]$  in equilibrium with NP in the dispersion does not depend on the concentration of dispersed NP or on the composition of the medium. This allows us to suggest a method to derive the solubility from AGNES measurements. This research has received funding from the EU (FP7/2007-2013) under grant agreement n° 229244 (ENNSATOX), from the Spanish Ministerio de Ciencia e Innovación (CTQ2009-07831 and CTM2009-14612), and from the Comissió Interdepartamental de Recerca i Innovació Tecnològica (2009SGR00465).

**558 NanoSilver in Urban Wastewater Systems: Transport, Analysis and Transformations** R. Kaegi, Eawag. Silver Nanoparticles (Ag-NP) are used as antimicrobial agents due to the well-known antimicrobial properties of Ag<sup>+</sup>. Currently, the major applications of Ag-NP are textiles and cosmetics, which eventually leads to a discharge of the Ag-NP to the municipal sewer system. In order to assess potential effects on the biology of the wastewater treatment plants (WWTP) and further on the downstream ecology after the WWTP, the physical (aggregation) and chemical (speciation) changes of the Ag-NP along their transport in the sewer system and in the WWTP have to be addressed. We thus performed lab-scale, pilot-scale and full-scale experiments in sewer systems and WWTP using different forms of Ag-NP (Ag(0) and AgCl) with different sizes (10nm, 100nm) and different coatings (citrate, PVP). Experimental samples were analyzed by a combination of bulk elemental (inductively coupled mass spectrometry, ICP-MS), particles specific (analytical electron microscopy, AEM) and element specific (X-ray absorption spectroscopy, XAS) methods. Results indicate a partial sulfidation of the Ag(0) and a full sulfidation of nanoscale AgCl particles along the transport in the sewer system. The sulfidation continues primarily during anaerobic stages of the WWTP with a strong concentration of the near completely sulfidized particles in the sludge.

**559 What are key processes that determine the environmental fate of engineered nanoparticles?** M. Scheringer, ETH Zuerich / Institute for Chemical and Bioengineering. Engineered nanoparticles (ENPs) undergo a wide range of processes in the environment, including homo- and heteroaggregation, loss of an initial surface coating, formation of a coating consisting of natural organic matter (NOM), dissolution, and others. Several of these processes have been investigated extensively in the last years, but which of the many potentially relevant processes actually determine the environmental fate of ENPs under given environmental conditions is not yet well understood. Multimedia environmental fate models are tools that can be used to address exactly this question. These models describe the mass flows of a chemical between different environmental compartments; they have been used in the environmental fate assessment of organic chemicals for several decades [1]. Application of multimedia fate models to ENPs is possible, but requires extensive modifications of the individual process descriptions in the models. A newly developed environmental fate model for ENPs in aquatic systems will be presented and results for titanium dioxide ENPs will be discussed [2]. A key finding is that heteroaggregation of the ENPs with suspended particulate matter is one of the most important processes and determines the mobility and water-sediment distribution of the ENPs. Implications for the characterization and environmental fate assessment of ENPs will be presented. **[1] MacLeod, M., Scheringer, M., McKone, T.E., Hungerbühler, K. (2010) The State of Multimedia Mass-Balance Modeling in Environmental Science and Decision-Making, *Environmental Science & Technology* 44, 8360-8364. [2] Praetorius, A.,**

Scheringer, M., Hungerbühler, K. (2012) Development of environmental fate models for engineered nanoparticles - a case study of TiO<sub>2</sub> nanoparticles in the Rhine River, *Environmental Science & Technology* 46, 6705-6713.

**560 Introduction** G. Morris; S. Reis, Centre for Ecology Hydrology.

**561 Ecosystem Services Approach and Valuation** T. Taylor, European Centre for Environment and Human Health, University of Exeter Medical School.

**562 The Ecological Public Health Approach and DPSEEA** L. Fleming, European Centre for Environment and Human Health, University of Exeter Medical School.

**563 Interactive Case study of complex environment and human health issue using eDPSEEA (I)** G. Morris; S. Beck, NHS Health Scotland.

**564 Predicting toxicity to fish based on in vitro data** J. Stadnicka-Michalak, EawagSwiss Federal Inst of Aquatic ScienceTechn; K. Schirmer, R. Ashauer, Eawag. Quantification of chemical toxicity is generally based on measurements of external exposure; however, in order to understand, interpret and extrapolate toxic effects, using internal concentrations of chemicals is more suitable. Also the quantification of the time course of internal concentrations (toxicokinetics) in cells and whole organisms facilitates a better understanding of toxicity and may improve *in vitro* to *in vivo* toxicity extrapolation. Finally, following the tissue-residue approach, one can derive the hypothesis that the concentration in a fish that causes toxicity must be similar to those concentrations that cause toxicity in a fish cell line. Therefore we aim to: (i) predict chemical concentrations in fish, (ii) measure and predict chemical concentrations in fish cells, (iii) link chemical concentrations in cells to the effect on cells, (iv) link the effect on cells to the effect on fish. We measured concentrations of eleven organic chemicals in exposure medium, fish cells (RTgill-W1) and plastic of the well plate for various time points. Based on the results, we created a toxicokinetic model to predict internal effect concentrations in cells which we compared with internal effect concentrations in fish gills predicted using a Physiology Based Toxicokinetic (PBTk) model. In addition, the cell proliferation under toxicant stress was investigated and compared with sub-lethal effect endpoints measured in rainbow trout. Our results show that in general, the difference between log LC50 and log EC50 is smaller than between log ILC50 and log IEC50; however, there was no visible relationship between the ratio of LC50 and EC50 values and log  $K_{ow}$  ( $R^2 < 0.2$ ,  $p = 0.17$ ), while the ratio of ILC50 and IEC50 values were correlated with log  $K_{ow}$  ( $R^2 > 0.7$ ,  $p = 0.0008$ ). Thus, it could be possible to predict effects on fish based on internal effect concentrations in cells. Fish cells were proliferating significantly slower ( $p < 0.0001$ ) under exposure to 1.5 mg/L Cyproconazole. That concentration in exposure medium corresponds to 86.4 µg/g of Cyproconazole in rainbow trout gill cells which was predicted for fish gills from 2.4 mg/L in water by using the PBTk model. This concentration reduces fish weight and length. Our study shows that modeling and experiments on fish cell lines can be used to obtain internal concentrations of various chemicals. These concentrations can be linked to concentrations in fish and to effects on cells and on fish.

**565 Primary gill cell cultures can be used for on-site environmental biomonitoring** M. Minghetti, Eawag / Environmental Toxicology; S. Schnell, C. Hogstrand, N.R. Bury, King's College London. The fish gill is an important multifunctional organ and is a primary target for toxicants dissolved in water. A primary Fish Gill Cell System (FIGCS) was developed to study the function of this organ at the cellular level. When cultured on inserts, FIGCS develops tight epithelia with formation of tight junctions, trans epithelial electrical resistance (TEER) values comparable to *in vivo* scenarios (over 20 k $\Omega$ ) and, most importantly, it is able to tolerate water on the apical surface. Moreover, we showed that

the response of this system to metals mimicked that of the whole organisms. An aim of the current study was to ascertain if this *in vitro* system could be taken from the laboratory to the field as a potential site-specific environmental monitoring tool. The river Hayle in Cornwall was chosen as a study area because it offers a gradient of polymetal contaminated natural waters. Three independent studies were conducted and water from five sites was taken. In the third field trip two sets of primary gill cell cultures were prepared. One set was transported to the site inside portable incubators and exposed directly to river water while the second set was exposed after 24 hours (h) to the same water in a cell incubator in the laboratory. In all other cases water was transported back to the laboratory and exposure were started 24 h after the sampling. Water chemistry was assessed and following 24 h of exposure TEER, cell viability and the expression of 11 genes was measured to assess their suitability as surrogate end-points for metal-specific toxicity. In all studies the expression of MTA and MTB correlated to the polymetal gradient and to the predicted metal toxicity (based on biotic ligand model for zinc and copper) of these waters indicating that these genes are useful indicators of metal contamination. In addition, primary gill cell could be taken from the laboratory to the field as a potential site-specific environmental monitoring tool as they can travel for 30 hours in a portable temperature controlled incubator and tolerate unfiltered river water. Moreover, this system reduces the numbers of fish used because it generates approximately 40 inserts from two fish. These results show that FIGCS has the potential to be used as an environmental monitoring tool and could form part of an investigative work package to identify reasons why water bodies fail the European Union Water Framework Directive's of good ecological status.

**566 Using passive dosing to assess the cytotoxicity and cytochrome induction potentials of polycyclic aromatic hydrocarbons (PAHs) on fish cell line** E.S. Emelogu, The Robert Gordon University Aberdeen / Engineering; S. Heger, Institute for Environmental Research RWTH / Department of Ecosystem Analysis; P. Pollard, The Robert Gordon University Aberdeen / Centre for Research in Energy and the Environment (CRE+E); C.D. Robinson, L. Webster, Marine Scotland Science; C. McKenzie, The Robert Gordon University Aberdeen; T. Seiler, H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V); C. Moffat, Marine Scotland Science. *In vitro* toxicity testing of hydrophobic organic contaminants (HOCs) is challenging due to their low aqueous solubility, compound losses through volatilisation, adsorption and absorption to plastic well plates and serum constituents. This leads to inaccurate and declining exposure concentrations and bias in the interpretation of toxicity data. Whilst, the use of co-solvents (e.g. methanol or dimethyl sulphoxide; DMSO) may increase the solubility of HOCs in cell culture medium, co-solvents may also interact with the test compounds and therefore interfere with the biological activity. For a realistic *in vitro* toxicity evaluation of HOCs, partitioning controlled delivery (passive dosing; PD) promises to overcome the highlighted challenges. PD involves the continual partitioning of test compounds e.g. HOCs, spiked into a biologically inert polymer that acts as a storage compartment and source. This eliminates test compound losses, provides known and stable amount of the freely dissolved concentrations of the test compounds and eradicate the need of dosing with co-solvents. The simplicity, cost effectiveness and sensitivity of using silicone rubber (SR) O-rings as the PD polymer during *in vitro* toxicity test of HOCs have been demonstrated. However, preparation of the SR O-rings including pre-extraction and loading with test compounds can still be improved and the dosing system can be extended to assays with adherent fish cell lines. In this study we assessed the feasibility of using SR O-rings for the toxicity testing of fluoranthene and chrysene with a fibroblast-like permanent fish cell line derived from rainbow trout liver (*Oncorhynchus Mykiss*; RTL-W1) in 24-well microtitre plates. The study is intended to set a foundation and provide insight to the assessment of environmental extracts in future studies.

**567 A 3D spheroidal fish liver model alternative to better understand bioaccumulation in ecotoxicology** M. Baron, Plymouth

University / School of Biomedical & Biological Sciences; K. Mintram, R. Cumming, AstraZeneca Safety, Health & Environment; W. Purcell, S. Jackson, Plymouth University / School of Biomedical & Biological Sciences; S. Owen, AstraZeneca; A. Jha, Plymouth University / School of Biomedical & Biological Sciences. The use of three-dimensional cell culture models (spheroids) are becoming more widely used in toxicological research, particularly in the fields of pharmacotoxicology, cancer and food research. This is due to their ability to simulate the *in vivo* environment more effectively than standard *in vitro* models, which lack the 3D microenvironment of intact tissue. We recently developed and characterised a method to produce primary hepatocyte spheroids from rainbow trout that provides long term cultures. This new model has the potential to pre-screen xenobiotic compounds and measure whether they can be metabolised by fish. The use of fish primary cells and cell lines are increasingly important in assessing the toxic action and bioaccumulation potential of xenobiotics. While these *in-vitro* systems do not fully replace the requirement for *in vivo* studies, they can be useful adjunct models with a higher throughput that can support the ecotoxicological evaluation of the potential risk of aquatic pollutants, offering an alternative to animal testing. Spheroid models offer additional functionality over conventional *in vitro* cultures, in particular defined *in-vivo* like tissue architecture and good cell-cell and cell-matrix interactions are longer-lived. As such they can be developed to offer a new *in vitro* screening tool for environmental toxicological monitoring. Here we report a study that examined the ability of fish liver spheroids to metabolise a range of 20 compounds; compounds were selected on the basis of their physicochemical properties, existing comparable datasets and known toxicity profile. Using a micro plate format we determined the rate at which the parent compounds were lost from the media over 24–48 h period, monitoring the appearance of some primary metabolites, toxicity and viability endpoints. Our results compare well with data from the literature, suggesting that this new model is worthy of further dedicated study for its application in accumulation studies.

**568 Adverse Outcome Pathways of Fish Reproduction: Integrating Cellular Assays of Trout Pituitary, Ovary and Liver Toxicity with Contaminant Exposures** L.R. Schultz, Battelle Northwest Laboratories / Marine Science Laboratory, Ecotoxicology Group; P. Swanson, NOAA Northwest Fisheries Science Center; G. Young, University of Washington / School of Aquatic and Fishery Sciences; J. Nagler, University of Idaho / Department of Biological Sciences. The integration of high throughput screening (HTS) assays with computational modeling of toxicant effects and dosimetry models of toxicant exposure, is central to the adverse outcome pathway (AOP) concept and future ecological risk assessments. However, two important challenges towards implementing an AOP have been identified: 1) The need to establish credible links between responses measured at the cell or tissue level and adverse outcomes traditionally measured at the whole-animal or population level. 2) The need to develop biologically based, quantitative extrapolation tools or models that allow us to apply cell- or tissue-level data to individuals, or individual-level data to entire populations. With regard to (1), cellular assay systems of the rainbow trout pituitary, liver, and ovary for use with HTS of environmental contaminants are well established. These assays can measure an essential reproductive endocrine function(s) of the corresponding organ. For example, pituitary cell assays measure follicle stimulating hormone (FSH) synthesis and secretion and permit derivation of the lowest observed effect and activation concentrations (LOEC, AC50) for a test contaminant. With regard to (2), recent development of computational models of the fish brain-pituitary-ovary-liver (BPOL) axis allows the results from *in vitro* assays to be used for estimating model parameters. In addition to the BPOL modeling is the need to determine environmental exposure levels that would correspond to target organ concentrations of the contaminant comparable to the various *in vitro* dose metrics (e.g. LOEC). This is sometimes referred to as reverse toxicokinetics. In this presentation, we will provide a description of the AOP process for fish reproduction with a focus on biological effects models for predicting reproductive outcomes, toxicokinetic models used to predict target organ dosimetry and applicability of freshly isolated vs.

cultured or cryopreserved cells in HTS assays.

**569 A quantitative and mechanism-specific analysis approach for the fish embryo toxicity test** V. Delov, Fraunhofer IME / Ecotoxicology; E. Muth Koehne, M. Fenske, C. Schaefer, Fraunhofer. To date, the application of the zebrafish embryo toxicity test (zFET) has focused on the assessment of acute toxicity, where only lethal morphological effects are accounted for. For applications beyond the acute toxicity, the test requires more refined and preferably quantifiable toxicological endpoints. Valuable tools in this context are fluorescent markers, which can either be expressed *in-vivo* or used for antibody-coupled labelling. The fluorescent signal facilitates the visualisation of specific cells or morphological structures and allows for quantitative measurements. Moreover, underlying toxicological mechanisms may be elucidated or the detection of adverse effects enhanced, what, in turn, increases the sensitivity and specificity of the conventional zFET. This project explores the benefits of fluorescent marker applications in the zFET for studying chemical toxicity and effects on the vascular, myotomal, and neuronal development. The transgenic line *Tg(fli1:EGFP)* expresses enhanced GFP in the entire vasculature and thus enables the visualisation of vascular defects in live zebrafish embryos. *Tg(gfap:GFP)*, expressing GFP coupled to the glial fibrillary acidic protein gene, promise the identification of potential neurotoxic chemicals. We assess the specific driven EGFP-expression qualitatively and quantitatively, and found an exposure concentration related increase in vascular respectively astrocyte damage for some exemplary chemicals. We additionally explored whole-mount immunofluorescence to visualise myotomal defects and performed an *in-vivo* staining for assessing chemical induced apoptosis. By the example of genistein exposure, we could demonstrate that the integration of different fluorescent signal based methods allowed a more specific identification of toxicity mechanisms and reduced the error rate of the non-lethal effect assessment. However, the fluorescent methods allow a sensitive and quantitative effect assessment, which will broaden the scope and minimize the data variability of the zFET.

**570 Thermodynamic bioaccumulation studies using PDMS-based passive equilibrium sampling in a Swedish lake** A. Jahnke, Stockholm University / Department of applied environmental science; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; M.S. McLachlan, Stockholm University; M. MacLeod, ITM Stockholm University / Department of applied environmental science. A variety of descriptors are in use to assess chemical enrichment in biota, including biota-sediment accumulation factors (BSAFs). A major drawback is oversimplification, e.g. normalization to idealized phases (i.e. lipids in fish, total organic carbon in sediment), and the neglect of other sorbing phases (e.g. proteins in biota, black carbon in sediment). An alternative thermodynamic approach, based on passive equilibrium sampling to sense the fugacity of a chemical in different media, has been proposed. This new approach utilizes a common reference phase, the silicone polydimethylsiloxane (PDMS), as a 'chemometer' for different environmental media (i.e., water, sediment and eel). The aims of this study were to i) explore these novel tools concurrently for the 'indicator' PCBs in the clean Swedish Lake Ången, and ii) to compare the obtained thermodynamically-based data to BSAFs. PDMS thin-films were used for eels, whereas PDMS-coated glass jars were used for sediment and water. The PDMS samplers were brought into contact with various media in separate experiments aiming for a thermodynamic equilibrium between media and PDMS, and the concentrations of the model PCBs in the PDMS were then determined and compared. Equilibrium sampling was achieved for sediment and eels, but not for water. For comparison, an exhaustive solvent extraction of eel and Soxhlet extraction of sediment were carried out. All extracts were analyzed by GC coupled to high-resolution MS. A comparison of the PDMS data for eel and sediment gave unexpected results: The levels in the PDMS equilibrated with the eels were a factor of 2.9–12.8 lower than in the PDMS equilibrated with the sediment, indicating a higher fugacity of PCBs in the sediment than in the eels from Lake Ången. In comparison, the calculated BSAFs on a lipid basis



ranged from 0.3 to 6.0. Our results using the BSAF approach to determine bioaccumulation in fish were thus consistent with the general expectation of BSAFs >1 (except for PCB 101 in one eel sample). However, the BSAF results indicating biomagnification of PCBs are inconsistent with the lower fugacity in eel than in sediment which we observed applying the passive equilibrium sampling approach. This surprising result challenges the conventional thinking about the thermodynamic controls on contaminants in aquatic food webs, and warrants further study. Additional experiments are underway to further investigate the apparent contradiction.

#### **571 Modelling the impact of the biofouling on DGT measurement in freshwater**

**E. Uher**, HBAN; M. Combe, Irstea; F. Mazeas, C. Bertrand, M. Tusseau Vuillemin, C. Compere, IFREMER; C. Gourlay-France, ANSES. Biofouling at the surface of passive samplers is a natural consequence when they are immersed in the water. Previous studies have highlighted that biofouling impacts the measurement by DGT, which is one of the most used passive sampler for metals and phosphate in the water. Nevertheless, little is known on how biofouling affects the transfer of the contaminant to the receiving phase of the passive sampler. Furthermore, previous studies took place in aquaculture ponds or in waste water. More data in freshwater are needed to establish a model of the impact of the biofouling on DGT measurement. To investigate this point, we deployed DGT in the Seine River, up stream of Paris, and we followed the accumulation of metals in the Chelex resin for 22 days. Biomass growth and metal bioaccumulation in the biofilm attached to the protective membrane of the DGT were also followed in parallel. The fluxes of metal were calculated for each DGT. Although the metal concentration in solution is relatively constant, the fluxes of metal decreased markedly throughout the deployment. Because metals are known to interact with biofilm through various processes of biosorption, precipitation, complexation... the decrease was attributed to the biofouling effect. A model is proposed to take into account the interactions of the metal with the biofilm during the course of the metals toward the resin. An expression of the effective flux  $J$  is proposed, which depends on the concentration of the metals in the biofilm, and which is linked to the kinetic constants of the chemical reactions between metals and biofilm, to the depuration process and the growth dilution, and to the transport toward the resin because of the diffusion gradient due to the Chelex resin. This model has been successfully applied to the data and allows the determination of characteristic times of the biofouling effect on the DGT measurement. Regarding the effect of the biofouling on the DGT measurement, all metals were impacted by the biofouling. Nevertheless, as it was already observed previously, the impact depends on the metal. Kinetic aspects may explain the difference observed between the metals.

#### **572 A comparison of lipid based concentrations of PCBs and PAHs obtained through passive sampling with lipid normalized concentrations in biota**

**F. Smedes**, DELTARES / RECETOX; T. Rusina, Masaryk University / RECETOX; P. Mayer, Technical University of Denmark / Department of Environmental Engineering. Passive samplers are widely used for measuring freely dissolved concentrations in the aqueous environment. Single phase samplers like silicone rubber can also be exposed to fish tissue. The equilibrium partitioning theory suggests that when a fish is in equilibrium with the surrounding water a passive sampler equilibrated with that water or with that fish tissue should show an equal concentration. Therefore transferring results from passive samplers to lipid based concentrations using a sampler-lipid partition coefficient should be a good predictor for lipid normalised concentrations in biota although such prediction does not include factors that caused biota to deviate from equilibrium. For this purpose sampler-lipid partition coefficients were measured, which were combined with sampler

Sciences, Environmental Technology; F. Smedes, DELTARES / RECETOX; P. Mayer, Technical University of Denmark / Department of Environmental Engineering. Silicone polymers and particularly polydimethylsiloxane (PDMS) are widely applied for passive sampling and passive dosing of hydrophobic organic chemicals (HOCs). In this study, we compared the partitioning properties of 13 silicone polymers and LDPE by determining polymer:polymer partition ratios of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and a selection of organo-chlorine pesticides (OCPs). Partitioning properties of all tested silicones varied up to factor of two, which is rather small compared to differences between polymer types (e.g. PDMS vs. LDPE) but still high enough to justify the need of calibration factors. For PCBs, polymer:polymer partition ratios showed in most cases a constant offset (0.0- 0.2 log-units) from identical partitioning behaviour with respect to a reference PDMS material among all congeners, whereas for PAHs some polymers exhibited higher affinities for the more hydrophobic substances, in which case polymer:polymer partition ratios could be described as a linear function of the log  $K_{ow}$ . Through a reference polymer with known polymer:water or polymer:lipid partitioning properties, polymer:polymer partition ratios facilitate a calibration of new polymeric materials and allow to derive polymer-specific polymer:water and polymer:lipid partition ratios.

#### **574 Passive dosing of hydrophobic organic mixtures - controlling exposure levels and compositions**

**S.N. Norgaard Schmidt**, Aarhus University / Environmental Chemistry and Microbiology; K.E. Smith, M. Holmstrup, P. Mayer, Aarhus University. Although mainly applied in experiments with individual hydrophobic organic contaminants (HOCs), passive dosing also offers possibilities for tightly controlled HOC mixtures exposure since the partitioning from silicone is characterized by a concentration independent partition coefficient and the absence of competition effects. This presentation gives an overview and update on passive dosing of HOC mixtures based on case studies with polycyclic aromatic hydrocarbons (PAHs). A wide range of passive dosing formats have been developed, including cast silicone at the bottom of test vessels and silicone O-rings, and the loading of these can be done in three different ways: (1) loading by equilibrium partitioning, (2) loading to saturation, and (3) loading by pushing. During a passive dosing experiment, the exposure of each mixture constituent is controlled independently of each other, by continuous equilibrium partitioning between a dominant and biologically inert silicone polymer and the exposure medium (e.g., water). In this way, defined mixture levels and compositions are controlled either at or below the saturation level during the experiment. Also, it is possible to control the same mixture composition within a dilution series. The exposure can be validated by equilibrating the silicone with pure water after ending the experiment and then measure the concentration of each mixture constituent. Exposure levels and mixture compositions can then be compared to estimated values. Results from a mixture toxicity experiment with 12 mixtures showed precisely controlled exposure levels between replicates (RSDs of < 2% for each PAH) and between treatments (RSDs < 3% for each PAH). This demonstrates that the exposure control of one PAH was unaffected by the presence of other PAHs, which is a prerequisite for the independent control of mixture levels and compositions. Also, the exposure in a dilution series was well produced and controlled by passive dosing. The effects of the 12 mixtures (after 7 days exposure) were plotted against sum chemical activities (?a), sum equilibrium lipid concentrations (?C<sub>lipid</sub>), and sum toxic units (?TU), respectively. In each case *one* exposure-response curve was fitted to *all* the data. Another mixture toxicity study with four mixtures demonstrated accurately controlled mixture compositions which were kept constant during a 7-day experiment. However, a moderate decrease for anthracene during the test was observed.

**575 Two "new" organophosphorus flame retardants detected in the indoor and outdoor environment** **S. Brandsma**, IVM institute for environmental studies / Faculteit der aard- en levenswetenschappen; C. de Wit, Stockholm University; U. Sellstrom, Department of Appleid

Scienc (ITM), Stockholm University / Department of Appleid Scienc (ITM); C. Pappa, P. Martinez Moral, Institute of environmental studies (IVM), VU university; P. Leonards, VU University Institute for Environmental Studies / Chemistry & Biology. The European Commission-funded project ENFIRO investigated halogen-free substitution options for some BFRs resulting in a comprehensive dataset on viability of production and application, environmental safety, risk assessment, and life cycle assessment. In total 15 halogen-free flame retardants (HFFRs), consisting of metal-, organic-, and nano-based FRs, as alternatives for decaBDE, TBBP-A, and brominated polystyrenes were selected. One of tasks was to investigate the exposure, fate and modeling of the HFFRs. A multi-media model was used to identify key matrices for environmental and human exposure Field monitoring of organic HFFRs was conducted in several European countries to assess the environmental levels arising from known sources (e.g. a wastewater treatment plant) as well as background contamination in a region. To assess indoor contamination, sampling was performed in microenvironments where products containing FRs are used (e.g. homes, offices). The focus of this study was on the analysis of the alternative flame retardants resorcinol bis (diphenylphosphate) (RDP) and bis phenol A bis (diphenylphosphate) (BDP) in dust, sediment and sewage sludge, including the analytical method development. RDP and BDP were detected in various dust samples from Sweden, The Netherlands and Greece. The RDP levels in Swedish dust range from 5-360 ng/g and as high as 83000 ng/g for BDP. BDP was the most predominant FR in the dust samples. RDP and BDP levels observed in the dust samples from Greece are similar to the levels found in Sweden. In the Dust samples from the Netherland the highest RDP and BDP levels were found. The dust was collected directly from the electronic equipment and from the floor and tables around the equipment. The highest concentrations were found in the dust collected on the electronic equipment with BDP levels up to 700 000 ng/g. The levels of RDP were 10-200 times lower than the BDP levels. Sediment samples from The Netherlands, Germany, Belgium, France and Norway were also analyzed for the presence of RDP and BDP. RDP and BDP were present in all sediment samples. The concentrations for RDP in sediment ranged from 0.21-2.21 ng/g and from 0.06-1.42 ng/g for BDP. In sewage sludge samples from the Netherlands the concentrations range from 0.7-3.4 ng/g for RDP and from 1.6-10.3 ng/g for BDP. To our knowledge this is the first time that RDP and BDP have been detected in the outdoor environment.

**576 Partition coefficients of flame retardants: Applications of polyparameter linear free energy relationships** A. Stenzel, Analytical Environmental Chemistry; K. Goss, Eawag / AUC; S. Endo, Helmholtz Centre for Environmental Research UFZ / Center for Applied Geoscience (ZAG). The ability to describe the partitioning properties of chemicals is essential to understand their environmental behavior and to assess potential risks, e.g., the bioaccumulation in organisms. Many current-use and alternative flame retardants are highly hydrophobic chemicals. The available experimental partitioning data for environmental phases are severely limited because the experimental determination can be challenging and time consuming. For these substances it may be useful to estimate equilibrium partition coefficients using a poly-parameter linear free energy relationship (pp-LFER), which has been repeatedly shown to provide accurate results. The advantage of the pp-LFERs is that the partition coefficients of various systems, including environmentally relevant ones, can be predicted easily once the pp-LFER descriptors have been determined. However, there are virtually no pp-LFER descriptors available for flame retardants. Hence, we experimentally determined the descriptors of 40 halogenated and non-halogenated flame retardants as well as degradation products thereof. The analyzed substances include alternative flame retardants such as allyl-2,4,6-tribromophenylether, tetrachlorobisphenol A, hexabromobenzene, 2,4,6-tribromophenol, triphenylphosphate as well as established flame retardants such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane. The pp-LFER descriptors were determined using GC-retention times, organic liquid/organic liquid and PDMS/water partition coefficients, which can be measured for

hydrophobic chemicals relatively easily compared to environmental partition coefficients. The total number of systems used for descriptor calculation exceeds the number of determined descriptors. This offers the possibility to evaluate the consistency of experimental partition coefficients used for descriptor determination. The determined pp-LFER descriptors have been evaluated through the prediction of experimental partition coefficients from the literature. The evaluation systems include octanol/water ( $K_{ow}$ ), air/water ( $K_{aw}$ ), organic carbon/water ( $K_{oc}$ ) and phospholipid membrane/water ( $K_{pm}$ ), whose partitioning data were predicted reasonably well. Moreover, we found that the H-bond acceptor properties of certain compounds (PBDEs, some phosphates) are depending on the solvent. This may be explained by steric hindrance of the H-bond acceptor sites. It is the first time that this feature is described for flame retardants.

**577 The bioaccumulation, environmental persistence, and degradation of several novel brominated flame retardants in aquatic mesocosms** B.P. de Jourdan, Department of Environmental Biology / School of Environmental Sciences; M.L. Hanson, University of Manitoba / Department of Environment and Geography; K.R. Solomon, University of Guelph / School of Environmental Sciences; D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division. Information on the environmental fate, persistence, and bioaccumulation potential of novel brominated flame retardants (NBFRs) in the environment is limited. While recent detection of some of these compounds in sediment cores and air samples provides insight into their persistence and potential for transport, there have been only a limited number of laboratory studies that have examined the fate and behavior of these compounds. Field based fate studies have been especially lacking. We conducted outdoor aquatic mesocosm experiments to assess the behavior of three NBFRs, bis(tribromophenoxy)ethane (BTBPE), tetrabromobisphenol A bis(2,3-dibromopropyl ether) (TBBPA-DBPE), and Firemaster® BZ-54 (a commercial mixture containing bis(2-ethylhexyl)tetrabromophthalate (BEHTBP) and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTeBB), in a ratio of 1:4). Analysis of biota (caged fathead minnows), filtered water samples, and sediments was performed by GC-ECNI-MS. BTBPE and TBBPA-DBPE were accumulated in the fathead minnows and did not show a significant decrease in concentration over the course of 70 d, despite a 28 d depuration period. The fathead minnows accumulated EHTeBB and BEHTBP to a lesser extent, and as with the BTBPE and TBBPA-DBPE treatments, there was indication of biotransformation products. The NBFRs partitioned into the particulate phase in the water column and into surface sediments. BTBPE, TBBPA-DBPE, and BEHTBP were found to be environmentally persistent in the mesocosms. The dissipation times differed in each compartment, with more rapid dissipation in the particulate as compared to the sediment compartment for each compound, where 50% dissipation times were greater than 100 days. The degradation products were more pronounced in the particulate compartment and corresponded to known photodegradation products. Several degradation products were identified based on full scan mass spectra. The ratio of EHTeBB to BEHTBP differed in the mesocosm compartments compared to the technical product used for treatment, indicating increased degradation of EHTeBB relative to BEHTBP. To our knowledge this is the first field-derived persistence data available for these NBFRs. While extrapolative estimates based on either singular lab or field studies are inadequate as a basis for risk assessment, the results of this study indicate these NBFRs are environmentally persistent.

**578 Wet deposition of Brominated Flame Retardants to the Great Lakes Basin** M. Robson, Department of Geography and program in planning; L. Melymuk, Research Centre for Toxic Compounds in the Environment RECETOX / RECETOX; L. Bradley, B. Treen, S. Backus, Environment Canada. The aim of this study was to examine the temporal and geographic trends in wet deposition of six brominated flame retardants: polybrominated diphenyl ethers (PBDEs), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenylethane (DBDPE), hexabromocyclododecane (HBCD) and

pentabromoethylbenzene (PBEB), in the Canadian Great Lakes region between 2004 and 2010. Concentrations of  $\Sigma$ BDEs ranged up to 100 ng/L, with an average of 2.0 ng/L. Concentrations of BTBPE ranged up to 22 ng/L, with an average of 0.16 ng/L; DBDPE up to 32 ng/L, with an average of 1.4 ng/L; HBCD up to 60, with an average of 1.9 ng/L; and PBEB up to 0.12 ng/L, with an average of 0.002 ng/L. Overall BDE-209 was the most prevalent flame retardant detected. Analysis of temporal trends revealed that there was a significant decline in BDE-209 at concentrations four of the sites. The data also revealed many sporadic high concentrations of in-use BFRs, and loadings calculations revealed that these episodic short-term high concentration events were largely responsible for driving the loadings of these chemicals to the Lakes. For instance, one month in 2007 was responsible for approximately 37% of the total HBCD loadings to Lake Ontario for the entire six-year study period. These results question current paradigms of how we believe such pollutants enter the environment. The results of this study highlight the need for more detailed spatial and temporal sampling to fully understand the deposition of these pollutants.

### 579 Bioaccumulation and biomagnification of “classical” and alternative flame retardants in dolphins from Southern Mediterranean Sea

**E. Barón**<sup>R. de Stephanis, J. Gimenez, Estación Biológica de Doñana (CSIC) / Department of Conservation Biology; E. Eljarrat, D. Barcelo, Institute of Environmental Assessment and Water Research Studies (IDAEA).</sup> Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants for many years and their concentration levels have been reported in different environmental and biotic matrices. The production and use of commercial PBDE mixtures are banned in Europe. Thus, alternative BFRs are being developed, such as pentabromoethylbenzene (PBEB), hexabromobenzene (hexaBBz) and decabromodiphenylethane (DBDPE). Moreover, Dechlorane 602 (Dec 602), 603 (Dec 603), 604 (Dec 604) and *syn*- and *anti*-Dechlorane Plus (*syn*-DP and *anti*-DP) are halogenated flame retardants introduced as substitutes of Mirex. Presence of these emerging pollutants has been observed in sediments and biota matrices, showing their bioaccumulation potential. Methoxylated PBDEs (MeO-PBDEs) are found in the marine environment. In contrast to PBDEs, MeO-PBDEs come from natural sources. The purpose of this study was to determine the occurrence of PBDEs, MeO-PBDEs, emerging BFRs and dechloranes in two species of dolphins (*D. Delphis* and *T. Trucatus*) from southern Mediterranean Sea. Moreover, the bioconcentration and biomagnification processes were also studied. Six different PBDE congeners, 3 different MeO-PBDE congeners and three halogenated norbornenes were detected. None of the non-BDE FRs were detected. BDE-47 and 6-MeO-BDE-47 were the most abundant compounds. PBDE levels were higher for individuals from the Strait of Gibraltar, while MeO-PBDE levels were higher in the individuals from the Gulf of Cadiz. Different halogenated norbornenes were detected for the first time in dolphin bubbler, but these levels were lower than those found for the “classical” FR Mirex and PBDEs.  $F$  value was studied to determine differences in the behaviour of both DP isomers.  $F$  value was lower than the reported value from commercial mixture (0.7) and was also different between species, with higher levels for *T. Trucatus*. Stable nitrogen and carbon isotope analysis were done, showing that the two species were in different isotope niches. Differences of concentration levels of the contaminants between the species were more influenced by the trophic position ( $\delta^{15}\text{N}$ ) than by the diet ( $\delta^{13}\text{C}$ ). Moreover, the stable nitrogen relationship with concentration level of PBDEs and MeO-PBDEs showed biomagnification capacity. Similar information was not available for dechloranes. In our study, Dec 603 showed a similar behaviour than PBDEs, whereas the biomagnification potential of DP is not clear.

### 580 Flame Retardant (FR) and Phthalate Spatial Variability

**Indoors** **E. Goosey**, University of Toronto; S. Chaudhuri, University of Toronto / Department of Chemical Engineering and Applied Chemistry; M.L. Diamond, University of Toronto / Department of Earth Sciences; A. Saini, University of Toronto / Department of Physical and Environmental Sciences. **Flame Retardant (FR) and Phthalate**

**Spatial Variability Indoors** Emma Goosey<sup>1</sup>, Sri Chaudhuri<sup>1</sup>, Miriam Diamond<sup>1</sup>, Amandeep Saini<sup>1</sup> <sup>1</sup>Diamond Environmental Group, 45 St George Street, University of Toronto, Ontario, M5S 2E5, Canada In the last decade PBDEs have been the focus of numerous environmental and health related studies because of their persistence, bioaccumulation and toxicity (PBT). Stringent flammability codes, coupled with recent voluntary and legislative bans (in N.America and Europe, respectively) have resulted in a broad range of FR compounds available to manufacturers. Plasticizers are also common within the indoor environment, due to their usage in myriad products. The volatility of these compounds allows them to off-gas from products. Although they are readily degraded outdoors, they can be persistent indoors and appear to be pseudo-persistent outdoors because of the sheer number of sources and continuous off-gasing. Here we assess the presence of 12 alternate FRs, 13 PBDEs and 6 common phthalates in Canadian homes, and identify variability between dust, indoor air and window film concentrations, as well as variability between homes, rooms and within-rooms and difference with outdoor air concentrations. Additionally dermal wipes were taken from residents’ hands and foreheads. Phthalate concentrations in air and dust were on par with concentrations measured in homes from USA and Europe. Dermal wipes contained traces of both PBDEs and NFRs. BDE 47 and BDE 99 were most abundant at 9 – 500 pg cm<sup>-2</sup> and 8 – 300 pg cm<sup>-2</sup> respectively, and TDCPP and PBEB concentrations ranged between 10 – 200 pg cm<sup>-2</sup> and 8 – 100 pg cm<sup>-2</sup> respectively. PBDEs and alternative FRs concentrations vary between rooms within homes. Their presence is much greater in living rooms and kitchens which correlates well with the number of electronic products in each of those rooms, compared to the bedrooms. The voluntary ban of deca-BDE use still has not been implemented in Canada, but the concentrations in homes appears to be slowly diminishing, whilst the alternative FRs are abundant in all samples. This suggests that a major proportion of manufacturers and industry have already begun using alternatives to BDE-209. However we expect PBDEs to be present indoors and outdoors for decades based on their large inventory in products still in use or “stored” in people’s homes.

### 581 Income inequality and infant mortality: An attempt to identify a new pathway to assess the social impact in LCA

**I. Bocoum**, IRSTEA Montpellier / UMR ITAP - ELSA; **C. Macombe**, Irstea; P. Feschet, CIRAD Montpellier; F. Benhmad, University of Montpellier 1. Our social LCA work encompasses the setting of impact pathways. We especially try to anticipate effects of changes in economic activities from life cycles in terms of public health. During the four past decades, there has been a lot of empirical works that have studied the role of various macroeconomic conditions on public health, e.g. wealth, wealth inequality, economic instabilities (wealth, exports of goods and services, agricultural productions, commodities prices) and their interactions. Whilst the correlations between different macroeconomic states and the multiple aspects of public health have been clearly demonstrated, there has been very little work that studied the existence of any causality. Using most recent dynamic panel data from 32 countries with 5 to 15 periods, we tried to bring out the evidence that a variation of income inequality may cause a variation of under one year infant mortality. The tests rely on dynamic econometrical models. A careful study of the data quality has led us to completing an important recovery work in order to minimize the estimations bias. To homogenize the dataset, we have used only Gini series obtained through disposable income. The simple OLS regressions showed a significant and strong positive relation between income inequality and infant mortality. This relation remains strong even after controlling for the level of GDP per capita. The country fixed effect models and the models that use GMM estimator without time lags, have not revealed any significant effect of a variation of income inequality on infant mortality. However, we found a significant and strong positive effect of the income inequality variable lagged by 7 periods (corresponding to 14 years). This time lag is very close to what Blakely *et al.* (2000) for instance, have suggested. The analyses we have done so far tend to show that a variation of income inequality may cause a variation of infant mortality, but the effect is not contemporaneous. In the light of these results it seems possible to build an income inequality

pathway to assess social impact of life cycles. However, the results we have obtained so far may be biased due to the narrow dataset used. The on going work aims at increasing its size (number of countries and length of time series) in order to get more robust results and to test and compare many time lags. **Keywords:** health, income inequality, social impact, life cycle.

**582 Towards the pathway "stress at work"** C. GASNIER, C. Macombe, s. grimbuhler, Irstea. In 2006, Bo Weidema issued a seminal paper suggesting several "impact pathways" in Social Life Cycle Assessment. Each one was liable to deliver quantitative estimations about the impacts of changes in the life cycles. One pathway was inspired by the work of Johannes Siegrist, who have measured the consequences of "stress at work" upon workers health, in many different situations. The work summarized here aims at producing a tool for decision makers, allowing them to compare and anticipate work's human cost caused by psychosocial risks of different organisation scenarios. Our starting hypothesis is that there are risk factors inherent in working conditions that are linked to working organizations. Those risk factors have likelihood to bring about - to short or long term - negative outcomes on human wellbeing (from absenteeism to death) of workers, whatever their personal characteristics may be (even though some risk factors are more dangerous for some sensitive groups). Some relationships between risk factors and health impairments are already documented, such as effort / reward imbalance (Siegrist) predicting mental distress and cardiovascular diseases. A French research group (CECILE, Inserm) also recently showed that night work increases the risk of breast cancer for women by 30%. We have reviewed scientific publications which establish qualitative and quantitative relationships between psychosocial risks and workers' health. We qualified each relationship for generalisation, that is to say we checked that the personal variables were controlled in each study, if wide range of workers were observed, if the places and sectors of original cases were clearly identified etc. To apply these relationships in another context than the one in which they were elaborated (original case) a method is needed to enable us to appreciate the proximity of a new case with the original case. We will propose a method to appreciate the proximity between the original case and the new case. We will eventually illustrate how the pathway "stress at work" can be implemented to a concrete situation, in order to anticipate human cost of work in terms of health impairments linked to psychosocial risk factors. Session :Social LCA (Arne Wangel) **Keywords :** SLCA- stress at work- prevision- impact assessment **Presentation preference :** platform presentation

**583 A new indicator framework for Life Cycle Sustainability Assessment considering safeguard subjects** R. Scheumann, TU Berlin / Chair of Sustainable Engineering; K. Wolf, Y. Chang, S. Neugebauer, Technische Universität Berlin; M. Finkbeiner, DaimlerChrysler AG. Life Cycle Sustainability Assessment has been defined as a combined approach of three assessment methods LCA, LCC and SLCA with a common goal & scope definition and a same set-up of boundary conditions (Klöpffer 2008; Finkbeiner et al. 2010). One other option to address sustainability is to integrate social and economic aspects into the LCA tool as proposed by Weidema (2006) to represent a strong and widely accepted sustainability assessment tool. With a look at the safeguard subjects (=area of protection, AoP) it is possible to identify the needed indicator, either as a top-down approach from the assessment frameworks (e.g. (Ness et al. 2007)) or as bottom-up from case studies. This work focuses on the development of a set of needed indicators from a top-down perspective. The commonly used AoPs in environmental LCA: human health, natural resources, natural environment, and man-made environment (Haes et al. 1999) are complemented with two new AoP to address to the social and economic dimensions of sustainability: **social integrity** and **economic stability**. Especially the AoP social integrity can cope very well with the aspect of intergenerational equity as one of the fundamentals in sustainable development, defined by the Brundtland report (1987). The following indicators are suitable to specify the new safeguard subject social integrity: equal opportunity, participation, educational value, child

labour, corruption, life and longevity, safety and security and mobility. For the safeguard subject economic stability, the following indicators can be used: ratio of GDP to national debt, innovation potential, economic prosperity and resilience as well as replacement cost of social function. In contrast to human well-being, the common discussed safeguard subject in sLCA (which is very broad and not very feasible), these new AoPs could even be used within LCA. The unit for effects on social integrity is SALY (social adjusted life years) in order to compare the results from damage to human health aspects measured in DALY (disabled adjusted life years). The unit for economic stability is any kind of currency, usually US\$ or €, per capita. The proposed framework makes it possible to measure sustainability within LCA. As work continuous, it is necessary to pay more attention on models behind the impact pathways. It is important to specify the relative reference points of the cause-effect modelling of social behaviour.

**584 Economic-Ecological for a Wood Based Product – an Empirical Study** R. Rieckhof, E. Guenther, TU Dresden. Life cycle thinking is still no mainstream in managerial decision-making. For this reason, we combine Life cycle Assessment with Material Flow Cost Accounting in order to support a more resource efficient decision-making by identifying economic-environmental optimization potentials. The focus of life cycle assessment is on environmental impacts rather than economic considerations. Hence, it is not self-evident that managers include the results in their decision-making. Material flow cost accounting considers economic efficiency with the unintended potential side-effect of ecological optimization. By combining both methodologies, it is possible to identify economic and ecological optimization potentials and support more resource efficient decisions. The present study includes the results of a combined Life Cycle Assessment and Material Flow Cost Accounting for the production of a wood-based product. Imminent research will also look at upstream and downstream parts of the life cycle. The functional unit is the amount necessary to cover 1 m<sup>2</sup> decorative surface. The case study represents an European production facility and firm data of 2010. Over several production steps the roundwood is processed to the wood-based product. Thereby, the by-products wood chips, industrial waste wood and bark are manufactured as well. The wood chips are further used to produce the heat required to cook and dry the wood product, while the waste wood and bark are sold. The results indicate that the material loss is the main contributor to the global warming potential. The material flow cost results show that the costs are split almost equally between the product and its by-products. While life cycle assessment can visualize environmental impacts, material flow cost accounting can demonstrate internalized environmental costs resulting from inefficiencies and reprocessing. Thus, a combination of both results focuses on the advantages of both methodologies to optimize the use of natural resources and environmental impacts. A social dimension could be added to the assessment in analogy to the environmental flows in order to perform an overall sustainability assessment, for instance by measuring impacts on health and safety. Future research should address multi-dimensional decision-making, allocation and internal pricing options which strongly influence the results of the study.

**585 LCSA application to evaluate the environmental innovations** K. Szita Toth, University of Miskolc. The LCA, especially the LCSA might an econometric model to analyse the environmental and economic benefit and risk of the environmental innovations. Recently lot of R+D aims to solve the environmental issues with developing a new technology or product from byproduct or waste, or tries to supplement non renewable resources with biodegradable materials, or alternative resources. If we combine and integrate the different evaluating methods (example market value estimating, cost-benefit analysis, LCA or IO-LCA) could get better picture a better picture of this real state of this innovative process. The overview of the environmental performance is especially important in discussion process with the stakeholders or policy makers. This paper focuses same "green development program" in Hungary and uses the LCSA an integrated method to evaluate the economic and environmental impact of these developments.

**586 Mixture toxicity of copper and zinc to barley root growth at low level effects can be described by the Biotic Ligand Model** L. Versieren, Department of Earth and Environmental Sciences; E. Smets, KU Leuven; K. De Schampelaere, Ghent University UGent / Environmental Toxicology and Aquatic Ecology; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology; E.E. Smolders, Katholieke Universiteit Leuven. It has been shown that the toxicity of metals is well described by the biotic ligand model (BLM). The BLM is a mechanistic bioavailability model based on the concept that toxicity depends on the amount of metal bound to a specific biological binding site, the so called biotic ligand (BL). Equal toxicity occurs when the same amount of metal is bound to the ligand. The model can take metal speciation and protective effects of competing cations into account. Here, we tried to use the BLM to interpret and explain mixture interactions between metals (Cu and Zn), and the effects of competing cations (Ca) on mixture interactions. A barley root elongation test (4 days) was performed in nutrient solutions at two contrasting Ca concentrations (0.4–11 mM). The solutions were buffered by a solid resin (Dowex®) to ensure constant free metal ion activities, unaffected by root exudates. At low Ca, the  $\text{Cu}^{2+}$  EC50 significantly ( $p < 0.05$ ) increased from 0.61  $\mu\text{M}$  to 1.14  $\mu\text{M}$  when solution Zn increased from background to its Zn EC20 level. Conversely,  $\text{Zn}^{2+}$  EC50 also increased from 252  $\mu\text{M}$  to 344  $\mu\text{M}$  with increasing Cu between background and its Cu EC20. Along the same lines, Cu EC10 also increased from 0.25  $\mu\text{M}$  to 0.46  $\mu\text{M}$  with Zn increasing between background and its Zn at EC20 level. Conversely, Zn EC10 was unaffected by Cu. At high Ca, the antagonistic interactions between Cu and Zn became insignificant at EC10 or EC50 values. The BLM was able to explain the interactions, i.e. the significant ( $\neq 0.05$ ) antagonistic (less than additive) interactions at EC50 level at low Ca with vanishingly low interactions at high Ca. This study showed that a part of the mixture interactions between metals on barley root elongation can be explained by competition effects. In the future, BLM's can be extended with factors that account for competition between metals, and may be used to predict and interpret mixture effects.

**587 Modelling toxicity of binary metal mixtures ( $\text{Cu}^{2+}$ – $\text{Ag}^{+}$ ,  $\text{Cu}^{2+}$ – $\text{Zn}^{2+}$ ) to lettuce, *Lactuca sativa*, using the Biotic Ligand Model** Y.T. Le, Radboud University Nijmegen / Environmental Science; M.G. Vijver, CML Leiden University; J.A. Hendriks, Radboud University Nijmegen / Department of Environmental Sciences; W.J. Peijnenburg, RIVM / Laboratory for Ecological Risk Assessment. Interactions between different metals affect the toxicity of their mixtures. In addition, metal toxicity is determined by the interactions between metals and the biological surface. However, these interactions are usually excluded or not properly addressed in the current approaches for metal assessment. By including competition between different ions for binding sites at the biotic ligands, the Biotic Ligand Model (BLM) has potential for assessing toxicity of metal mixtures, taking into account both interactions among metals and interactions between metals and organisms. The BLM was combined with the Toxic Equivalency Factor approach in estimating toxicity of  $\text{Cu}^{2+}$ – $\text{Ag}^{+}$  and  $\text{Cu}^{2+}$ – $\text{Zn}^{2+}$  to lettuce, *Lactuca sativa*. Particularly, the fraction of the biotic ligands occupied by metal ions was used as the basic unit for calculating the toxic equivalency factor of metals in mixtures as well as the toxic equivalency quotient of metal mixtures. As such, interactions among metals at the biological surface and interactions between metals and organisms at the water-organism interface are included in estimating mixture toxicity. The BLM parameters were found to be better indicators of toxicity of single metals, providing a mechanistic understanding of metal binding and toxicity. More importantly, the BLM principles might be applicable to metal mixtures. Around 64–84% of the variability in the toxicity of mixtures of  $\text{Cu}^{2+}$ – $\text{Ag}^{+}$  and  $\text{Cu}^{2+}$ – $\text{Zn}^{2+}$  was explained by the model. The pattern of metal mixtures depended not only on the toxic equivalency quotient of the mixture, but also on the specific composition of the mixture.

**588 Toxicity of metal mixtures to *Daphnia magna*: Competitive**

**binding to dissolved organic matter and biotic ligand can explain non-additive interactions** J.S. Meyer, ARCADIS; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; R.C. Santore, HydroQual Inc; A.C. Ryan, HDR HydroQual; J.W. Gorsuch, Copper Development Association Inc; W. Adams, Rio Tinto; E.R. Garman, NiPERA / Ecotoxicologist; C.E. Schlekat, NiPERA; R. Dwyer, International Copper Association / Health, Environment and Sustainable Development Program; K. Delbeke, European Copper Institute. In real-world aquatic systems, organisms are usually exposed to metal mixtures instead of individual metals. To provide a coherent dataset for modeling the toxicity of metal mixtures, we exposed *Daphnia magna* to Cd–Cu, Cd–Zn, Cu–Ni, Cu–Zn, and Cd–Cu–Zn mixtures in moderately hard reconstituted water containing dissolved organic matter (DOM; added as Suwannee River fulvic acid) at 3 mg DOC/L. We then compared observed 48-h mortality to the null assumption of additive mortality, as predicted from results of concurrent Cd-only, Cu-only, Ni-only, and Zn-only toxicity tests. This research has revealed several metal-metal interactions that, superficially, suggest metals interact in non-additive ways. Simple geochemical speciation can explain these interactions, leading to alternative conclusions. For example, the toxicity of Cu–Ni and Cu–Zn mixtures always appeared to be more-than-additive or additive when based on dissolved metal concentrations, whether Cu was varied while Ni or Zn was held constant, or vice versa. In contrast, the toxicity of Cd–Cu and Cd–Zn mixtures appeared to be less-than-additive or additive when based on dissolved metal concentrations; and the less-than-additive interactions also occurred in Cd–Cu–Zn mixtures. Use of mechanism-based biotic ligand models, however, shows that the apparently more-than-additive toxicity was explained by competition among metals for binding to DOM, whereas the apparently less-than-additive toxicity was explained by competition among metals for binding to the biotic ligand. Both mechanisms can occur concurrently in metal mixtures, and mechanistic models that take both processes into account to predict tissue residues of metals can improve predictions of metal-mixture toxicity.

**589 Modeling the toxicity of metal mixtures: a bioavailability-based approach using the Biotic Ligand Model framework** A.C. Ryan, HDR HydroQual; R.C. Santore, HydroQual Inc; J.S. Meyer, ARCADIS; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry. A bioavailability-based approach for predicting toxicity of metal mixtures is advantageous over approaches that utilize dissolved metal concentrations for the same purpose. Characterization of mixture toxicity on the basis of dissolved metals ignores important interactions at chemical and biotic ligand (BL) sites that occur as a result of varying exposure conditions. The effects of solution chemistry on the concentrations of bioavailable forms of metals and competitive interactions among cations and metal species are well-described in the scientific literature, and provide the basis for the biotic ligand model (BLM). Our goal here was to extend the BLM framework to describe the toxicity of metal mixtures. The resulting model is a multi-site BLM that combines previously developed single-metal BLMs. This mixtures model assumes that each metal in a mixture can bind at all available BL sites, but that each metal is toxicologically active at only one BL site that is specific to that metal. This formulation accommodates apparent competitive effects between toxicologically active and inactive metals at BL sites. Toxicity due to each metal is related to the accumulation of each metal at its toxicologically active BL, and mixture toxicity is calculated by combining the effects from single metals in a response-additive fashion. An additional element considered during model development was uncertainty in toxicity due to single metals. To address this, confidence bounds for single metal toxicity predictions were developed based upon repeated single metal toxicity tests. A bootstrap approach was then used to develop confidence bounds for toxicity predictions of metal mixtures. The multi-metal BLM was primarily developed with data from single metal *Daphnia magna* toxicity tests, and refined after application of the model to binary and ternary mixtures of cadmium, copper, and zinc. Generally, the model performed reasonably well with the *D. magna* tests, as it predicted the patterns of responses in toxicity data. For example, the model accounted for the

apparent beneficial effect of copper and zinc on cadmium toxicity (i.e. cadmium toxicity decreased with sub-lethal concentrations of copper or zinc). In addition, the multi-metal BLM accounted for some of the apparently more-than or less-than additive effects of mixtures by consideration of competitive interactions at BLs or competition for binding with inorganic or organic ligands in solution.

**590 WHAM-FTOX: an aquatic cation mixture toxicity model** E. Tipping, Centre for Ecology and Hydrology / Lancaster Environment Centre; S. Lofts, Centre for Ecology Hydrology / Shore Section. Aquatic organisms are subject to toxic effects from the cations of numerous metals and the proton ( $H^+$ ). The effects have largely been studied in laboratory experiments, usually with only one toxic cation. But in the natural environment, mixture toxicity is probably more often the case. Moreover one should also take into account “medium effects”, i.e. variation in toxicity with water chemistry, for example the dependence of the toxicity of a metal cation with pH or Ca concentration. These complexities can in principle be taken into account with the Biotic Ligand Model, through competitive chemical reactions at one or more biotic ligands that mediate the toxic effect. We propose an alternative, but still chemically-based, approach, in which the exposure of an organism to potentially-toxic cations is expressed by their “active body burdens” ( $n \text{ mol g}^{-1}$ ). Toxicity is quantified empirically through the product of the active body burden and a toxicity coefficient,  $\alpha$ , providing a straightforward means of combining the toxic effects of different cations, including  $H^+$ . Key to the calculations is the use of the WHAM chemical speciation model to quantify the competitive reactions of cations with living organisms, thereby estimating the values of  $n$ . Evidence to justify this comes from both field and laboratory studies with bacteria, bryophytes and macroinvertebrates. The toxicity function  $F_X$  is obtained from the equation:  $F_X = \alpha n$ . For values of  $F_X$  less than a lower threshold ( $F_{X-LT}^{TOX}$ ), there is no toxic response, while maximum toxic response occurs when  $F_X$  exceeds an upper threshold ( $F_{X-UT}^{TOX}$ ). In between, the toxic response changes linearly with  $F_X$ . To apply the model to a set of laboratory toxicity data, values of  $n$  are calculated from the water chemical composition, then the values of  $\alpha$ ,  $F_{X-LT}^{TOX}$  and  $F_{X-UT}^{TOX}$  are optimised by minimising the sum of squared residuals between observed and calculated toxic response. In practice the value of  $\alpha$  can be set to unity, since the toxicity coefficients are only relative numbers. WHAM- $F_X^{TOX}$  has been applied to the results of laboratory metal mixture toxicity experiments with bacteria, algae, duckweed, zooplankton, invertebrates and fish. The model comes close to achieving the objective of condensing all the data into a single trend, i.e. toxic response as a function of  $F_X^{TOX}$ .

**591 Modeling impacts of metal mixtures on macroinvertebrates in stream microcosm experiments: humic acid as a proxy of exposure** Y. Iwasaki, Department of Civil Engineering; P.J. Cadmus, Colorado State University Fish Wildlife Conserv / Grad Research Teaching Assistant; W.H. Clements, Colorado State University / Fish, Wildlife and Conservation Biology. River benthic macroinvertebrates are frequently used to evaluate ecological impacts of metal mixtures in natural environments. However, knowledge about which predictors of metal exposure are best to model the impacts remain uncertain. Although the use of total and dissolved concentrations of metals has been dominant as the predictors of metal exposure in the field observations, a new predictor based on the amount of metals binding to humic acid, which is assumed to be a proxy of non-specific biotic ligand sites, has been proposed. The amount can be calculated using Windermere Humic Aqueous Model; we will refer to this method as WHAM-HA approach. We aimed to test the hypothesis that the predictor based on the WHAM-HA approach was a better predictor of metal effects than three other measures: total concentrations, free ion concentrations, and cumulative criterion unit (CCU) which is a measure of the ratios of measured metal concentrations to the hardness adjusted criterion values. We used macroinvertebrate data on abundance and richness obtained from three microcosm experiments with macroinvertebrates exposed to metal mixtures (Zn alone, Zn + Cd, and

Zn + Cd + Cu). For each of four predictors, we performed multiple linear regression with variables corresponding to the three metal concentrations or CCU. In each predictor category, we selected the model with the smallest value of a second order bias correction for Akaike's information criterion (AICc) as the “top” model. Then, by comparing AICc values of the four top models, we selected the “best” model among the predictor categories. For all of the macroinvertebrate metrics affected by metals, the WHAM-HA approach was constantly selected as the best among the top models, followed by the model with total concentration, although the differences in AICc values between them were not large particular for richness metrics (0.8?2.1). Either of the models with free-ion concentration and CCU was the third best model. Our results suggest that the estimated amount of metals binding to humic acid is a better predictor for the effects on macroinvertebrate richness and abundance observed in microcosm experiments than total or free ion concentrations of metals and CCU. To our knowledge, this is the first study that demonstrated the superiority of the WHAM-HA approach, supporting the use of the amount of metals binding to humic acid as a predictor of metal impacts on river macroinvertebrates.

**592 Phytoremediation using trees: Lessons from the field** M. CHALOT, Université de Franche-Comté / UMR 6249 Laboratoire Chrono-environnement.

**593 Long-term efficiency of gentle soil remediation - the Greenland Project** M. Puschenreiter; M. Mench, Institut National de la Recherche Agronomique (INRA); V. Bert, Institut National de l'Environnement industriel et des Risques; J. Kumpiene, Luleå tekniska universitet; P. Kidd, Consejo Superior de Investigaciones Científicas; A. Cundy, University of Brighton. Gentle remediation options (GRO) include various and in general plant-based approaches to remediate trace element contaminated soils at low cost and without significant negative effects for the environment. Although GRO comprise very innovative and efficient technologies, they are still not widely used as practical site solution due to several reasons of hindrance. Contamination of soils with trace elements is worldwide still one of the major environmental problems. Conventional technologies for soil remediation are usually very expensive and may negatively affect or destroy soil structure and functions. GRO, however, comprise environmentally friendly technologies that have little or no negative impact on the soil. The main technologies are phytoextraction, in situ immobilization and assisted phytostabilization. Although major progress has been achieved on the lab scale, success stories obtained in the field are still limited. In addition, data on the long-term efficiency of GRO under various conditions are needed to fully assess the application potential. Therefore, the EU-FP7 project GREENLAND (KBBE-2010-4) with 17 partners from 11 countries has been launched on January 1 2011 to address these issues based on data of 13 long-term case studies (up to 20 years duration) and to make GRO ready for use as practical site solution.

**594 Phytomanagement of Cr polluted sites with *Silene vulgaris*** A. Pradas del Real, P. García, M. Lobo, IMIDRA; A. Perez Sanz, IMIDRA / Agroenvironmet. Chromium is a commonly metal found in two forms in the environment: trivalent chromium (chromium III) and hexavalent chromium (chromium VI). Chromium (III) is the most stable form of the element, and occurs naturally in animals, plants, rocks, and soils. Chromium (VI) rarely occurs in nature, and is usually the product of anthropogenic activities. In 2011, EU member states have reported increased chromium emissions by 12.6% compared to 2009. Although this metal can be released through natural forces, the majority of the environmental releases of chromium are from industrial sources. Chromium compounds are highly toxic to plants and are detrimental to their growth and development. Although some crops are not affected by low Cr concentration ( $3.8 \times 10^{-4} \mu\text{M}$ ), Cr is toxic to most higher plants at  $100 \mu\text{M} \cdot \text{kg}^{-1}$  dry weight. The poor translocation of this element from roots to shoots is a major hurdle in using plants and trees to apply phytotechnologies. Mycorrhizae and organic acids (citric and oxalic) have been reported to play an important role in phytoremediation of Cr-contaminated soils by enhancing Cr uptake and increasing translocation

to shoots. *Silene vulgaris* is a metallophyte facultative which present a high concentrations of organic acids in vegetal tissues, specially oxalic acid. Wild species and native from polluted sites are the best candidates to be used in phytotechnologies. Taking in account this considerations, the talk will provide quick access to aspects related to the toxicity of Cr in the global environment, specially from industrial activities, and possible strategies to reduce “in situ” Chromium toxicity. A case of study on a greenhouse experiment will be presented as practical case to remediate Cr polluted soils with *S. vulgaris* focused on organic acid role in Cr availability.

**595 Can riparian vegetation be used in phytotechnologies applied to mining districts?** M. Lominchar, EIADES - Ciemat; E. García-Ordiales, University of Oviedo; L. Bueno, J. Rodríguez-Alonso, M.

Lacal, M. Sierra, CIEMAT – DMA; J. Loreda, University of Oviedo; R. Millan, CIEMAT / Dpto. Medio Ambiente. The use of plants as alternative to decontaminate areas affected by heavy metals is growing, and it is mainly due to its ease to implement and to be environmentally friendly. Although to carry out greater development of these technologies, it is necessary to increase the knowledge about the behavior of different plant species in the presence of heavy metals. And for that, it should not be only done by laboratory tests but completed with field trials. The aim of this experimental work is to study the accumulation of mercury in the aerial part of five different riparian plant species for their possible use in phytoremediation technologies. Three of these studied plants were shrubs (*Tamarix canariensis*, *Flueggea tinctoria* L. and *Nerium oleander* L.) and the other two were macrophytes (*Typha domingensis* and *Phragmites australis*). The selected study area for this work is located in riverbanks of Valdeazogues River which flows through the Almadén mercury mine district (Ciudad Real, Spain).

**596 Phytomining of Ni: how hyperaccumulation traits can be used in green mining?** G. Echevarria, University of Lorraine; A. Bani, T.

Sterckeman, Laboratoire Sols et Environnement, Université de Lorraine – INRA; E. Montargès-Pelletier, Laboratoire Interdisciplinaire des Environnements Continentaux, Université de Lorraine – CNRS; J. Morel, Laboratoire Sols et Environnement, Université de Lorraine – INRA; M. Simonnot, Laboratoire Réactions Génie des Procédés, CNRS – Université de Lorraine. One of the challenges that drive current research on the extraordinary behaviour and physiology of hyperaccumulator plants is their adaptation as phytomining crops. Recent studies have proven that it is possible, thanks to their particular Ni content and speciation, to produce high purity Ni salts from hyperaccumulator biomass. Therefore we aimed at developing specific crop systems for the wild *Alyssum murale* and other hyperaccumulator species from Europe. We contributed to the study of the physiological mechanisms involved in Ni hyperaccumulation (elemental distribution, Ni speciation in plant parts, ecophysiology,...) and adapted our knowledge to agronomic applications. We started investigating (field experiment in Albania) the performance of a low-cost phytoextraction with limited agronomic actions adapted to the Balkan context. We have carried out a number of field and laboratory trials to define the optimal harvest time according to the phenology of *A. murale* and the evolution of Ni distribution in plant parts, the effect of fertilization practices, weed control and defined optimal cropping systems. Despite high productivity of native stands controlled by fertilization and selective pesticides (70 kg Ni ha<sup>-1</sup>), sowing was the best alternative to improve phytoextraction yields (105 kg Ni ha<sup>-1</sup>).

**597 A successful phytoremediation strategy in a TE polluted mine soil under semi-arid conditions** P. Bernal, CSICR. Clemente,

Soil&Water Conservation&Organic Waste Management; T. Pardo, D. Martínez-Fernández, D. Walker, CSIC. The application of phytotechnologies to real field situations is still a pending matter in the phytoremediation world, especially in those areas of unfavourable climatic and soil conditions for plant growth. The semi-arid climate, common in the Mediterranean area, is characterised by long hot and dry summers and low annual rainfall (< 300 mm) with occasional heavy

storms. These conditions together with unfavourable soil characteristics, high TE concentration, soil salinity, and low organic matter and nutrient concentration in the soil make the use of plants for the remediation of such areas of an extra difficulty. A field experiment was carried out under these conditions in a TE polluted soil in the mining area of La Unión-Cartagena (Murcia, SE Spain). The selected technology was phytostabilisation by using the halophytic shrub *Atriplex halimus* in combination with organic amendments to improve soil fertility and to help plant establishment. During 2.5 years, soil and plants were tested and a self-sustainable and permanent vegetation cover was established in the area. The efficiency of the soil remediation process was tested through an ecotoxicological evaluation, which confirmed the recovery of the soil functions.

**598 Working towards standardized tests for assessing ecotoxicology of nanoparticles** T.F. Fernandes, HeriotWatt University / School of Life Sciences.

**599 Effects of Carbon Based Nanoparticles in Aquatic**

**Environments.** J.V. Kukkonen, University of Jyväskylä / Biological and Environmental Science; K. Pakarinen; G.C. Waissi-Leinonen, University of Eastern Finland; J. Akkanen, University of Eastern Finland / Department of Biology; M.T. Leppanen, Finnish Environment Institute; E.J. Petersen, National Institute of Standards Technology. Nanoparticles are receiving more attention in the field of environmental science. Among carbon based nanoparticles (CBNs), fullerenes and carbon nanotubes have attracted significant research attention as a result of their potential for breakthroughs in a broad range of applications, a number of which have or are approaching their respective commercialization stages. One reason that the environmental behavior of CBNs is not more fully understood is that quantifying or even identifying them in environmental or biological systems remains a substantial challenge. This presentation will give an overview of the bioaccumulation and some potential effects CBNs may cause. In aquatic exposures the accumulation of CBNs by *Daphnia magna* was rapid during the first 20 hours, and the steady-state concentration was reached within a day. However, after transfer to the clean water the depuration was slow. The microscopy results suggest that the vast majority of the accumulated CBNs remained in the organisms' guts and were not absorbed into cellular tissues. Sediment assays were developed and tested for *Lumbriculus variegatus* and *Chironomus riparius*. In these assays no lethal effects were observed. However, chronic effects on growth or behavior of the organisms were observed. Further, histological changes were clear. In exposed *C. riparius* the gut microvilli were damaged and were significantly shorter compared to control organisms. Similarly, in *L. variegatus* exposure the epidermal cuticle fibers of the worms were damaged. It is speculative but this kind of damages may make organisms more susceptible to infections, parasites or contaminants in long run.

**600 To describe and control exposure in nanoecotoxicology tests**

A. Baun, Technical University of Denmark - Department of Environmental Engineering. There is an urgent need for reliable and reproducible results of ecotoxicological experiments in order to identify, rank, and classify the environmental hazards of nanomaterials. Standardized methods and guideline tests traditionally used for chemicals are today recommended for the testing of nanomaterials. While the database of results from these tests is rapidly expanding, problems with controlling the exposure are often reported, e.g. due to the particle behavior in the test media used. Problems encountered include uncontrollable aggregation, agglomeration, sedimentation, or dissolution. The interpretation of the test results may further be complicated by the fact that these processes are ongoing during the testing period and may be affected by the organisms themselves. Therefore, the total concentration reported in concentration-response relationships in these tests encompass a range of possible alterations of the bioavailable part of the nanomaterial added. These alterations, which may be determining for the biological effects found, are difficult, if not impossible, to control in a standard test setting. This raises the question



whether the results generated in standardized test systems are fulfilling the purpose they originally were intended for (ranking and classification). The validity of extrapolations made from these data (e.g. predicted no-effect concentrations for environmental risk assessments) is therefore further questionable. Based on own experiences these fundamental problems for testing of engineered nanoparticles in aquatic toxicity tests and their implications for risk assessment will be addressed in this presentation.

**601 Panel discussion: Measurement and methods in environmental nanotechnology** E.J. Petersen, National Institute of Standards Technology. Each presenter in this session will give a short recapitulation of the major issues and formulate the needs to go forward.

**602 Interactive Case study of complex environment and human health issue using eDPSEEA (II)** G. Morris; S. Beck, NHS Health Scotland.

**603 Audience discussion of next steps and new directions** S. Reis, Centre for Ecology Hydrology.

**MO001 Review on the status and challenges of soil ecotoxicology in the tropics** O.J. Owojori, Obafemi Awolowo University / Zoology; P.C. de Silva, University of Ruhuna, Matara; M.A. Daam, Technical University of Lisbon, Portugal; J. Roembke, ECT Oekotoxikologie GmbH; J.P. Sousa, University of Coimbra; A.V. Waichman, Federal University of Amazonas; C.A. van Gestel, Free University of Amsterdam. Ecotoxicology as an emerging field arising from ecology and toxicology has helped to properly evaluate the risks of natural and anthropogenic substances released into the environment but most of these efforts are lopsided to the temperate regions, as opposed to tropical areas. It is well known that the tropical regions of the world are rich in biodiversity and natural resources suggesting that adequate care should be taken to protect this fragile ecosystem from the imminent contamination with pollutants. Thus, a review of the status and challenges of soil ecotoxicology in the tropics was conducted. The review starts with a short historical reflection of ecotoxicology in general and delineates the major differences between temperate and tropical environments. It further identifies the contaminants of concern in tropical areas, and assesses their degradation and toxicity in studies conducted under tropical conditions. The review also describes the use of case studies, by comparing the toxic response of tropical species with mediterranean/temperate species where possible. The challenges of extrapolating data from temperate environment to tropical environment are discussed. In addition, recent attempts to establish native tropical species for standardized testing as well as the possibility of the development of a standard artificial soil for the tropics are discussed. Regulatory procedures for chemical use and their risk assessment in tropical areas are compared with data collected from other geographical zones. Areas of immediate concern in the protection of the tropical soil biodiversity are identified and suggestions for the future development of tropical ecotoxicology are proposed.

**MO002 Effects of deltamethrin, dimethoate and chlorpyrifos on survival and reproduction of the mite *Hypoaspis aculeifer* using African and European soils** O.J. Owojori, Obafemi Awolowo University / Zoology; K. Jegede, Obafemi Awolowo University / Dpt of Zoology; I. Jaabiri, National School of Engineering of Sfax, Tunisia / Laboratory of Water, Energy and Environment; J. Roembke, ECT Oekotoxikologie GmbH. Indiscriminate use of pesticides is rampant in most parts of Africa but only scanty data exist on their impact on soil organisms. In order to assess the site-specific effects of three commonly used pesticides on soil fauna in Africa, non-contaminated surface soils were collected from Nigeria and Tunisia, and used in the predatory mite (*Hypoaspis aculeifer*) reproduction tests following the standard OECD guideline. Results were compared with data gathered in tests with European LUFA 2.3 soil. For deltamethrin, no significant effect on survival up to 10 mg a.i./kg soil dw was found in all three soils, while significant effects on reproduction started at 3.2 mg a.i./kg soil dw

(LUFA) or 10 mg a.i./kg soil dw (other soils). For dimethoate, the toxic effect on survival was clearly higher in the Tunisian and LUFA soil (LC50= 4.6-6.6 mg a.i./kg soil dw) than the Nigerian soil (LC50 > 10 mg a.i./kg soil dw). For reproduction, higher toxicity was obtained for Tunisian soil (EC50=2.23 mg a.i./kg soil dw) than in the Nigerian and LUFA soil which both had similar EC50 values (4.8-6.2 mg a.i./kg soil dw). For chlorpyrifos, similar LC50 values were obtained for all three soils and ranged from 3.7-6.6 mg a.i./kg soil dw. The reproduction EC50 values of 1.7 and 2.5 mg a.i./kg soil dw obtained in Tunisian and Nigerian soils, respectively, were clearly lower than those for LUFA (EC50 = 5.3 mg a.i./kg soil dw) soil. Soil specific patterns of toxicity of the tested pesticides to *H. aculeifer* were therefore evident and might be related to the physicochemical properties of the soils and not the origin of the soils. Although data of tests with other soil micro-arthropods are still underway, the present results indicate that ecological risk assessment of pesticides in Africa should be done on a case by case basis using tests simulating the local environmental conditions.

**MO003 A comparison of organism and sub-organism responses of an indigenous earthworm species and *Eisenia andrei* to copperoxychloride** S. Reinecke, Stellenbosch University / Dept Botany and Zoology; F. Fourie, Stellenbosch University / Botany and Zoology; A.J. Reinecke, Stellenbosch University / Department of Botany and Zoology - Stress Ecology Research Group. To date research into the environmental toxicology and chemistry of soil pollutants has focused almost exclusively on temperate countries. Environmental risk assessors and regulators in non-temperate regions and countries rely very strongly on data generated in the temperate eco-zones in spite of the fact that the fate of chemicals and their potential side-effects in soils may be very different between such climatic distinct regions. In this study we compared responses of an indigenous earthworm species (*Chilota sp.*, family Acanthodrilidae) with the standard USDA and EPA test species *Eisenia andrei* (family Lumbricidae). Responses at both the organism and suborganism levels were measured after experimental exposure of specimens of both species to a range of concentrations of the widely used fungicide copperoxychloride. The aim was to compare differences in sensitivity between the typically temperate species (which is not a typical soil dwelling species but nevertheless used as benchmark internationally) and an indigenous South African soil dwelling species in order to gain an understanding on how the results of internationally standardised toxicity tests and various other biomarker tests will relate between the two species. The eventual aims are to use these study outcomes to develop and implement sound ERA schemes for non-temperate soils. Apart from measuring effects on feeding activity, body mass and reproduction we used the neutral red retention assay (NRR) and the comet assay (single-cell gel electrophoresis) as biomarkers to measure responses of both species to the fungicide. The results of this study showed that the indigenous *Chilota sp.* differed markedly from *Eisenia andrei* in various of its responses to copperoxychloride. The former being more sensitive in various instances than the latter but the pattern was not consistent for all measured parameters. We conclude that simply adjusting techniques developed in temperate regions may not suffice, particularly for the testing of local more sensitive indigenous species in non-temperate regions, unless a reliable means of extrapolation can be shown

**MO004 Ecotoxicological evaluation of the sugarcane vinasse disposal in soil under tropical conditions using earthworms** R. Lopes Alves, University of São Paulo / Soil Science; E. Jurandy Bran Nogueira Cardoso, University of São Paulo. In Brazil, the sugarcane alcohol production generates large amounts of vinasse, a liquid waste. Nowadays, this waste is mainly disposed in agricultural soils because of its great potential as fertilizer. There is a national specific standard for vinasse disposal onto the soil, to prevent salt saturation, however, this document does not take into account certain biological properties of the soil. In this study, using an ecotoxicological assay with *Eisenia andrei* (Oligochaeta), we evaluated the impact of three vinasses on earthworm reproduction. Two vinasses, called A and B, were collected from different distillery plants, while a third vinasse (C) was obtained from a

laboratory production, without using antibiotics nor other additives during the fermentation process. This bioassay, adapted from ISO/OECD, carried out in an Oxisol, was set up in a climate-controlled room with a temperature of  $23 \pm 2$  °C and a photophase of 12 hours. At the beginning of the test, the soil was artificially contaminated with 5 increasing concentrations of each vinasse, each one with 5 replicates. The concentrations used were based on the norm about field applications: 0 (control), 15, 30, 60, 120 and  $240 \text{ m}^3 \text{ ha}^{-1}$ . After filling the test containers with the soils, 10 adult *E. andrei* were added to each one and the bioassay was run for 56 days. After 28 days all adult worms were removed, and for the next 28 days only the soils, juvenile worms, and cocoons remained in the containers. On day 56, we recorded the number of individuals generated during the period in which the adults had been present and observed that, in general, for all vinasses, the increase of the concentration showed a trend to increase the number of juveniles. However, at the highest concentration of the vinasses A and B the average number of juveniles ( $39.8 \pm 1.5$  and  $43 \pm 1.8$ , respectively) was reduced, in comparison to the control ( $70.8 \pm 0.9$ ), while at the highest concentration of the vinasse C the increase of juveniles was still maintained. We attribute the toxicity of the vinasses A and B to the antibiotics and/or other compounds added in the industrial fermentation process. Although these results indicate that high vinasse concentrations may be toxic to earthworms, other soil organisms must also be evaluated, complemented by chromatographic analyses to detect and quantify the presence of antibiotics in vinasse. These studies may reduce the uncertainties about the eventual risks of vinasse disposal in soils.

**MO006 Fish pond disinfection after outbreaks of virus epidemics: Assessment of pollutive effects** S. Elbers, S. Classen, gaiac Institute for Environmental Research, RWTH Aachen; U. Hommen, Fraunhofer IME; R. Marschang, University of Hohenheim, Institute of Environmental and Animal Hygiene; C. Schlechtriem, Fraunhofer IME / Oekotoxikologie. The disinfection of fish ponds is carried out routinely as an important hygiene measure, as well as after outbreaks of infectious diseases. The bottom and walls of natural ponds are usually covered with soil and thus particularly difficult to disinfect because most standard disinfectants are readily exhausted in contact with organic matter. Quicklime (CaO) and slaked lime are most frequently used for the disinfection of fish ponds. In the course of the treatment of whole ponds with lime the disinfectant action is achieved by raising the pH of the water to pH 12. The discharge of the treated water into surrounding water bodies may occur only when pH has dropped to pH 8.5. However, the environmental compatibility of these measures, in particular with regard to the macro-zoobenthos community of streaming water bodies below fish ponds is mostly unknown. As part of the project „Disinfection after outbreaks of infectious virus epidemics in fish ponds” funded by the German Federal Office for Agriculture and Food, the impact of the short-term pH increase following the discharge of water from ponds previously treated with quicklime was examined. Besides the pH of the water, further physical and chemical parameters were continuously monitored. Macro-zoobenthos communities were evaluated before and after the disinfection measures in the receiving water course above and below the treated fish ponds, to determine the impact of increased pH values on the abundance of single populations and the general community structure. The results show that the discharge of water from limed ponds leads to degraded water quality due to increased nutrient loading. No major effects on ecosystem health could be observed.

**MO007 Disinfection of aquaculture facilities: Acute toxicity of disinfectants to aquatic macroinvertebrates** C. Schlechtriem, Fraunhofer IME / Oekotoxikologie; S. Classen, D. Becker, gaiac Institute for Environmental Research RWTH Aachen; S. Rinke, U. Hommen, Fraunhofer IME; R. Marschang, University of Hohenheim, Institute of Environmental and Animal Hygiene and Veterinary Medicine. The disinfection of aquaculture facilities is an important measure to protect fish against diseases and to maintain the productivity of fish farming systems. Hygiene measures are carried out routinely and straight after outbreaks of infectious diseases. However, only a few chemical substances used as disinfectants in aquaculture have been

extensively tested with regard to their environmental compatibility. In this project the acute toxicity of two disinfectants often used in aquaculture were examined in laboratory studies with aquatic macroinvertebrates. Quicklime (CaO) is the substance most often used for the disinfection of fish ponds. Halamid® (Chloramine-T) is commonly used for the disinfection of fish tanks and hatchery equipment. Toxicity tests were carried out with the amphipod crustacean *Gammarus pulex* and mayfly nymphs (*Habrophlebia lauta*), which are commonly found in brooks in hilly countryside and mountain areas. Disinfection effects following application of quicklime are induced by rising the pH of pond water to pH12. Only after a significant decrease of the pH value to pH 8.5 the pond effluents are allowed to be discharged into the aquatic environment. Therefore, in the toxicity studies with quicklime, test media with pH values ranging from 8.0-10.5 were tested to investigate the potential impact of highly alkaline pond effluents on aquatic macro-invertebrates. Clear effects were only observed for the highest treatment (pH10.5). The concentration recommended for disinfection measures using Halamid® is 3 mg/L. Concentrations ranging from 0.5 to 50 mg/L were tested in the toxicity studies over a period of 96 h. The mortality and immobility of the laboratory animals was estimated every 24 h and compared with an untreated control group. In the studies with *H. lauta* an EC<sub>50</sub> (96 h) of 4.2 mg/L was determined for Halamid®, which is lower than the value obtained for *G. pulex* (9.2 mg/L). The lowest LC<sub>50</sub> (96 h) for fish described in the literature is 7.2 mg/L. Considering the further dilution of effluents discharged into the aquatic environment, the correct use of quicklime and Halamid® for the disinfection of aquaculture facilities should lead to only a limited risk for aquatic macro-invertebrates.

**MO008 Use of veterinary medicinal products in freshwater aquaculture: Exposure scenarios for the assessment of environmental concentrations** C. Schlechtriem, Fraunhofer IME / Oekotoxikologie; A. Hein, GKSS Research Centre Geesthacht / Pharmaceuticals, washing and cleansing agents, nanomaterials; M. Klein, Fraunhofer IME; W. Koch, Federal Environment Agency (UBA). Modeling exposure plays a key role in the environmental risk assessment of veterinary medical products (VMP). The framework for the assessment of environmental concentrations is presented in the “Revised Guideline on Environmental Impact Assessment for veterinary medical products” (EMA) supporting the VICH guidelines GL6 and GL38. Default values for “Intensively reared animals” and “Pasture Animals” are available for the prediction of VMP concentrations in the terrestrial environment (PECsoil). Models are also available for the environmental risk assessment of pharmaceutical compounds used in marine cage fish farming (e.g. SEPA). However, standardized exposure scenarios and default values for inland freshwater aquaculture are still missing. Therefore, fish-farming systems commonly found in Germany were investigated with respect to the theoretical maximum levels of pharmaceutical compounds leaving the aquaculture facilities following administration of chemotherapeutic regimes. “Realistic worst case” exposure scenarios with corresponding default values for PEC calculation were defined for flow-through systems (e.g. trout production), fish ponds (e.g. carp production) and recirculatory systems.

**MO009 Ecotoxicological testing strategy for ERA of Veterinary medicinal products vs. Plant Protection products** V. Croce, ChemService srl Controlli e Ricerche / Dossier; M. Neri, ChemService s.r.l. Controlli e Ricerche; B. Lugoboni, VETSPIN; O. Schifanella, ChemService s.r.l. Controlli e Ricerche. Environmental Risk Assessment (ERA) is one of the regulatory requirement for both Veterinary Medicinal Products (VMPs) and Plant Protection Products (PPPs). A similar approach is adopted in the two cases, based on the comparison of the Predicted Environmental Concentrations (PEC) with the ecotoxicological endpoints. However, a main difference exists regarding the ecotoxicological testing strategy, as basic ecotoxicity information is always required for PPPs, while for VMPs the ERA is carried out in a phased manner and the ecotoxicological testing is only necessary if the PEC soil calculated in Phase I exceeds the trigger value. In Phase II, the environmental fate and effects of the VMP are

considered in more detail through appropriate laboratory studies (Tier A and Tier B). Therefore, a key component of the ERA process for VMPs is the determination of the exposure concentration, which can be used as a starting point in the selection of the test concentrations. In the present work, 4 VMPs with antibiotic mode of action and failing the Phase I assessment, were tested for terrestrial and aquatic ecotoxicological endpoints. The concentrations for the preliminary tests were chosen based on the PEC and were then refined for the aquatic endpoints of 2 substances out of 4, i.e. in those cases were, for different reasons, it was not possible to find the EC<sub>50</sub> or the NOEC. When the chosen concentrations were too high, only a qualitative comparison with the PEC can be carried out, but this result cannot be used for the calculation of the Risk Quotient (RQ). In the PPPs approach, ecotox testing is not triggered by the PEC and the test concentrations are directly selected in such a way to obtain the EC<sub>50</sub> or the NOEC, possibly through preliminary tests. The difference in testing strategy between PPPs and VMPs, which can be summarized in an extensive ecotoxicological testing for PPPs and in a stepwise process for VMPs, is justified based on the consideration that PPPs are deliberately applied to the environment and therefore a risk for the different compartment cannot be excluded based on preliminary considerations. On the contrary, the environmental contamination from VMPs is more linked to many different factors that can be analysed prior to the testing phase, thus allowing to avoid unnecessary use of test animals and to save financial resources.

**MO011 Gaps in the environmental risk assessment of veterinary medicines: poorly extractable compounds** R. van Herwijnen, Centre for Safety of Substances and Products; P.v. Vlaardingen, RIVM / Expert Centre for Substances; C. Moermond, RIVM / Centre for Safety of Substances and Products. Like in many other frameworks, for registration of veterinary medicines the environmental risk is assessed through the PEC/PNEC approach. One of the most important parameters for calculating the Predicted Environmental Concentration (PEC) is the half-life (DT50) for aerobic transformation in soil determined according to OECD 307. This parameter, with others, is used to calculate surface- and groundwater concentrations. However, several active substances like tetracyclines, avermectins, pyrethroids or fluoroquinolones display very high soil adsorption coefficients. Because of the strong sorption, the substance cannot be fully extracted and analysed to generate a mass balance as required in OECD 307. As a consequence of extraction problems, the mass balance as required in the OECD guideline cannot be generated and a required mass balance between 70 and 110% can often not be achieved. In order to build a strong case, we strongly support the inclusion of an extraction scheme in the study report. With this scheme, the applicant shows that all available and relevant techniques have been used to obtain the best extraction results for the parent and possible metabolites. If this does not result in extraction efficiencies according to the OECD guideline, this can also be solved by using a <sup>14</sup>C labelled compound. The radio active label enables that the amount of substance remaining in the soil as unextractable residue can be determined. However, <sup>14</sup>C labelled compounds are often not available for e.g. antimicrobials because they are produced through fermentation processes. We like to highlight this problem, which hampers a proper risk assessment, and present a pragmatic approach to work around the problem. Furthermore we invite people to suggest alternative methodologies.

**MO012 A comparison between the ecological effects of ivermectin in pony and cattle dung** J. Lahr; D. Lammertsma, R. van Kats, A. van der Hout, Alterra, Wageningen UR; A. Siepel, Unifarm, Wageningen UR; W. Blanckenhorn, Institute of Evolutionary Biology and Environmental Studies, University of Zurich; J. Roembke, ECT Oekotoxikologie GmbH. Experiments in the past have shown that anthelmintics such as ivermectin excreted in dung from domestic animals are toxic to dung insects that are using the dung for food, reproduction and shelter. It has also been reported that the loss of insect activity can lead to a reduced rate of dung breakdown. The major part of this research has focused on dung of cattle. Over the past years field

experiments have been conducted in The Netherlands with both cattle and pony dung. In the early summer, ponies were treated with an oral paste at a dose of or 0.2 mg ivermectin/kg body weight and cattle were treated with a pour-on formulation of ivermectin at 0.5 mg/kg b.w. Ivermectin was found in the dung of both species following treatment. In both cases emergence of groups of flies from dung collected in the field was significantly reduced. In pony dung, affected fly families were Sphaeroceridae (lesser dung flies), Muscidae (house flies or stable flies) and Stratiomyidae (soldier flies), in the case of cattle dung Sphaeroceridae and Sepsidae (black scavenger flies) were reduced. However, the total number of Diptera was only significantly affected in cattle dung because, contrary to pony dung, the affected groups also were the most abundant flies. The half-life in the field for the degradation of organic matter in uncontaminated pony and cattle dung was similar: 8-10 weeks. There was no effect of ivermectin on the dung degradation rate. The results show that in terms of impacted groups of fauna and (no) effect on dung degradation results may be similar for pony and cattle dung. However, because certain groups of flies may be more numerous in one type of dung than in the other, effects on the whole taxonomic group of dipteran flies may be different.

**MO013 Degradation of the veterinary ionophore lasalocid in broiler manure and compost** S. Zizek, M. Dobeic, S. Pintaric, M. Gombac, M. Pogacnik, S. Kobal, University of Ljubljana / Veterinary Faculty. Lasalocid is a veterinary ionophore antibiotic used in the poultry industry as a coccidiostat. It enters the environment with the use of chicken manure on agricultural soil. Due to a lack of literature data, a study was undertaken to measure the rate of degradation of this coccidiostat in chicken faeces under two different conditions – in a manure pile and in compost. There were marked differences between the two treatments. Lasalocid in manure degraded with a half-life of 61.8 days and did not fall below 45% of the initial concentrations even after 84 days, whereas its half-life in compost was 17.5 days and the concentrations fell below the limit of detection (10 ng/g) after 80 days. Broiler manure is usually aged for approximately three months before application on land. In most cases on Slovenian farms, it is simply left in a pile with no additional treatment such as aeration, moistening or mixing with plant material. We can therefore expect that about one half of the initial lasalocid levels reach the environment. At initial concentrations of about 10 mg/kg, which can be expected in broiler excreta and which were measured for monensin, which is structurally similar to lasalocid, the PEC for lasalocid in soil after three months of aging would be 0.031 mg/kg. The lowest EC<sub>50</sub> value reported for soil organisms was 4.9 mg/kg soil for isopod avoidance. Using an assessment factor of 100, the PNEC would be lower than PEC and the risk quotient for lasalocid in agricultural soil would be below 1 (0.63). We can therefore conclude on the basis of our preliminary results that the current practice of manure storage is sufficient to pose no risk to soil organisms. However, continual use of lasalocid-contaminated manure on the same area could increase the risk and composting would be recommended.

**MO014 The application via manure – A better choice for a more realistic exposure** N. Graf; B. Foerster, ECT Oekotoxikologie GmbH (ECT); J. Roembke, ECT Oekotoxikologie GmbH; M. Simon, Fraunhofer IME; M. Herrchen, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME); U. Kuehnlen, Federal Environment Agency; I. Ebert, Umweltbundesamt / Pharmaceuticals Department. The application of veterinary pharmaceuticals via manure has recently been discussed as an opportunity for a more realistic exposure in plant tests according to OECD-Guideline 208. To determine whether the application via manure changes the effect of veterinary antibiotics on higher plants, tests were performed with various monocotyle and dicotyle species. A veterinary antibiotic was applied in different concentrations: either directly to the soil or via pig and cattle manure after incubation for various time periods. The endpoints were emergence rate, survival, shoot length and shoot fresh weight. Results will be shown and discussed. This work is part of the research project „Development of a concept for the advanced plant testing and evaluation

in the environmental risk assessment of veterinary medicinal products“ (FKZ 3711 63 424) of the federal environmental agency.

**MO015 Physical and chemical characteristics of soils can influence antibiotics action on microbiota?** L.A. Figueiredo, University of São Paulo / Ecotoxicology; D.H. Silva, Secretaria da Agricultura e Abastecimento do Estado de São Paulo / Coordenadoria de Assistência Técnica Integral; P.A. Andrade, T. Gumiere, Escola Superior de Agricultura Luiz de Queiroz/University of São Paulo / Department of Soil Science; V.L. Tornisielo, Center for Nuclear Energy in Agriculture/University of São Paulo. Antibiotics are used in large quantities in livestock. Many of these molecules are not fully metabolized by the animals, leaving detectable residues in soil. The broiler (*Gallus gallus domesticus* L.) production is one of the activities that most employ the use of these veterinary antibiotics so, the contact of poultry litter with soils may contaminate them. The occurrence of these residues in the environment can favor microorganism resistance to antibiotic agents. The objective of this work was to determine the toxicological effects caused by veterinary antibiotics on the microbiota of tropical soils of Salinho, Tietê and Piracicaba regions (São Paulo, Brazil). Five farms, that work with veterinary antibiotics, were selected (fluoroquinolones: norfloxacin - NOR, enrofloxacin - ENR and ciprofloxacin - CIP; sulfonamides: sulfamethazine - SMT). For the present study, 10 samples were collected in two distinct points: 1) Soil without poultry litter and 2) soil with constant application of manure originated from chickens treated with veterinary antibiotics. Subsequently, the total DNA of soil microorganisms were extracted and amplified from soils samples. Changes in community structure of bacteria, evaluated through the separation of 16S rRNA gene fragments by *Denaturing Gradient Gel Electrophoresis* (PCR-DGGE), were observed. The antibiotics affected negatively soil bacterial diversity in all samples with high concentration of  $Mg^{2+}$  and  $Ca^{2+}$ . DGGE profiling revealed a lower number of bands in samples with high levels of  $Mg^{2+}$  ( $> 350 \text{ mmol dm}^{-3}$ ), indicating that these antibiotic molecules were strongly adsorbed and kept immovable on soil surface layers (0 - 10cm) causing toxic effects on bacterial community, since NOR, ENR, CIP and SMT are *broad-spectrum antibiotics*. These antibiotics performance may be associated to the cation exchange capacity (CEC) because in sandy soils, with low CEC, the microbiota was not influenced by the application of poultry litter containing these veterinary drugs. Probably these antibiotics are leaching in the soil profile. We conclude that NOR, ENR, CIP and SMT found in soils cause ecotoxicological effects in the structure of bacteria community, depending on physical-chemical factors (Mg, Ca concentration and soil texture).

**MO016 Testing toxicity of Vermophyt and Vermox nematocides by acute test with nematode *Caenorhabditis elegans*** L. Skulcova, J. Hofman, Masaryk University / Faculty of Science; P. Behensky, NEOFYT, spol. s r.o.. Nematodes are abundant and fundamental group of organisms and they are important for keeping soil quality. They are also suitable organisms for environmental toxicity testing. The goal of this study was to test acute toxicity of two nematocides - Vermophyt and Vermox with nematode *Caenorhabditis elegans* in aquatic and agar medium and investigate how the food addition changes the toxicity. Testing of these nematocides run generally without complications for all concentrations except the highest one. Evaluation of the surviving nematodes at the end of test was influenced by insoluble particles from nematocides at the highest concentration. Toxicity of both nematocides was influenced by addition of the food (*E. coli*). It influenced the toxicity in each test and independently on the used matrices. When the lowest concentration of Vermophyt was applied, mortality of nematodes was 62 % in 24 hour test without food. Whereas with addition of the food it was 74 %. In case of adding food to test with Vermox, mortality increased approximately of 10 %, too. Regarding 48 hour testing, influence of the toxicity is comparable to 24 hour testing. Generally, aquatic test appeared more sensitive. Comparing toxicity of the two nematocides, Vermophyt was shown more toxic.

**MO017 Pharmaceuticals in sewage sludge compost** E. Haiba, Tartu

College of Tallinn University of Technology / Department of Environmental Protection; K. Kipper, Testing Centre of University Of Tartu; L. Nei, Tallinn University of Technology Tartu College / Department of Environmental Protection; M. Lillenberg; K. Herodes, Tartu University / Institute of Chemistry. Large quantities of different drug residues enter the soil via fertilizing with sewage sludge. In the current study the selection of pharmaceuticals was made considering their resistance in soil and the scale of their use. The studied pharmaceuticals included fluoroquinolones: ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL); and sulfonamides: sulfadimethoxine (SDM) and sulfamethoxazole (SMX). These drugs end up in agricultural soils, where they can accumulate and have adverse effects on living organisms. Different sewage sludge composting technologies were performed as model experiments under laboratory conditions and the change in the concentrations of pharmaceuticals was followed. These experiments showed that the level of degradation of pharmaceuticals was strongly time-dependent and less correlated to the applied technology. The level of degradation after 4-month period from the beginning of the experiment was 90 - 98 % for CIP, 80 - 98 % for NOR, 75 - 100 % for OFL, 83 - 100 % for SMX and 73 - 99 % for SDM. The results clearly proved that the degradation of the studied pharmaceuticals did not have sufficient dependence on composting technologies.

**MO018 Transformation Products of Carbamazepine and Acetaminophen in Soil** J. Gan, University of California, Riverside / Department of Environmental Science; I. Li, University of California. Carbamazepine (CBZ) and acetaminophen (ACM) are heavily used human pharmaceuticals that have been found in wastewater effluents and biosolids. Soil is a primary environmental compartment for these contaminants and a source for secondary pollution, such as accumulation into plants or leaching to groundwater. When in soil, these chemicals may undergo incomplete microbial transformations, producing intermediates with altered biological activity and mobility. Here we explored the degradation kinetics and identified transformation products of CBZ and ACM in soil by using both  $^{14}\text{C}$ -labeling and LC-MS/MS. Through 120-d incubation under aerobic conditions, CBZ dissipated rather slowly, and mineralization did not exceed 2% of the spiked rate. Most of the spiked CBZ appeared in extractable residues. Five degradation intermediates were identified, including carbamazepine-10, 11-epoxide and acridine with known human toxicity. In contrast, ACM degraded more rapidly under similar conditions, and up to 16% of the spiked activity was mineralized after 120 d and a majority of the remaining residue was bound to soil. A total of 7 degradation intermediates were identified, including 4-methoxybenzylamine and 4-methoxyphenol. The persistence of parent compound and formation of incomplete intermediates suggest that CBZ has a high risk for off-site transport from soil, such as accumulation into plants and contamination of groundwater.

**MO019 Ecotoxicity and biodegradability of oxytetracycline and ciprofloxacin on terrestrial and aquatic media** E. Martí, Universitat de Barcelona / Productes Naturals Biologia Vegetal i Edafologia; C.E. Parente, J. Sierra, M. Garau, R. Cruanas, Universitat de Barcelona. Antibiotics are designed to be effective even at low doses and to be resistant to biodegradation. These substances, for various reasons, can reach different environmental matrices such as surface water, groundwater, soil or sediment. Recently, concerns about potential ecological impacts of synthetic antibiotics increased as they can inhibit key environmental processes mediated by microorganisms, as the cycles of the elements carbon, nitrogen and degradation of contaminants. Antibiotics are frequently found in the environment, so its biodegradability and ecotoxicological effects need to be studied. For this work, ciprofloxacin (CIP), prescribed for human and veterinary medicine and oxytetracycline (OTC), commonly used for veterinary purposes, were chosen. Ecotoxicity test of both antibiotics was assessed on terrestrial and aquatic organisms determining toxicity values: LOAEL,  $EC_{10}$ ,  $EC_{50}$  and  $EC_{50}$ . For the effects on terrestrial organisms, the inhibition of soil C and N mineralization, according to

the OECD guidelines were determined, as well as the inhibition of germination and elongation of *Raphanus sativus*, *Lolium perenne* and *Allium cepa* seeds. The incubation for C mineralization test was used to study the biodegradability of the substances in soil media. For the aquatic environment, bioluminescence test with *Vibrio fischeri*, and algal inhibition growth with *Pseudokirchneriella subcapitata* were performed. Biochemical Oxygen Demand after 5 and 28 days of incubation was determined and used to assess the biodegradability in aquatic media. The analytical quantification of the compounds by HPLC techniques in the soil and aqueous media after the incubations was also done to supplement the respirometric tests regarding biodegradability.

**MO021 Environmental relevance of pharmaceuticals – the global perspective** I. Ebert, Umweltbundesamt / Pharmaceuticals Department; S. Hickmann, Federal Environment Agency UBA / Pharmaceuticals Department; A. Kuester, German Federal Environment Agency (UBA); J. Koch-Jugl, Federal Environment Agency / International Chemical Management; F. Weber, IWW Rheinisch-Westfälisches Institut fuer Wasser / Bereich Wasserressourcen-Management; A. Bergmann, IWW Rheinisch-Westfälisches Institut fuer Wasser / Wasserressourcen-Management. Pharmaceuticals are known to occur ubiquitarily in the aquatic environment of industrialized countries. An analysis of monitoring data (IWW 2011) showed that in Europe nearly 300 pharmaceutical substances and degradation products were detected in different environmental compartments. Considering the fact that consumption and availability of medicines is increasing worldwide, it is obvious that drug residues in the environment are of global relevance as well. In addition, production sites for active pharmaceutical substances are by now predominantly located in emerging countries. Although some information on the global occurrence of pharmaceuticals in the environment has become available increasingly, a concise picture on the prevailing concentrations and potential effects on human and ecosystem health is still elusive. The International Society of Doctors for the Environment has recently proposed the topic “Environmentally Persistent Pharmaceutical Pollutants” for nomination as an emerging issue under the Strategic Approach on International Chemicals Management (SAICM) of the United Nations Environmental Programme (UNEP). The Open-ended Working Group OEWG 1 encouraged further development of the proposal. In order to define the state of knowledge on the global relevance of pharmaceuticals in the environment, the German Federal Environment Agency has initiated a research project in 2012 ([www.pharmaceuticals-in-the-environment.org](http://www.pharmaceuticals-in-the-environment.org)). As first step the project partners IWW Water Centre and Adelphi Consult Germany compile an inventory of monitoring data from each of the five UN regions: African Group, Asian Group, Eastern European Group, Latin American and Caribbean Group, Western European and Others Group. This global database is used to assess the range of pharmaceuticals found in the environment, the measured concentrations in different regions and the relevance of different emission pathways (production, use, disposal) on a global scale. Based on regional consumption data the role of infrastructure, population, pharmaceutical availability and agricultural practice on emissions of pharmaceuticals into the environment is assessed. Additionally, specific strategies for further action e.g. to reduce problematic emissions will be evaluated. The project provides a platform to encourage policy makers from agriculture, infrastructure, health care sector and scientists to discuss key issues and possible activities to be included in the global plan for action.

**MO022 Pharmaceutical concentration remediation as an ecosystem service provided by natural microbial communities** P. Grenni, National Research Council CNR / Water Research Institute; L. Patrolecco, N. Ademollo, M. Pirredda, A. Barra Caracciolo, National Research Council / Water Research Institute. Many pharmaceutical residues (such as carbamazepine, ibuprofen, gemfibrozil, naproxen, diclofenac, ketoprofen and estrone) have been found in rivers, including the River Tiber (Rome, Italy). Along its urban stretch, the anti-inflammatory drug Naproxen is one of the most commonly found in high concentration, ranging from 200 to 1000 ng/L depending on the

sampling point (e.g. upstream or downstream of the effluents of the wastewater treatment plants around Rome). In order to assess the ecosystem service consisting of natural attenuation of this drug in the river, the capability of the autochthonous microbial community to biodegrade Naproxen was evaluated. For this purpose, we conducted microcosm experiments using undisturbed river water samples collected in two different seasons (Spring and Autumn). The first experiment consisted of Microbiologically Active Water (MAW) microcosms (50 mL) treated with 100 µg/L of Naproxen. The biotic degradation was evaluated comparing the MAW microcosms with the microcosms containing previously sterilized river water. The second experiment was performed in the same conditions as the first one; moreover, some additional microcosms were used in order to evaluate Naproxen degradation in the co-presence of another pharmaceutical, the lipid regulator Gemfibrozil. The degradation of Naproxen occurred in all the MAW microcosms, showing a DT<sub>50</sub> of about 20 days. However, the co-presence of Gemfibrozil initially affected Naproxen degradation by lengthening the degradation lag phase. The bacterial abundance decreased immediately after the treatment, but this toxic effect was transient. The analysis of the bacterial community structure by the Fluorescence In Situ Hybridization method (FISH) made it possible to highlight the increase of some bacterial groups such as Gamma- and Alpha-Proteobacteria near the time of the DT<sub>50</sub>, suggesting their active role in the drug degradation. On the contrary, in the sterile microcosms no decrease of Naproxen concentration was observed during the experimental period. The overall results showed that the autochthonous river microbial community had a natural ability to remediate pharmaceutical contamination. The fact that Naproxen residual concentrations are commonly found in the river can be ascribed not to its intrinsic persistence (after 35-40 days Naproxen was completely degraded in the microcosms), but to its pseudo-persistence due to the spread in its use among the human population.

**MO023 Untreated sewages as source of antibiotics resistances in fresh water bodies in Buenos Aires, Argentina** D. Castineira, N. GOMEZ N, M. RODRIGUEZ MOREYRA, A. MONZON, S. DEMICHELIS, NATIONAL UNIVERSITY OF LANUS / Department of Productive Development and Technology. Amoxicillin is a commonly used antibiotic in Argentina, 60% of the ingested is excreted in the urine within 6 to 8 hours, while 30-40% of clavulamic acid is also eliminated in urine. Ciprofloxacin and cephalosporines are excreted in more than 60% in the un-metabolized form after therapeutic use. In consequence and due to the lack of sewage treatment most of these antibiotics are expected to be present in the freshwater bodies of the Río de la Plata basin in significant quantities. We evaluated the 3300 surveys that were conducted in adults, who were asked about the use of drugs in a period of 30 days before the survey. 67% of respondents said they had used at least one drug during the period and 7.3% used different class of antibiotics. An estimation of the annual contribution in Buenos Aires area is presented. An extrapolation was performed, considering the population size, the age pyramid, the average use of each antibiotic in one month and the un-metabolized excretion rates. The estimated quantities of antibiotic entering annually to this fresh water system are expressed in Kg: amoxicillin 14570, cephalexin 12868.9 clavulamic acid 2380, penicillin G 2040, sulfatiazol 1900, ampicillin 1430, claritromicin 1360, cefadroxile 953.3, sulfatoxazol 763, levofloxacin 476, azitromicin 425, sulfamicillin 357, ceftriaxone 272, cefuroxim 238 and eritromicin 48. The first consequence of antibiotic release in a natural water environment will be the selection of resistant bacteria. Since drinking water is obtained by treatment from water coming from the same river will not be surprising a rise in antibiotic-resistant infections incidence.

**MO024 Removal of sulfamethoxazole in continuous flow rolling tube biofilm reactors and analysis of the biofilm bacterial community** R. Oliver, Brixham Environmental Laboratory / Brixham Environmental Laboratory; G.H. Panter, Brixham Environmental Lab; G.C. Roberts, Astrazeneca UK Ltd / Brixham Environmental Laboratory; A. Bartram, E. Pratt, R. Sutcliffe, Y. Zhang, Astrazeneca UK Ltd; R.E. Hannah, Temple University / NSF WET Center. The

occurrence of pharmaceuticals in sewage effluents and river water is well documented. However, only a few studies have investigated the removal of pharmaceuticals by microbial biofilms in sewage treatment plants and in the natural environment. Likewise, there have been many investigations into the microbial community in activated sludge plants and in pelagic water bodies, but few have looked at the biofilms in these manmade and natural environments. A study was undertaken to examine the biodegradability of the bacteriostatic antibiotic sulfamethoxazole (SMX), in rich and poor nutrient environments. Continuous flow rolling tube biofilm reactors were seeded and established using either primary settled sewage or river water, dosed with SMX at environmentally relevant concentrations (primary settled sewage: 0, 1.0 and 1.0 to 10 µg/L, river water: 0, 0.1 and 0.1 to 1.0 µg/L; nominal concentrations). The operation of the rolling tube biofilm reactor was monitored by measuring the removal of dissolved organic carbon and ammonia. Microbial community status of the rolling tube biofilm reactor was monitored by measuring heterotrophic plate counts with and without the addition of SMX. Removal of SMX by the rolling tube biofilms was quantified using LC/MS. Throughout the study biofilm samples were taken, DNA extracted, 16S rRNA amplified by polymerase chain reaction (PCR) and separated by denaturing gradient gel electrophoresis (DGGE) to further characterise bacterial community diversity in response to the presence of SMX as well as increasing doses. Removal of SMX was high, but not 100%, in biofilms formed from primary settled sewage (nutrient rich, high bacterial numbers, higher bacterial diversity). SMX removal in the river water biofilm was much lower, as expected, given the conditions (nutrient poor, low bacterial numbers, lower bacterial diversity). Microbial community diversity was similar in primary settled sewage biofilm samples (dosed and undosed). Whereas, the biofilms formed from control river water were not as similar (60% similarity) to the SMX dosed river biofilms. This work shows that SMX exposures at relevant concentrations to trickling filter type biofilms had little effect on performance or community diversity. There appeared to be possible SMX-related differences in diversity (increasing) in the river water biofilms but no strong evidence of increased SMX resistance as a result of long-term low concentration exposures.

**MO025 Aquatic toxicity of the macrolide antibiotic clarithromycin and its metabolites** K. Weiss, M. Baumann, W. Schuessler, W. Kopf, Bavarian Environment Agency; D. Maletzki; C. Kussatz, Federal Environmental Agency / Ecotoxicological Laboratory. Many antibiotics, although on the market for many years, have never been assessed for their environmental exposure and risk. Especially their human metabolites had scarcely been monitored in surface waters nor involved in environmental risk assessments. The human macrolide antibiotic clarithromycin is widespread present in surface waters. Our study shows that its major metabolite 14-hydroxy(R)-clarithromycin is found in surface waters in comparable amounts. This metabolite is known to be also pharmacological active. Additional, clarithromycin is metabolised in lower amounts to N-desmethyl-clarithromycin, which shows no antimicrobial activity. Ecotoxic effects of clarithromycin and its two metabolites on the zebrafish *Danio rerio* (embryo), the microcrustacean *Daphnia magna*, the aquatic macrophyte *Lemna minor*, the green algae *Desmodesmus subspicatus* (Chlorophyta) and the blue-green algae *Anabaena flos-aquae* (Cyanobacteria) were investigated. The environmental risks were assessed in compliance with the TGD-EQS (technical guidance document for deriving environmental quality standards).

**MO026 Direct and indirect effects of the antibiotic enrofloxacin on tropical freshwater microcosms** A. Rico, Wageningen University / Aquatic Ecology and Water Quality Management Group; R.P. Van Wijngaarden, M.R. Dimitrov, Wageningen University and Research Centre; K. Satapornvanit, Kasetsart University; P.J. Van den Brink, Wageningen University and Research Centre. Antibiotics used in human medicine, livestock and aquaculture production are released into the environment by untreated waste water effluents or by the leaching/runoff of agricultural fields, posing a potential risk for aquatic ecosystems. The objective of the present study was to assess the

potential toxic effects of the fluoroquinolone antibiotic enrofloxacin on the structure and functioning of tropical aquatic ecosystems. Enrofloxacin was applied at a concentration of 1, 10, 100 and 1000 µg/L for 7 consecutive days in 600L outdoor plankton-dominated microcosms in Thailand. The experiment was run in duplicate with two untreated controls and had a duration of 4 weeks after the first antibiotic application. The effects of enrofloxacin were assessed on seven ecosystem structural (i.e., macroinvertebrates, zooplankton, phytoplankton, periphyton and bacterial community) and functional (i.e., organic matter decomposition, nutrient cycling) endpoints. The results of the chemical analysis showed that enrofloxacin had a very low persistence in the water column (calculated first-order half-dissipation rate = 15-16h), and about 10% of the applied dose was transformed into its main by-product ciprofloxacin. The evaluation of the dynamics of the invertebrate and primary producer communities did not reveal significant differences between the treated and untreated microcosms. However, antibiotic-related effects were demonstrated in the structure and abundance of bacterial communities inhabiting the water column (NOEC = 1 µg/L) and in the ammonia (NOEC = 100 µg/L) and nitrate (NOEC = 1 µg/L) concentrations during the treatment period, indicating a clear dose-response effect on the abundance and structure of the microbial community and a potential impairment of the related (de-)nitrification processes. The results of this study suggest that enrofloxacin is not likely to result in direct or indirect toxic effects on the invertebrate and primary producer communities at environmentally relevant concentrations. However, alternative endpoints such as nutrient cycling should be taken into account in the aquatic risk assessment of antibiotics.

**MO027 A rapid screen for the assessment of antibiotic toxicity to 3 species of cyanobacteria** G. Le Page, R. Maunder, R. Swarbrick, B. Brown, AstraZeneca, Brixham Environmental Laboratory; J. Snape, Astrazeneca UK Ltd. Antibiotic compounds play a vital role in modern society, notably in human and veterinary medicine, aquaculture and farming. They have been detected in the environment at concentrations expected to cause toxic effects to non-target organisms as well as potentially promote antimicrobial resistant communities. It is therefore crucial that their effects upon ecological health are well understood and carefully considered. Cyanobacteria (blue-green algae) are particularly sensitive to antibiotics and regularly drive the environmental risk assessment of antibiotic compounds. As cyanobacteria are important primary producers in aquatic ecosystems there is a requirement to understand their toxicological response to antibiotics. A rapid 96 well microplate screening method for the determination of the ecotoxicity of chemicals to 3 species of freshwater cyanobacteria, *Anabaena flos-aquae*, *Microcystis aeruginosa* and *Synechococcus leopoliensis* was developed. The screen has been validated and gives highly comparable results with the regulatory approved standard OECD 201 (shake-flask) test guideline and enabling efficient ranking of compounds in order of toxicity. Using standard environmental risk assessment endpoints (NOEC, LOEC, EC<sub>10</sub>, EC<sub>50</sub> and EC<sub>100</sub>), we present the effects of a range of antibiotic compounds, across a variety of antibiotic groups upon the 3 species of blue-green algae. Differences in species sensitivity are also highlighted and discussed.

**MO028 Summary of a Comprehensive Program to Evaluate the Risk of Triclosan in Aquatic and Terrestrial Environments** J. Fort, Fort Environmental Laboratories Inc; M. Mathis, G. Fent, C. Fort, H. Fort, Fort Environmental Laboratories; S. Pawlowski, S. Champ, BASF SE; R. Peter, Intertek Expert Services. A comprehensive program to evaluate the risk of the antimicrobial personal care product triclosan (TCS) in both terrestrial and aquatic environments was performed as an extension of initial ecological risk assessments (ERAs). The terrestrial phase of the evaluation included effects of TCS on soil microflora respiration and nitrification, impact on terrestrial arthropods, acute and chronic toxicity to earthworm (*Eisenia fetida*), earthworm bioaccumulation and effects on emergence and growth terrestrial plant species. The aquatic phase of the evaluation included effects on amphibian (*Xenopus laevis*) development, metamorphosis, and

reproduction; and fish (*Pimephales promelas*) short-term reproduction assay (FSTRA). No Observed Effects Concentrations (NOEC) and Lowest Observed Effects Concentrations (LOEC) for soil respiration and soil nitrification were 2 and >2 mg/Kg (dw), respectively. NOEC and LOEC values for acute and chronic toxicity to earthworms were 1,026 and >1,026 mg/Kg (dw) [survival], and 100 and >100 mg/Kg (dw) [survival and reproduction], respectively. NOEC and LOEC values in terrestrial plants ranged from 75 and 100 mg/Kg (dw) [biomass in lettuce] to 1,000 and >1,000 mg/Kg (dw) [emergence and phytotoxicity in vetch and phytotoxicity in cucumber]. NOEC and Lowest Observed Effects Concentrations (LOEC) values for predatory mite survival and reproduction were 10 and 32 mg/Kg (dw) and 3.2 and 10 mg/Kg (dw), respectively. Steady state tissue residues were achieved on uptake d 14 and resulted in BAFss value of 59.4 and 50.0 (per g tissue dw). The Biota-Soil Accumulation Factor (BSAF) was 69.1 and 41.7 (per g lipid/g TOC) for the 0.1 and 1.0 ?g TCS/g soil treatments. A polynomial fit was applied to the data sets and BAFk values of 0.59 and 1.40 for the 0.1 or 1.0 ?g TCS/g soil treatments, respectively. Based on a 21-d exposure period during the uptake phase, a 14-d uptake of TCS to steady state would result in would in a BAF ranging from 8.3 to 19.6. The NOEC values for the FSTRA, AMA and AGRA studies were 50 ?g/L. Overall, these studies demonstrate that TCS poses minimal risk in either the aquatic or terrestrial environment.

**MO029 Influence of temperature on the toxicity of the veterinary antibiotic florfenicol to *Daphnia magna*: life cycle effects** A. Martins, ICBAS CIIMAR University of Porto / Laboratory of Ecotoxicology and Ecology; L. Guilhermino, Instituto de Ciências Biomédicas de Abel Salazar / Laboratory of Ecotoxicology. Recent studies have been showing that temperature modifies the toxicity of some chemicals, a most important question considering the actual scenario of global climate changes and the increase use of chemicals. Here, the influence of temperature on the effects induced by the veterinary antibiotic florfenicol (FLO) on *Daphnia magna* life cycle was investigated. Chronic (21-day) bioassays were carried out at 15, 20 and 25°C in laboratorial conditions with organisms being exposed to several concentrations of FLO (0.8 to 50.0 mg/L). Effect criteria were somatic growth, reproduction and the intrinsic rate of population natural increase. FLO reduced the somatic growth, reproduction, and the population growth rate of *D. magna* at all the temperatures tested, with more pronounced effects at 15 and 25°C than at 20°C. These results indicate that temperature changes modify the effects of FLO on *D. magna* life cycle highlighting the need of considering temperature variation in the risk assessment of antibiotics. This work was supported by EU-FEDER and national MEC funds through a PhD grant from the Portuguese Foundation for the Science and Technology (FCT) to Alexandra Martins (SFRH/BD/65436/2009).

**MO030 The interaction between food availability and Triclosan in a *D. magna* life cycle test** K. Hammill, P.K. Sibley, University of Guelph / School of Environmental Sciences. Triclosan (TCS) is a ubiquitous compound in wastewater effluents and biosolids and is commonly detected in surface waters throughout North America and Europe. Our current understanding of the potential risk that TCS poses in aquatic systems has largely been informed by individual acute and chronic toxicity studies and little emphasis has been placed on evaluating environmental stressors that could interact with TCS to modify its toxicity. The objective of the present study, therefore, was to evaluate the toxicity of triclosan in relation to food availability in a 21-d chronic test using *Daphnia magna* under laboratory conditions. Neonates (< 24-hr old) of *D. magna* were exposed to three concentrations of TCS (0.2, 1.0, and 5 µg/L) and three levels of food (a mixture of two algal species, *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* at 10 (1.25x10<sup>5</sup> cells/mL), 25 (3.13x10<sup>5</sup> cells/mL), and 100% (1.25x10<sup>6</sup> cells/mL) in a fully crossed completely random design with 10 replicates per combination. Solutions were renewed every other day at which time survival and reproductive output were recorded. No significant effect was observed on survival after 21 d or time to first brood. Fecundity (average neonates per female) was

significantly affected by feeding level (up to 70% reduction) but not TCS. However, there was a significant interaction term indicating that the effect of food availability was dependent on TCS concentration. These data suggest that the toxicity of TCS in aquatic systems is likely to be affected by its interaction with other environmental stressors and that understanding the potential risks of this antibiotic warrants more detailed investigations to elucidate the nature of these interactions.

**MO031 Mixture toxicity of pharmaceuticals detected in treated municipal wastewater to three aquatic organisms (algae, daphnids and fish)** N. Tatarazako, National Institute for Environmental Studies / Environmental Risk; H. Watanabe, R. Abe, National Institute for Environmental Studies; A. Nakamura, Graduate School of Frontier Sciences; H. Takanobu, NIES / Center for Environmental Risk Research; T. Suzuki, Department of Pharmaceutical and Environmental Sciences; A. Hirose, National Institute of Health Sciences / Division of Risk Assessment, Biological Safety Research Center; T. Noshimura, Teikyo Heisei University. A number of pharmaceuticals have been detected in surface waters and their impacts on aquatic organisms have become increasing concern. However, there are no effluent standards and environmental quality standards for pharmaceuticals and guideline for environmental risk assessment is still under development in Japan. To develop testing and assessment framework, selection of appropriate toxicity test for pharmaceuticals and considering the mixture toxicity of pharmaceuticals are important issues. In this study, we used three short-term chronic toxicity tests: algal growth inhibition test (*Pseudokirchneriella subcapitata*), a daphnid reproduction test (*Ceriodaphnia dubia*), a fish short-term toxicity test on embryo and sac-fry stages (*Danio rerio*) to evaluate the biological impact of pharmaceuticals and treated municipal wastewater which contained pharmaceuticals. To evaluate the mixture toxicity of pharmaceuticals, we prepared mixed solution of 14 pharmaceuticals which mainly detected in six treated wastewater samples in Tokyo: mefenamic acid, ketoprofen, diclofenac, etodolac, salicylamide, indomethacin, clofibrate, benzaifibrate, fenofibrate, eponastine HCl, phenytoin, carbamazepine, amantadine, sulpiride. Each pharmaceutical was mixed at 10,000 times concentration detected in the treated wastewater samples, and a series of diluted solutions (1, 10, 100, 1,000 times the concentration) was also subjected to the toxicity test. In the result, reproduction of *C. dubia* and survival of *D. rerio* were affected in the mixture solution at 10,000 times the concentration. On the other hand, algal growth was not inhibited even at 10000 times concentration mixture although the concentrations of mefenamic acid and fenofibrate in the mixture solution were higher than each NOEC values. In addition, we also subject the treated municipal wastewater samples to the toxicity test and chemical analysis in order to estimate the actual contribution of pharmaceuticals to biological impact of treated wastewater. We collected six treated wastewater samples just at the outfall in the urban river. Concentrations of pharmaceuticals were determined by LC/MS and GC/MS. The predicted mixture toxicity of detected pharmaceuticals was calculated by the sum of the individual MEC/NOEC and compared to the whole toxicity of treated wastewater to assess whether the whole toxicity of treated wastewater could be explained by the predicted toxicity of pharmaceuticals.

**MO032 OCCURRENCE OF TETRACYCLINES RESIDUES IN SURFACE SEDIMENTS COLLECTED IN POLISH COASTAL ZONE** g. siedlewicz, IO PAN / Marine Chemistry and Biochemistry Department; M. Borecka, University of Gdansk; K. Pazdro, Institute of Oceanology PAS. Tetracyclines are commonly used for human and animal therapy and for animal breeding. Natural features of the Baltic Sea, like water residence time of around 30 years, its shallowness and particularly large catchment area, make it susceptible to the accumulation of hazardous substances, including new emerging contaminants like pharmaceutical residues. Antibiotics are bioactive compounds acting as antimicrobials thus they may influence the processes carried by marine microorganisms. These microorganisms play a crucial role in nutrient cycling, organic matter mineralization and pollutant degradation. Despite this, the knowledge on antibiotics



occurrence, behavior and fate in the Baltic Sea is very limited. This study reports first measurements and identification of tetracycline class residues occurring in sediments from the southern Baltic Sea. Sediments were collected in years 2010-2012 during s/y "Oceania" cruises along the polish coast (Vistula, Slupia and Oder estuary, Gulf of Gdańsk near discharges of Gdańsk- Wschód and Gdynia Dębogórze wastewater treatment plants, Puck Bay and in the profile from Vistula river to the Gdańsk Deep). The method for tetracyclines residues determination was optimized to achieve the best validation parameters. Finally the extraction with mixture of EDTA and McIlvaine's buffer and SPE technique with Discovery SAX and Oasis HLB was used. Quantitative and qualitative determination of analysed antibiotics were performed with the use of LC-MS/MS. Tetracycline and oxytetracycline were identified in 19% of analysed sediment samples at concentration level in kg of dry weight of the sediment. The obtained results revealed that the residues of tetracycline and oxytetracycline were present in coastal sediments in the investigated area. Particular emphasis should be placed on further investigations concerning the influence of identified compounds on bacterial communities living in Baltic Sea sediments.

**MO033 Preliminary risk assessment of the pharmaceuticals occurrence in two marine ecosystems - Cadiz Bay and Gulf of Gdańsk** g. siedlewicz, IO PAN / Marine Chemistry and Biochemistry Department; E. Kotlarska, Institute of Oceanology PAS; M. Borecka, University of Gdansk / Chemistry Department; K. Pazdro, Institute of Oceanology PAS; I. Moreno, M. Hampel, C. Trombini, J. Blasco, Institute for Marine Science of Andalusia CSIC. Pharmaceuticals are biologically active and relatively persistent substances which have been recently recognized as a continuing threat to the aquatic environment. Numerous adverse effects may arise for aquatic non-target organisms from the presence of pharmaceutical residues. Nevertheless, the knowledge about sources, fate and behaviour of medicines in the environment including marine and estuarine waters is still sparse and limited. The behaviour and thereby ecotoxicity of pharmaceuticals could be influenced by several environmental parameters differing significantly in individual local ecosystems. Hence, in the presented study, the concentration of numerous pharmaceuticals were analysed in abiotic environmental compartments (sediment and seawater) of two different marine coastal ecosystems - Gulf of Gdańsk (Baltic Sea) and Cadiz Bay (SW Iberian Peninsula). The antibiotics were identified as the most relevant compounds in the Gulf of Gdańsk, while carbamazepine and representatives of nonsteroidal antiinflammatory and beta-blocking agents were the most important in the case of the Cadiz Bay area. Following-up these results, the first approach about the risk related to the presence of identified pharmaceuticals in both ecosystems was carried out. The results of microbiological assays applying bacteria isolated from the baltic sediments indicate that the antibiotic concentrations found in the Baltic Sea can affect the bacteria community and thus the functioning of the ecosystem. In the case of Cadiz Bay relevant contaminants toxicity tests for individual and mixtures compounds were applied for the copepod *Tisbe battagliai* and the clam *Ruditapes philippinarum*.

**MO034 A multi-species study to assess the acute toxicity of three pharmaceutical products spiked on sediment samples** L.A. Maranhão, Universidad de Cadiz / Department of Chemistry and Physics; M. Garrido-Perez, Andalusian Center for Marine Science and Technology (CACYTMAR), University of Cádiz; T. DelValls, University of Cadiz / Department of Physical Chemistry; M. Martín-Díaz, University of Cádiz, Center for Marine Science and Technology (CACYTMAR) / Química Física. Pharmaceutical products are potential environmental pollutants and have been found at trace concentrations in water and sediment samples. Sediments might be important as source of pharmaceutical products to the biota and the water. In this study, a series of bioassays was done in a way to evaluate the possible acute toxicity of pharmaceutical products associated to sediments. Clean sediment samples were spiked with five concentrations of three pharmaceutical products: Carbamazepine, Ibuprofen and Propanolol (50?g·L<sup>-1</sup>, 50?g·L<sup>-1</sup>, 5?g·L<sup>-1</sup>, 0.5?g·L<sup>-1</sup>, 0.05?g·L<sup>-1</sup>). Whole sediment samples were

assessed by the bioluminescence inhibition of the bacteria *Vibrio fischeri* (Microtox® Basic Solid Phase Test - BSPT) and mortality rate of 10-d amphipod *Ampelisca brevicornis*. Elutriate samples were evaluated applying the Microtox® Basic Test, the growth rate of the marine microalgae's *Isochrysis galbana* and *Tetraselmis chuii* and the fecundation ratio and abnormal larval development of the sea-urchin *Paracentrotus lividus*. Statistical differences were tested using ANOVA ( $p < 0.05$ ). Concerning the whole sediment, the toxic samples were classified according to the Microtox® BSPT: Carbamazepine>Ibuprofen>Propanolol. Mortality of amphipods was significantly different from the control for Carbamazepine (500?g·L<sup>-1</sup>, 5?g·L<sup>-1</sup>). For elutriate samples, Microtox® Basic Test might represent hormesis. The fecundation ratio of sea-urchin eggs was significantly different from the control for Carbamazepine (5?g·L<sup>-1</sup>, 0.05?g·L<sup>-1</sup>), Ibuprofen (500?g·L<sup>-1</sup>, 50?g·L<sup>-1</sup>, 0.5?g·L<sup>-1</sup>) and Propanolol (all concentrations tested). The abnormal development of sea-urchin larvae was significantly different from the control for Propanolol (50?g·L<sup>-1</sup>, 5?g·L<sup>-1</sup>, 0.5?g·L<sup>-1</sup>, 0.05?g·L<sup>-1</sup>). *I. galbana* and *T. chuii* microalgae's inhibition was significantly different from the control for Carbamazepine (50?g·L<sup>-1</sup>, 5?g·L<sup>-1</sup>, 0.5?g·L<sup>-1</sup>, 0.05?g·L<sup>-1</sup>). *T. chuii* microalgae's inhibition was significantly different from the control for Propanolol (50?g·L<sup>-1</sup>). Based on a wide assessment of potential spiked sediment acute toxicity, the use of different species and sediment phases can be considered as an useful tool for the sediment quality assessment related to pharmaceutical products.

**MO035 A comparative in situ study on ecotoxicological effects of pharmaceuticals in Ireland, using marine mussels (*Mytilus* spp.)** W. Schmidt, European Centre for Environment and Human Health ECEHH / Galway-Mayo Institute of Technology; L. Rainville, Proteomic Research Groups / Department of Biochemistry & Environmental Research, University College Cork.; G. McEneff, Irish Separation Science Cluster (ISSC) / Dublin City University; D. Sheehan, Proteomic Research Groups / Department of Biochemistry & Environmental Research, University College Cork; B. Quinn, Irish Centre for Environmental Toxicology / Galway-Mayo Institute of Technology. In recent decades the amount of pharmaceuticals used and released has constantly increased. One of the main sources of this pollution is through the release of wastewater effluent, both treated and untreated, into the aquatic environment. These novel contaminants can be found now through the developed world, including Ireland. In our field study, we collected blue mussels (*Mytilus* spp.) from a pristine site in the west of Ireland (Lettermullan, Co. Galway). They were caged and exposed at three sites around Ireland for three months in 2010. Cages at site one were based above the wastewater diffuser pipe of Mutton Island wastewater treatment plant (WWTP), a secondary treatment facility serving Galway city with a population equivalent (p.e.) of 91,600. This WWTP treats both municipal and industrial waste. The second location was at the North Bank Lighthouse in Dublin Bay. This lighthouse is based 700m downstream of the Ringsend WWTP, which is a tertiary treatment facility. Wastewater from homes, industries, commercial premises as well as rainfall water is treated in Ringsend, which serves a p.e. of 1,7 million. Omey Island, Co. Galway was the control site for this study. It is a remote island on the Galway coast, with little anthropogenic input. The protein expression signature and other general stress responses were determined by measuring changes in a suite of biomarkers comprising glutathione S-transferase, lipid peroxidation, DNA damage and vitellin-like proteins. Our results contribute to the understanding of whether chronic exposure of pharmaceuticals causes a real threat to aquatic organisms, their habitat and, ultimately, to humans health.

**MO036 The effects of two antimicrobial chemicals, Ciprofloxacin and Triclosan, on target organisms and other marine species** A.J. Smith, Cefas / Ecotoxicology; C. Askem, T. Fisher, Cefas; T. Hutchinson, School of Biological Sciences Plymouth University; J. Brant, Cefas. Until recently there was a shortage of good marine data for assessing the toxicity of chemicals in the marine environment. This study looks at two pharmaceutical/ personal care product chemicals

designed to kill microbes and assesses their effects on other non-target organisms. This research has investigated the acute toxicity of Ciprofloxacin to five marine species: 7 day growth in the red algae *Ceramium*; 96 hour growth in the brown algae, *Fucus*; 72 hour growth in the green algae *Isochrysis*, 72 hour growth in the diatom, *Skeletonema* and 24 hour embryo development in the bivalve mollusc, *Crassostrea*. MARA and LumiMARA bacterial tests were also run on these chemicals to look at effects on target organisms. Tests were carried out according to standard OECD or ISO protocols where available, or from peer reviewed research papers. Ciprofloxacin was not particularly toxic at environmentally relevant concentrations, requiring high mg/l concentrations to reach EC50s. Its effect on microbes was much stronger, but still not particularly worrying compared to measured marine concentrations. Triclosan was much more active at lower concentrations, having effects at low µg/l concentrations for some non target species. These concentrations have been measured in rivers and may be possible in the marine environment. Mode of action is discussed and the relevance of the chemicals ability to affect bacterial populations.

**MO037 Sublethal endpoints for the environmental risk assessment of four selected pharmaceuticals dissolved in aquatic system** G.V. Aguirre-Martinez, Universidad de Cadiz / Chemical Physical; M.O. Awour, South Eastern University College / Department of Applied Limnology and Marine Sciences; M. Garrido-Perez, Andalusian Center for Marine Science and Technology (CACYTMAR), University of Cádiz; T. DelValls, University of Cadiz / Department of Physical Chemistry; M.J. Salamanca, University of Cadiz / Química Física; M. Martin-Diaz, University of Cádiz, Center for Marine Science and Technology (CACYTMAR) / Química Física. The presence of pharmaceutical products in aquatic environment is not only common, but is also of significant concern regarding the adverse effect that they may produce to aquatic biota. In order to determine the adverse effects of caffeine (CAF), ibuprofen (IBU), carbamazepine (CBZ) and novobiocin (NOV), at environmental occurring concentrations, different endpoints were evaluated in four organisms: bioluminescence response in bacteria *Vibrio fischeri*, fertilization and embryogenesis in sea urchin *Paracentrotus lividus* and growth rate in microalgae *Selenastrum capricornutum* (fresh water) and *Isochrysis galbana* (marine). To reach these aims bioassays were performed by exposing organisms to water spiked with drugs dissolved in DMSO (0.001 %). Significant decrease in bioluminescence of *Vibrio fischeri* ( $p < 0.05$ ) with increased concentrations was observed, with pharmaceuticals expressing EC<sub>50</sub> in the following decreasing order CBZ > CAF > IBU > NOV. Regarding sea urchin fertilization results obtained after, exposure to environmental concentrations of drugs (0 - 15 µg·L<sup>-1</sup>) resulted in no significant effect compared to control organisms, whereas fertilization of *P. lividus* significantly decreased with higher range concentrations (0 - 50 g·L<sup>-1</sup>) ( $p < 0.05$ ). Teratogenic effect was observed on *P. lividus* after exposure to environmental concentrations of drugs at 15 µg·L<sup>-1</sup>. Moreover, CBZ was found to cause a 50 % risk to embryo development at 0.01 µg·L<sup>-1</sup>. The growth inhibition rate of microalgae species was observed at concentrations that are not likely to occur within the environment. Growth rate of *S. capricornutum* decreased significantly when exposed to 500 mg·L<sup>-1</sup> of CBZ, CAF and IBU, while *I. galbana* growth rate was significantly inhibited by CAF at 500 mg L<sup>-1</sup>, IBU at 100 mg L<sup>-1</sup> and NOV at 50 mg L<sup>-1</sup>. *I. galbana* is significantly more sensitive to CAF, IBU and NOV than *S. capricornutum* ( $p < 0.05$ ). Endpoints applied in this study showed the necessity of using more sensitive responses, as sea urchin larval development, when assessing risk of pharmaceuticals in aquatic environments, since endpoints applied in current guidelines may not be suitable.

**MO039 Histological changes in the three-spined stickleback after diclofenac exposure** J.M. Naslund, Swedish University of Agricultural Sciences / Department of Biomedical Sciences and Veterinary Public Health; J. Fick, Umea University / Department of Chemistry; E. Ekman, Swedish University of Agricultural Sciences / Department of Biomedical Sciences and Veterinary Public Health; D. Larsson, University of Gothenburg / Department of Infectious Diseases;

S. Orn, L. Norrgren, Swedish University of Agricultural Sciences / Department of Biomedical Sciences and Veterinary Public Health. The non-steroidal anti-inflammatory drug diclofenac has recently received much attention for its presence and associated effects in the environment. Diclofenac exposure and resulting kidney failure is responsible for the well-studied dramatic decline of vultures in India and Pakistan over the past years. In Europe, diclofenac concentrations of around 1 µg/L are frequently found in treated sewage effluents. From laboratory studies in salmonid fish, a range of histological changes in gills, liver and kidney have been reported at exposure concentrations found in the environment. This has led to a widespread concern for detrimental effects of diclofenac in European waterways. As a result, diclofenac has recently been included in the list of priority substances within the European Water Framework Directive. If Environmental Quality Standards for diclofenac come into place, then major investments will be required in wastewater infrastructure. Therefore additional, independent data on effects in other fish species would be valuable to make informed decisions. The three-spined stickleback (*Gasterosteus aculeatus*) is a commonly used fish model in ecotoxicological research. The kidney of sticklebacks plays an important role in male reproduction, as the male kidney produces a glue ("spiggin") used for building a nest for the eggs. Changes of normal kidney function could thus very well lead to reproductive disturbances in this species. We have commenced an exposure study of wild-caught female and male three-spined stickleback to diclofenac under flow-through conditions (17-18°C). No carrier solvents are being used and the nominal concentrations are 0, 0.5, 5, 50 and 500 µg/L. Each aquarium is stocked with 40 fish with three replicate aquaria per concentration. The fish will be sampled after four weeks, and gills, kidney, liver and reproductive organs will be analysed by light microscopy and transmission electron microscopy. Actual exposure concentrations together with whole body levels of diclofenac will be analysed as well using mass spectrometry.

**MO040 Histopathology to assess the health status of wild fish downstream from pharmaceutical manufacture discharge** Cardoso, INERIS / Unité d'écotoxicologie in vitro et in vivo; E. Chadili, INERIS; S. Paris-Palacios, Université de Reims; J. Porcher, W. Sanchez, INERIS. Pharmaceutical Active Ingredients (APIs) are frequently detected in surface waters at levels from 1ng/l up to a few µg/l. Several sources of APIs are identified including effluents from pharmaceutical factories that contain high concentrations of various APIs and are able to disturb the health of aquatic organisms. The aim of present work was to assess adverse effects induced by pharmaceutical factory discharges in wild fish. For this purpose, a multi-organ histopathological approach was performed in liver, gonads, gill, kidney and spleen of fish collected upstream and downstream from four pharmaceutical manufactures. Preliminary results indicated a high level of intersex in gudgeons living downstream from steroid synthesis manufacture that could support disturbance of reproductive function associated to population effects. To complete, these fish exhibited also a set of changes in gill ultra-structure such as epithelial lifting and necrosis, secondary lamellae fusions, erythrocytes stases and mucous cells proliferation that could induce disruption of iono and osmoregulation process. Necrosis was also observed in kidney. Results of histological analysis performed in other investigated sites will be presented here and relationship with industrial production and individual or population adverse effects will be discussed. These first results confirm that chemicals contained in industrial effluents can induce histological adverse effects in wild fish from receiving area. Moreover, this work argues for the implementation of monitoring programs using effect-based monitoring tools such as histopathological approaches. *This work was supported by the French Ministry for Ecology and Sustainable Development (MEDDE-Program 181).*

**MO041 Assessing the impacts of a 5?-reductase inhibitor on reproduction and sexual development in medaka (*Oryzias latipes*)** M.R. Lee, J. Loux-Turner, Smithers Viscient; K. Oliveira, University of Massachusetts Dartmouth / Department of Biology; M.A. Cafarella, Waterborne Environmental Inc / Ecotoxicology. There is no known

published data on the impacts of steroid 5- $\alpha$  reductase (SRD5 $\alpha$ ) inhibitors on fish development and reproduction. The lack of data is likely due to the differences in androgenic sex steroids between fishes and other vertebrates. Steroidogenesis in vertebrates is evolutionarily conserved and initiates with the conversion of cholesterol, by various enzymes, to testosterone (T). T will then either act as an androgen, be metabolized by SRD5 $\alpha$  to dihydrotestosterone (DHT), or be converted by aromatase to estradiol. In most vertebrates (e.g. mammals and amphibians) DHT is a more potent androgen than T. DHT binds to the androgen receptor with a stronger affinity and is responsible for male phenotypic sexual differentiation and maturation. Men deficient in SRD5 $\alpha$  will exhibit partial masculinization at puberty (e.g., testicular descent, penile enlargement, muscle development) with less facial and body hair and smaller prostates. SRD5 $\alpha$  also plays a role in female physiology. In gestating female mice, a SRD5 $\alpha$  deficiency will lead to an increase in the conversion rate of androgens to estrogens. This increased rate will lead to excess estrogen production and ultimately result in fetal death. Also, chronic aqueous exposure to a potent SRD5 $\alpha$  inhibitor (SRD5 $\alpha$ I) has induced intersex (presence of testicular oocytes) and a skewed sex ratio towards females in amphibians. In fishes, 11-ketotestosterone (11-KT) is the androgen analog to DHT and one of the 11-oxygenated androgens responsible for male sexual differentiation. We will utilize a modified partial life-cycle study with medaka to evaluate whether a potent SRD5 $\alpha$ I pharmaceutical impacts the steroidogenesis of 11-KT in fish by evaluating development and reproduction endpoints. The design and results of a modified partial life-cycle study with medaka under continuous exposure to a SRD5 $\alpha$ I will be presented. The primary data endpoints presented will be F0 reproduction, F0 fertilization rate, F1 hatching rate, F1 growth, F1 phenotypic and genotypic sex ratio, F1 whole body histological analysis. This information should be useful in delineating the potential endocrine disruption response in fishes for chemicals which test positive for SRD5 $\alpha$  inhibition in the Hershberger assay.

**MO042 Effects of fluoxetine on the reproductive axis and aggression of the cichlid fish *Cichlasoma dimerus* (Teleostei, Perciformes).** E. Lo Nostro, R. Da Cuña, UBA & CONICET; G. Rey Vázquez, Lab. Ecotoxicología Acuática, DBBE, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires; F. Meijide, Fac. de Ciencias Exactas y Naturales, Univ. de Bue; R. Honji, Instituto de Biociências, Universidade de São Paulo; L. Dorelle, Universidad de Buenos Aires - Facultad de Ciencias Exactas y Naturales / Lab. Ecotoxicología Acuática / Lab. Ecotoxicología Acuática; R. Moreira, Instituto de Biociências, Universidade de São Paulo; G. Genovese, Universidad de Buenos Aires. Pharmaceuticals are increasingly detected in a variety of aquatic systems. Fluoxetine (FLX) -the active ingredient of the antidepressant Prozac<sup>®</sup>- is a selective serotonin reuptake inhibitor which can bioaccumulate in wild-caught fish. This has raised concerns over potential disruptive effects of FLX because of the known role of serotonin in the modulation of neuroendocrine function in teleost fish. Some fish exhibit a wide range of behavioral responses associated with their reproduction. *Cichlasoma dimerus* is a freshwater species that presents social hierarchies, biparental care and complex stereotyped breeding activities; thus it has become an interesting model for reproductive, behavioral and ecotoxicological studies. The aim of this study was to describe the effects of FLX on the reproductive axis and aggressive behavior during breeding of *C. dimerus*. Male and female fish were housed in individual aquaria and received injections of 2  $\mu$ g/g FLX (i.p) every 48 h during 2 weeks. Estradiol, testosterone and cortisol levels were measured in plasma using ELISA. Gonadotropin levels were semi-quantified by Western blot from pituitary homogenates. Liver and gonads were processed for histology. Additionally, aggressive behavior was evaluated using the resident-intruder paradigm. Males of a reproductive pair were injected with saline or 2  $\mu$ g/g FLX (i.p) daily for 3 days after spawning and subjected to the temporary presence of an intruder male. Aggressive interactions from the male and female of the reproductive pair towards the intruder were recorded and quantified. In males, FLX caused a reduction of plasma estradiol. In these individuals no estrogenic effect was evidenced since FLX failed to induce

vitellogenin, a common endpoint for estrogenic disruption. Pituitary FSH and LH content remained unchanged in males; on the contrary, female FSH content was reduced significantly after treatment with FLX. Liver and gonad histology was normal in both males and females. In the behavioral study, both male and female increased aggressive interactions after spawning, regardless of FLX injection. However, of all the behavioral interactions studied, biting and tail beating behavior from males showed variation upon FLX injection, the former increasing and the latter decreasing. Since hatching induces changes in parental care behavior, it would be interesting to evaluate the reproductive axis and the aggressive pattern differentially during each reproductive stage.

**MO043 Back from the brink: Genetics of a recovered fathead minnow population following an estrogen-induced collapse.** L. Postma, Fisheries and Oceans Canada / Freshwater Institute; M. Docker, University of Manitoba / Department of Biological Sciences; R. Bajno, Fisheries and Oceans Canada / Freshwater Institute; L. Johnson, Fisheries and Oceans Canada / Freshwater Institute; K.A. Kidd, University of New Brunswick. A whole-lake experiment in Lake 260 at the Experimental Lakes Area in Ontario, Canada showed that chronic exposure of fathead minnow (*Pimephales promelas*) to low concentrations of the synthetic estrogen used in birth-control pills led to feminization of males and near extinction of the species from this lake (Kidd et al. 2007 PNAS 21: 8897– 8901). The population collapsed in 2002, after the second season of estrogen addition, because of recruitment failure. Reproductive failure was also observed in the third and final season of amendments and for the next two years, with only a few individuals caught each year. By 2006, however, appreciable numbers of fathead minnow were again found. We therefore conducted genetic analysis to determine: 1) the source of these fathead minnow; and 2) whether the population experienced a genetic bottleneck effect. Using 12 microsatellite loci, we analyzed over 450 specimens from Lake 260 and two reference lakes (neither of which are connected to Lake 260) prior to the collapse (1999–2001) and in 2010. This also included samples collected in 2010 from a fourth lake that has the greatest potential to provide a source for immigration of fathead minnow into Lake 260. The genetic results clearly show that the existing fathead minnow in Lake 260 represent recovery of the estrogen-treated population (rather than recolonization from elsewhere). Furthermore, it appears that the observed demographic bottleneck did not produce a lasting genetic bottleneck effect.

**MO044 Evaluation of surface water exposure with Ethinylestradiol by geo-referenced modeling** N. Kehrein, Institute of Environmental Systems Research; J. Berlekamp, J. Klasmeier, University of Osnabrueck / Institute of Environmental Systems Research. Large quantities of pharmaceuticals and personal care products (PPCP) are produced within the European Union. The active ingredients or their metabolites are excreted or washed off in varying quantities and enter the environment mainly via household wastewater. Therefore, PPCPs or their residues are regularly detected in surface and drinking water. Although these residues are usually found in low concentrations in the ng•L<sup>-1</sup> range or even lower, it is largely unknown if they pose a risk to the aquatic environment or human life. Of special concern in this context are endocrine disrupting substances such as the artificial estrogen 17 $\alpha$ -Ethinylestradiol (EE2) which is mostly used in contraceptive drugs. About 47.5 kg of the synthetic hormone EE2 were sold in Germany in 2001. Detecting EE2 in water is a challenge to chemical analysis technologies because of its small environmental concentrations. Predicted environmental concentrations of EE2 are generally in the pg•L<sup>-1</sup> range. Peak concentrations of more than 2 ng•L<sup>-1</sup> in drinking water and groundwater have been reported. Only recently, an Environment Committee of the European Union added three pharmaceuticals including EE2 to the priority substance list of the Water Framework Directive. An environmental quality standard (EQS) in surface water as low as 0.035 ng•L<sup>-1</sup> EE2 is currently under controversial discussion due to uncertainties regarding toxicology and financial concerns. The commonly promoted course of action to reduce environmental concentrations of PPCPs is the refitting of sewage

treatment plants with additional treatment processes. Tertiary sewage treatment steps such as activated carbon filtration or ozonation have been reported to achieve additional substance removal rates of 60 to 80% for EE2. It is therefore important to know whether such a target value could be met area-wide by end-of-the-pipe solutions. Simulations with the geo-referenced exposure model GREAT-ER revealed that such measures are feasible to reduce environmental exposure of EE2 below the proposed EQS under mean flow conditions. However, it is virtually impossible to meet this criterion area-wide in low flow situations. Therefore, we suggest using the model for critical evaluation of action alternatives - both in terms of reduction measures and political parameters such as legally binding target values.

**MO045 Chronic tests of potent anticancer drug metabolites on *Daphnia pulex*** M. Borgatta, Institute of Earth Sciences / Geosciences and Environment; P. Waridel, University of Lausanne, Protein Analysis Facility, Center for Integrative Genomics / Faculty of Biology and Medicine; L. Decosterd, T. Buclin, Division of Clinical Pharmacology and Toxicology / Department of the Faculty of Biology and Medicine; N. Chevre, Img / Faculty of Geosciences and Environment. The 4-hydroxy-tamoxifen (**4-OH-Tam**) and 4-hydroxy-N-desmethyl-tamoxifen (**endoxifen**) metabolites are the result of tamoxifen metabolism by several polymorphic cytochromes P450 enzymes in human. Tamoxifen is an anticancer agent as well as a hormonal disruptor that is largely prescribed worldwide for the prevention and treatment of hormone receptor-positive breast cancers. It can be regarded as a pro-drug, since the pharmacological activity of the 4-OH-Tam and endoxifen metabolites is 30- to 100-fold more potent than tamoxifen. The pro-drug and its metabolites ultimately undergo fecal excretion (through biliary route). As other pharmaceuticals, tamoxifen is known to escape degradation process in wastewater treatment plant (WTP) and enters continuously into surface waters, being therefore considered as a pseudo-persistent chemical. Tamoxifen was measured in WTP effluents and surface water, but to the best of our knowledge no literature exists on the occurrence and effects of the metabolites on the environment despite their high pharmacological activity. In a previous study, tamoxifen and 4-OH-Tam appeared more toxic than endoxifen in acute tests carried out with the *Daphnia pulex* microcrustacean (*D. pulex*). Moreover, after 21 days of exposure, tamoxifen induced abnormalities in offspring and reduced *D. pulex* fecundity. In the study presented here, chronic tests were performed to assess the ability of 4-OH-Tam and endoxifen to induce effects on *D. pulex*. Two generations of daphnids (F0 and F1) were exposed to increasing concentrations of endoxifen or 4-OH-Tam for 21 days and the number of offspring was recorded daily. The fecundity of F0 generation was reduced at concentrations ranging from 11 to 20 µg/L of 4-OH-Tam and from 11 to 39 µg/L of endoxifen. Interestingly, the concentrations reducing the fecundity of F0 generation impacted seriously the survival of F1 generation, in both endoxifen and 4-OH-Tam experiments. The size of mothers was also decreased at these concentrations. These findings underscore the importance of evaluating the effects of metabolites as well as the need of long-term experiments.

**MO046 Environmental fate and toxicity of mitoxantrone and chlorambucil in water** C. Gómez-Canela IDAEA-CSIC; B. Campos, C. Barata, S. Lacorte, IDAEA-CSIC / Environmental Chemistry. The combination of liquid chromatography coupled to mass spectrometry (LC-MS) and toxicity studies with *Daphnia magna* was used to elucidate the behaviour of two cytostatic compounds, mitoxantrone and chlorambucil, in water. Both compounds were rapidly hydrolyzed in water with the subsequent formation of bioactive degradation products. Results obtained evidenced a fast and exponential degradation of chlorambucil within the first 12 h, degradation that was also related with an exponential loss of toxic activity. Nevertheless, despite its fast degradation rates chlorambucil was able to impair *Daphnia magna* survival in a dose related manner at 48 h, which indicates that this compound has delayed toxicity. Conversely, mitoxantrone suffers a rapid change in its conformation losing the two 2-(ethylamino)ethanol chains as evidenced by mass spectrometry but the active toxic

transformation products were unaltered and stable in water along the studied two day period. The present results indicate that neither the concentration of the parental structure, neither dose response toxicity assays allow to correctly assess exposure and toxicity of the studied cytostatic compounds. Both of them rapidly degraded in water thus LC-MS spectrometry methods should target degradation products. Chlorambucil was toxic to *Daphnia magna* even when no residues of the compound neither its degradation products were detected, thus in the field concentrations of this compounds measured in water may not reflect its toxicity.

**MO047 Effect of human pharmaceuticals on hepatic cytochrome P450-mediated reactions in fish** V. Zlabek, Faculty of Fisheries and Protection of Waters; V. Burkina, University of South Bohemia in Ceske Budejovice; T. Randak, University of South Bohemia Ceske Budejovice / Faculty of Fisheries and Protection of Waters; G. Zamaratskaia, Swedish University of Agricultural Sciences / Department of Food Science. Many recent investigations showed presence of wide range of pharmaceuticals in the aquatic environment. However, little is known about the effects of human drugs on metabolic processes in fish. First three families of CYP450 involved in phase I metabolism are oxidative enzymes which play an important role in the metabolism of endogenous and xenobiotic compounds such as drugs, environmental chemicals and etc. *In vivo* or *in vitro* effects of 5 drugs which belong to 4 distinct groups of human pharmaceuticals were studied on fish hepatic microsomes. The *in vivo* effects of sublethal concentrations of atenolol (ATN - beta-blocker) and verapamil (VRP - calcium channel blocker) were studied on CYP450-mediated reactions in juvenile rainbow trout (*Oncorhynchus mykiss*). The following reactions were studied in hepatic microsomes: O-dealkylation of ethoxyresorufin (EROD), methoxyresorufin (MROD) and pentoxyresorufin (PROD), hydroxylation of coumarin (COH), tolbutamide (TB0H), and p-nitrophenol (PNPH), and O-debenzylation of 7-benzyloxy-4-trifluoromethylcoumarin (BFCOD). The measured amounts of products of these reactions did not differ among fish exposed to different levels of VRP or ATN and control fish. This suggests that the levels of ATN and VRP used did not alter catalytic activity of the selected CYP450 enzymes. *In vitro* effects of clotrimazole (CLO - antifungal imidazole) and dexamethasone (DEX - glucocorticoid) were assessed on CYP450 in hepatic microsomes from rainbow trout by investigating the activity of EROD, BFCOD and PNPH. CLO decreased the activity of EROD and BFCOD. EROD activity was non-competitively inhibited and BFCOD activity revealed competitive inhibition. PNPH activity was not affected by CLO. DEX showed no inhibitory potency on any investigated reaction. CLO, but not DEX, interacted with CYP450 and inhibited the catalytic activity by different inhibitory mechanism. Moreover, the effect of antifungal drug ketoconazole (KET) on *in vitro* metabolism of several substrates was studied. KET was a potent competitive inhibitor of BROD (7-benzyloxyresorufin O-dealkylase) and BQOD (7-benzyloxyquinoline O-debenzylation) and non-competitive inhibitor of BFCOD metabolism in the microsomes of Atlantic salmon (*Salmo salar*).

Acknowledgements: This study was supported by the project CENAKVA CZ.1.05/2.1.00/01.0024, Grant agency of USB GAJU 047/2010/Z, Grant agency of Czech Republic P503/11/1130, and Swedish University of Agricultural sciences, NL Faculty.

**MO048 Effects of spironolactone on fish immune capacities** A. Bado-Nilles; S. Betoulle, URCA; B. Gagnaire, IRSN; R. Techer, INERIS; A. Geffard, URCA; J. Porcher, W. Sanchez, INERIS. Spironolactone, which could be shown in aquatic ecosystem, is a pharmaceutical product used to treat many different disorders, from high blood pressure to fluid retention. This molecule could alter the endocrine system, which is in charge of maintain of principal physiological functions such as growth, reproduction, behavior and/or defense capacities. So, the destabilization of endocrine function, by natural and synthetic estrogens and androgens, could modify organism health, especially by impact on immune function due to important bi-directional interactions between immune and endocrine system. Moreover, recent

studies suggest that immune parameters may be used as biomarkers of contamination by endocrine disrupting contaminants. Nevertheless, the specificity of these immune markers must be verified due to many external/internal factors influencing the response. In fact, for example, fish seems to be more sensitive to pathogens during gametogenesis due to seasonal variation in plasma sex

**MO049 Toxic effect of 4 drugs on the macrophyte *Lemna gibba* L.** A.S. Sobrino-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia, Laboratorio Alejandro Villalobos; J. Morales-Torres, Universidad Autonoma Metropolitana Iztapalapa / Ciencias de la salud Lab. Nutricion vegetal. The presence of drugs in wastewater is a problem that has been intensifying since 20 years, because in countries like the U.S., Canada, Switzerland and France have been detected in ppb concentrations. Because these products are designed to be active in ppm or ppb concentrations, can cause deleterious effects to aquatic organisms. The aim of this study was to evaluate the toxic effect of four commonly used drugs (aspirin, paracetamol, naproxen and diclofenac) on the macrophyte *Lemna gibba*. bioassays of 96 hours duration were conducted to determine the EC<sub>50</sub> (effective concentration 50), is the concentration which inhibits population growth, were also evaluated chlorophyll and carotene production and the level of lipoperoxidation by assessing the concentration of MDA (Malondialdehyde). The EC<sub>50</sub> varied from 17.42 to 1471.6 mg/L the most toxic compound was<sup>50</sup> naproxen. It was observed a decrease in the levels of chlorophylls, 31%, 18%, 3.18% and 8.5% for the tests with acetylsalicylic acid, paracetamol, naproxen and diclofenac respectively. MDA levels were high in organisms exposed to drugs compared to the control group. Because in Mexico only 14% of the wastewater produced receive some form of treatment, and has not evaluated the concentrations of these drugs in these, it is important to continue to make assessments of the effects of these drugs for the purpose of proposing appropriate management measures to reduce the risk for the presence of these compounds in aquatic systems.

**MO050 Endocrine Disruptive Effects of the feedlot contaminant 17 $\alpha$ -trenbolone on the Australian Murray River rainbowfish (*Melanotaenia fluviatilis*)** A. Miranda, S. Admane, J. Rodrigues, RMIT University; V.J. Pettigrove, The University of Melbourne / Zoology; D. Nugegoda, RMIT University / School of Applied Sciences. Trenbolone acetate (TBA) is a synthetic androgenic steroid hormone administered as a subcutaneous implant for growth promotion in beef cattle. The primary metabolite excreted in manure from implanted cattle is 17 $\beta$ -trenbolone with lesser amounts of 17 $\alpha$ -trenbolone and trenbolone also present. The aquatic environment, where these metabolites are known to be very stable, is potentially exposed via direct discharge, runoff, or both. The androgenicity of 17 $\alpha$ -trenbolone on adult Murray River Rainbowfish (*Melanotaenia fluviatilis*) was evaluated in a laboratory study. Adult female and male rainbowfish were exposed for 21 d to two different concentrations of 17 $\alpha$ -trenbolone: 40 and 400ng/L. Assessment endpoints included measurement of vitellogenin (Vtg), estradiol and testosterone concentrations in female and male adult fish plasma, aromatase mRNA expression and gonad and liver morphology as endpoints at the end of the 14 day experiment. Vitellogenin mRNA and plasma protein concentrations were evaluated using both q-PCR and an indirect ELISA. Concentration of both estradiol and testosterone was measured using a commercially available EIA kit. In females exposed to 17 $\alpha$ -trenbolone a reduction of plasma vitellogenin and steroid concentrations was observed as well as morphological changes with intensification of colour i.e. a darker band on the dorsal and ventral fins, usually characteristic of males. Analyses of gonadal morphology revealed increased testicular area and sperm percentage in trenbolone exposed male fish, and an increased number of atretic oocytes in exposed female fish.

**MO051 Ecotoxicological study of micropollutants through several bioassays** E. Zuriaga, Universidad San Jorge; B. Giner; L. Lomba, R. Pino, Universidad San Jorge. Nowadays, there are at least 3000 Pharmaceuticals Active Compounds (PhACs) available for human

medicine. The presence of drugs in the environment has become a recent research topic because they have been found in different kinds of aquatic compartments such as groundwater, surface and drinking water [1]. Due to the concentrations which have been found, pharmaceuticals are a class of emerging environmental contaminants called micropollutants. The main problem of the presence of these drugs in the environment is their stability, persistence, and capacity to act on different organism. [2] The knowledge of ecotoxicity is very limited in most of drug families. Only some PhACs have been measured for acute toxicity but there are no systematic and complete studies [3]. For this reason, we have been studied several PhACs belonging to two different families (beta-blockers and estatines). These products are widely consumed at present due to an increase of cardiovascular diseases or hyperlipidemias. Some of these drugs, as propranolol or metoprolol, has been detected in rivers in maximum concentrations of 0.59 and 2.2 ug/L, respectively [4]. However, there is no enough information about their toxic effects in aquatic and terrestrial ecosystems. In this work, we present a part of our research project which is based on the physicochemical and ecotoxicological characterization of several products such as solvents or drugs. In this case, we are focusing on the ecotoxicity of several drugs. To carry out this study, different bioassays have been used. Firstly, the standardized toxicity test using *Vibrio fischeri* [5] and secondly, the standardized toxicity test using earthworms *Eisenia fetida* [6]. Furthermore, it is worth mentioned that the standardized bioassay with earthworms has not been used to evaluate ecotoxic effects of human pharmaceuticals before. From the experimental results, we can conclude that betablockers are less toxic than estatines in terrestrial organism, being the most toxic simvastatine followed by lovastatine. In case of aquatic media, the results obtained for betablockers show a big variability depending on the drug structure being the most toxic the propranolol.

**MO052 Assessment of the effect of 3 analgesics on *Daphnia magna*.** Straus A.S. Sobrino-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia, Laboratorio Alejandro Villalobos. The pain medications are products that are sold freely and they are more often substances eliminated aquatic systems. In countries like USA, Canada, Sweden, France have been detected in wastewater at concentrations of ppb. These medications can cause adverse effects on aquatic organisms, due to they are designed to have a physiological effect at very low concentrations. The aim of this study was to evaluate the toxicity of 3 analgesics (Acetylsalicylic acid, paracetamol and naproxen) in *Daphnia magna*. Static Bioassays were carried out with a duration of 48 hours, where 5 drug concentrations were tested to determine the LC<sub>50</sub>, to the surviving organisms were evaluated your recovery, to move them to cameras without xenobiotics. LC<sub>50</sub> values varied from 0.02 to 0,150 mg / L. The most toxic compound was<sup>50</sup> the Ac. Acetylsalicylic. Organisms exposed to sublethal concentrations (less than CL<sub>10</sub>) during the recovery period, showed high levels of lipid peroxidation compared to those observed in the control group, they were not reproduced and died between the second and fourth days. The results show that the tested drugs have harmful effects in sublethal concentrations.

**MO053 Bioconcentration of Pharmaceuticals and Personal Care Products in blood plasma of Zebrafish (*Danio Rerio*)** F. Chen, National University of Singapore / Graduate School of Integrated Sciences and Engineering; Z. Gong, B.C. Kelly, National University of Singapore. The occurrence of pharmaceuticals and personal care products (PPCPs) in the environmental has received increasing interest in recent years. The bioconcentration behavior of PPCPs in aquatic organisms is not well documented. This study involves laboratory investigations to assess the bioconcentration behavior of PPCPs in zebrafish (*Danio rerio*). We conducted a continuous flow-through exposure experiment to assess the bioconcentration kinetics of several PPCPs in blood plasma of adult female zebrafish. Bioconcentration experiments involved 8 days of aqueous exposure, followed by an 8-day depuration phase, at high and low exposure concentrations. For exposure experiment, fish were collected at six time-points during uptake phase and five time-points during the depuration phase. Blood were collected

from individual fish and pooled into composite samples (five fish per composite). The blood samples were extracted and cleaned up by sonication and solid phase extraction (SPE). Determination of test compound concentrations was conducted by analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS). Observed bioconcentration factors (BCFs) varied among test compounds and ranged from approximately 0 to 116 for the various PPCPs investigated. The results are further evaluated to assess the role of key biological constituents (proteins, phospholipids) and influence of octanol-water and protein-water distribution coefficients (Dow, Dpw) on bioaccumulation potential of PPCPs in aquatic organisms.

**MO054 Seasonal Monitoring of Emerging Substance in the Danube River and Risk Assessment** N. Grujic Letic, Pharmacy; N. Milic, M. Milanovic, Medical Faculty, University of Novi Sad; M. Turk Sekulic, J. Radonic, M. Vojinovic Miloradov, I. Mihajlovic, Faculty of Technical Sciences, University of Novi Sad. Caffeine, an emerging substance, is found to be a good indicator for human sewage because of its relatively high concentrations in surface water and its unambiguous anthropogenic origin. It is considered to be one of the most commonly consumed drugs with more than 80 percent of the world's population consuming caffeine daily. The Europeans are found to be the world's largest consumers of caffeine intake of approximately 4.6 kg/person/year. The objectives of this study were to monitor seasonal caffeine concentrations and to evaluate potential risk on aquatic organisms during a one-year period. The samples were collected from seven representative locations of the Danube River on the territory of Novi Sad, Serbia. Seasonal caffeine concentrations were determined by reversed phase High-performance liquid chromatography (RP HPLC) and Maximum Risk Indexes (MaxRIs) for each sampling site were calculated. The mean caffeine concentrations for summer, autumn, winter and spring periods were 24.78 ng/L, 26.83 ng/L, 24.61 ng/L, and 86.29 ng/L, respectively. The highest caffeine concentrations were found at sampling sites near direct human impact (15.91-306.12 ng/L). The levels of potential risk for the river system were divided into three classes: Class I or high risk with MaxRI < 10; Class II or sublethal effects on the aquatic organisms with 10100. The results showed that sampling sites far away from direct human impact had no potential risk for the aquatic organisms. MaxRIs calculated for sampling sites 100 meters downstream of each sewage discharge ranged 20.27-71.66 (Class II). It can be concluded, according to MaxRIs, that the potential risk for the chronic effects may occur in the resident organisms in the long-term period. The work was financially supported by Ministry of Education and Science, Republic of Serbia (III46009) and NATO Science for Peace Project (ESP.EAP.SFP 984087).

**MO055 Rational selection of alternative, environmentally compatible detergents for biotechnological production of pharmaceuticals** J. Straub, F. Hoffmann, La Roche Ltd / Roche Group Safety Health Environmental Protection; R. Shearer, Genentech Inc., a member of the Roche Group; M. Studer, F. Hoffmann La Roche AG. Biotechnological production of pharmaceutical active substances needs ancillary substances for various purposes. Detergents are used at the end of the cell growth and synthesis phase, to lyse the producing cells, so as to liberate the synthesis products for isolation and purification, and at the same time as a protection against potential viral or bacterial pollution. In order to replace a detergent that had raised environmental concern we proceeded in the following fashion. Potential alternatives were tested at a contract lab according to OECD test guidelines and under GLP quality assurance: ready biodegradability, algal growth inhibition, both acute daphnid immobilisation and chronic daphnid reproduction toxicity, fish acute toxicity (in a limit-test/step-down procedure to use a lower number of fish) and activated sludge respiration inhibition test. The results were used to assess biodegradability and model removal in the wastewater treatment plants serving three Roche Group biotechnological production sites. Predicted no-effect concentrations (PNECs) were derived following the European Technical Guidance Document, both for fresh and marine surface waters, as the treated wastewater of one of the production sites is

discharged to a coastal receiving water. Last, predicted environmental concentrations (PECs) were modelled using realistic amounts of both detergents, total wastewaters, modelled removals for the respective wastewater treatment plants and site-specific dilution factors by the receiving waters. This resulted in a spreadsheet showing PECs, PNECs and PEC-PNEC risk characterisation ratios for the wastewater treatment plants and receiving waters for the three sites. This spreadsheet, with the alternative detergents ordered by environmental compatibility and site, now serves as the final decision base for the biotechnological producers.

**MO056 Formation of stable transformation products of pharmaceuticals in the water treatment processing.** Weiss; R. Bolek, Leuphana University; V. Boireau, Veolia / Environment Recherche et Innovation SNC; B. Roig, Advanced School of Public Health / LERES; M. Lamoree, Chemistry & Biology. After human consumption, pharmaceutically active substances can be excreted and enter the effluent treatment facilities. Often degradation in sewage and water treatment and the environment is incomplete, resulting in the formation of stable transformation products. In only a few cases, full mineralization of the parent compounds is achieved. This is even more important as advanced oxidation techniques employing e.g. ozone, hydrogen peroxide, light or electro-coagulation are subject of discussion for the removal of pharmaceuticals in effluent treatment and drinking water treatment. Treatments using these techniques may even lead to the formation of transformation products that may be more toxic than the parent compound. Within the Pharms project (EU grant agreement no. 265346) a selection of drugs of a pharmaceutical class which has been poorly investigated, i.e. the anticancer drugs, were evaluated for the formation of transformation products. The three compounds selected are 5-FU, Imatinib and Cyclophosphamide. The formation of stable transformation products was investigated in various stages of the water treatment cycle (both drinking and sewage) in laboratory scale studies. Treatment processes include mainly chlorination, ozonation and UV-disinfection for drinking water treatment and advanced oxidation, photolysis/photocatalysis/photo-fenton for sewage treatment. For structure elucidation of the stable transformation products formed, different LC-MS/MS approaches as well as high resolution MS techniques were implemented. In addition, target analysis methods will be developed and the presence of these stable transformation products assessed in the environment. The knowledge on specific transformation product formation pathways, the molecular identity and (eco)toxicological behavior is expected to ultimately lead to recommendations for the targeted design of pharmaceuticals with improved degradation and elimination properties, whilst maintaining their therapeutic value.

**MO057 Development and application of a focused microwave assisted extraction protocol for the quantification of pharmaceuticals in solid matrices** y. aminot, UMR CNRS 5805 EPOC-LPTC; P. Pardon, University of Bordeaux / UMR CNR 5805 EPOC-LPTC; H. Budzinski, University of Bordeaux / UMR CNRS 5805 EPOC-LPTC. Pharmaceuticals have now been clearly identified as emerging contaminants of the water compartment. Numerous publications present analytical methods for the determination of drug residues in water, giving the possibility to study the occurrence and the fate of these molecules from their source to the receiving environment. However, considering their assumed hydrophilic properties, only a little number of studies takes into account the solid phase (sludge, particulate matter, sediment or soil), that can lead to an under-estimation of the contamination. For instance, Silva et al. (2011), showed a 100% speciation in particulate matter of 8 of the 30 detected pharmaceuticals in the Ebro river. Complete occurrence and fate studies have to characterize the speciation of the target molecules, at least on preliminary analyses. In this communication, a method for the determination of 53 pharmaceuticals in solid matrices is proposed. Solid matrix is freeze-dried straight after sampling and extracted by focused microwave assisted extraction with a mixture of pH2 deionized water and acetonitrile. After evaporation of the organic part, the extract is considered as a water sample and undergoes a purification on Oasis

MCX SPE cartridge. The final extract is analysed on LCMSMS, quantification being achieved by isotopic dilution. This protocol was applied to particules and sediments from Jalle de Blanquefort river, a low-flow 34 km river located in the Bordeaux urban area, France. This river, impacted by a 85 000 population-equivalent Waste Water Treatment Plant (WWTP) was sampled upstream and downstream the discharge point in order to qualify the impact of the continuous release of pharmaceuticals. Speciation between water, particulate matter and sediment has been evaluated. In sediment, the  $\beta$ -blockers exhibit the highest concentrations: propranolol is quantified up to 60 ng.g<sup>-1</sup> and acebutolol up to 20 ng.g<sup>-1</sup> after the discharge point. A simple ratio between sediment concentration and water concentration straight after the discharge point gives the molecules likely to bind to sediment and particules: among them, ritonavir, propranolol, imipramine, amitriptyline, fluoxetine and sildenafil are the most notable. Acknowledgement: The Etiage program (Agence de l'Eau Adour-Garonne, the CUB (Communauté Urbaine de Bordeaux) and Lyonnaise des Eaux), ARS (Agence Régionale de la Santé) and CPER A2E (FEDER and Région Aquitaine) are acknowledged for financial support.

**MO058 External genitalia development in male and female rats given flutamide in utero** Y. Ohta, Tottori University; S. Miyagawa, Okazaki Institute for Integrative Bioscience; T. Iguchi, Nat'l Institutes of Natural Science. Hypospadias is the most common malformation in males, which may be related to abnormalities of androgen production and timing or receptor function during male sexual differentiation during pregnancy. Flutamide, a potent nonsteroidal androgen receptor antagonist, has been used therapeutically to treat androgen-dependent prostate cancer and as a tool to study male reproductive development. Since flutamide is known to induce hypospadias when given pregnant rats, development of external genitalia was examined in male and female rats given oral administrations of flutamide (45 mg/kg/day) from pregnant days 12 to 22. External genitalia dissected out from males and females (embryos, neonates and adults) were fixed in Bouin's solution and cut in paraffin for histological examination. Each part of the genitalia in serial sections was measured with a micrometer. Measurement of a major component of phallus revealed that males given flutamide mimicked the control females in fetal development of the genitalia, the urethra development involving urethral openings in males given flutamide being similar to that of the control females. Although adult males aged 60 days given flutamide in utero showed severe hypospadias, male neonates showed no sign of hypospadias, as well as females given flutamide *in utero*. These results indicate that flutamide exposure in utero induces female type genitalia in male neonates. Hypospadias seems to develop after puberty in males given flutamide in utero.

**MO059 Investigation of the accelerated degradation of atrazine in soil using next generation sequencing technology** R.L. Yale, University of York / Department of Biology; C. Sinclair, The Food and Environment Research Agency / EcoChemistry; R. Thwaites, The Food and Environment Research Agency / PPPA; J. Moir, University of York / Department of Biology. Following the repeated application of pesticides to soil, the rate of their dissipation has been shown to increase, this process is termed accelerated degradation (AD). AD has implications for the environmental exposure of pesticides. During the risk assessment process, dissipation rates are determined from soils which have previously not been treated with pesticides. AD has been proven to occur for a large number of pesticides, although its microbial basis has not been fully investigated. In this study multiple applications of the herbicide atrazine to an agricultural soil that had not previously been treated with the pesticide or its analogues demonstrated the occurrence of AD. The rate of dissipation was more rapid after each application with the first atrazine application having a DT<sub>50</sub> of 15.9 days and third application DT<sub>50</sub>. In parallel the microbial communities between control and treated soil sub-samples was assessed by 454 pyrosequencing of the 16S rRNA gene of bacteria present after the third atrazine spike. Sequencing results suggest that atrazine treatment may have had an effect on the structure of the bacterial community.

Specifically there was an increase in abundance of the gammaproteobacteria, members of which are known atrazine degraders. These changes could imply that atrazine is acting as a selective pressure in favour of the bacterial classes which are capable of degrading atrazine. Analysis of additional time points during the AD process will enable the community shifts that are most instrumental in mediating AD to be assessed. Keywords: Accelerated Degradation; Microbial community; Adaptation

**MO060 Organic pollutant effects to *Prochlorococcus* sp. at the gene expression level** M. Fernández-Pinos, M. Casado, Environmental Chemistry; E. Zinser, University of Tennessee / Department of Microbiology; B. Pina, J. Dachs, IDAEACSC / Environmental Chemistry. Hydrophobic, persistent, and semivolatile organic pollutants have the potential for long range atmospheric transport and are eventually deposited to the ocean, where they can bioaccumulate and be toxic for aquatic organisms. In the case of phytoplankton decrease of abundance, growth rate and chlorophyll a concentration have been highlighted, thus there is a potential influence of organic pollutants on net primary productivity, thus on ecosystem function. In addition, these effects have been demonstrated to be directly related to the cell volume, being smaller organisms more sensitive due to a higher surface to volume ratio. Therefore the cyanobacterium *Prochlorococcus*, which is the smallest known photosynthetic organism, is considered one of the more affected organisms by a variety of organic pollutants. The fact is that this genus is numerically dominant in the photosynthetic community of the tropical and subtropical regions of the world's oceans and its contribution to primary production is very important. The goal of the present study was to assess the possible effects of different organic pollutants mixtures at sub-lethal levels on the regulation of some genes responsible of photosynthesis. These experiments are part of a larger effort to evaluate the impact of organic pollutants in the oceanic ecosystem functions mediated by phytoplankton, specially primary production. For this aim, we performed lab experiment using pure cultures of two different strains of *Prochlorococcus* that were challenged with either a PAHs mixture or a HCB+HCH mixture. Gene expression of *rbcl* and *psbA* were quantified by quantitative Real Time PCR (qRT-PCR) technique. The results show a systematic decrease on *rbcl* transcripts due to the pollutant exposition in both strains, whereas *psbA* transcript levels remained unaltered. Any effect on cell density or division rate is observed. The data could be interpreted as a drop of phytoplankton carbon fixation caused by the exposition to organic pollutant present in seawater, which could mean together with the previously observed drops in cell abundance and growth rate an important impact on the global carbon cycle. Key words: organic pollutants, carbon cycle, *Prochlorococcus*, gene expression. **Acknowledgements** – This work was funded by the Spanish Government through the Malaspina project. MC Fernández-Pinos acknowledges a predoctoral fellowship from the Spanish National Research Council (CSIC).

**MO061 Gene expression analysis of the genes involved in oxidative stress to explain DNA damages in organisms exposed to cadmium/lead contaminated soils** E. BERNARD, LGCgE Université de Lille / LGCgE, University of Lille 1; S. Lemiere, University of Lille / Nord; S. DUMEZ, F. BRULLE, PRES Lille University North of France / LSVF, University of Lille 2; C. COCQUERELLE, PRES Lille University North of France / LGCgE, University of Lille 1; A. PLATEL, F. NESSLANY, PRES Lille University North of France / PASTEUR Institute of Lille, Toxicology Laboratory; D. CUNY, PRES Lille University North of France / LSVF, University of Lille 2; A. Deram, University of Lille / LSVF/ILIS University of Lille 2; F. VANDENBULCKE, LGCgE Université de Lille / LGCgE, University of Lille 1. Concerns about soil and sediment pollution are recent. Degradation of these matrices may present risks to human and environmental health, including possible contaminant transfers in food webs or to groundwater. Effects of pollutants on living organisms can also spoil DNA structure and thus generate damage. For instance, cadmium is a well-known genotoxic and mechanisms



explaining its clastogenicity may be mainly indirect: induction of Reactive Oxygen Species (ROS) and/or inhibition of DNA repair mechanisms. In order to better understand the potential link between ROS and DNA damages *in vivo*, we performed comet assay and analysed the gene expression level of most genes involved in oxidative stress in 3 test species living in close contact with soil and often used in ecotoxicology as model. Test species (the worm *Eisenia fetida*, the white clover *Trifolium repens* and the cabbage *Brassica oleracea*) have been exposed *in vivo* (3, 10, 56 days) in a well-characterised reference soil spiked with cadmium or/and lead at environmental concentrations. DNA damage has been estimated using comet assay which is a technique of microelectrophoresis of single isolated cell nuclei highlighting single and double strand breaks, alkali-labile sites in a cellular population. Comet assay has been developed and validated on cœlomocytes (immune cells) of *Eisenia fetida* and on leave cells of *Trifolium repens* and *Brassica oleracea*. In complement to comet assay, some oxidative stress enzyme activities have been measured. Therefore, compared to enzymatic approach, gene expression allows distinction between individual enzyme involvement in oxidative stress. The newest part of the work was the analysis of the gene expression level of most genes involved in oxidative stress in the 3 species. The comet assay allowed detection of DNA damage in organisms exposed to a soil spiked with cadmium or/and lead. The tools used (comet assay and measurements of gene expression) have shown interest in studying the effects of metal stress in several species. A major interest of this work lies in the measurement of most genes involved in oxidative stress at the same time and in several species. Indeed, a detailed analysis of the expression level of genes involved in oxidative stress and in detoxification mechanisms can be used to gain knowledge about the mechanistic response of organisms to stress and to better understand the link between damage to DNA and oxidative stress.

**MO062 Global protein expression in liver tissues of non-human primates exposed to arsenite** D. Ryu, College of Veterinary Medicine Seoul Natl Univ / College of Veterinary Medicine; J. Park, Chung-Ang University / College of Medicine; S. Kim, Seoul National University / College of Veterinary Medicine. Chronic exposure of arsenic in drinking water is associated with an increased risk of developing diseases, such as diabetes and cancer. However, the underlying molecular mechanisms have not been fully elucidated thus far. In this study, effect of arsenic on protein expression was studied using a proteomic approach in the liver tissues of cynomolgus monkeys (*Macaca fascicularis*). Monkeys were administered with sodium arsenite in their drinking water for 4 weeks. Proteins differentially regulated by arsenic treatment were identified using two-dimensional gel electrophoresis in combination with mass spectrometry. Over 1000 protein spots were separated by the electrophoresis and visualized by Coomassie Blue. Among them, four proteins were found to be significantly up- or down-regulated in liver tissues following arsenic treatment. The proteins down-regulated were phosphoenolpyruvate carboxykinase (PEPCK-M), cystathionine beta-synthase (CBS) and selenium binding protein (SBP), while mortalin (heat shock 70 kDa protein 9) was up-regulated in the liver tissues of arsenic-treated monkeys. Regulation of the four protein expression was confirmed by western blots. In addition, those proteins were also found to be similarly regulated in human hepatoma cells following treatment with sodium arsenite. Based upon functional roles of the proteins, it is suggested that regulation of the protein expression is involved in arsenic-induced disturbance of glycometabolism and increased risk of cancer.

**MO063 Development of Transgenic Green Fluorescent *Daphnia magna* to Detect Toxic Materials and Its Application to Portable System** T. Le, Chonbuk National University / Dept. of Bioprocessing Engineering; Y. Kim, Chonbuk National University / School of Life Science; J. Min, Chonbuk National University / Division of Chemical Engineering. A transgenic water flea, *Daphnia magna*, expressing a green fluorescent protein has been created to rapidly detect various types of environmental toxicity, especially heavy metals. A 32bp-DNA promoter of daphnia 18s ribosome was generated by *in vitro* annealing

the commercial primers at 80°C. This promoter fragment (pD18s) was inserted and replaced the CMV promoter that was located ahead to the *gfp* gene in the EGFP vector (Enhanced Green Fluorescent Protein). The successful recombinant plasmid confirmed by the DNA sequencing was introduced into the earlier developing-state daphnia eggs (round shape) using microinjection technique. After 48h incubation in the 20°C chamber, the injected eggs able to develop to juveniles were observed at the 488nm wavelength by the confocal fluorescent microscope to confirm the presence of green fluorescent protein. Our results show that the green fluorescent protein was substantially found in the upper part of the transgenic organisms (e.g., head, back) while no fluorescent signal was detected in the control organism. To develop a portable detection kit, a LED system, a Fluorescent Photomultiplier tube (PMT) and a fluorometer are basically employed for supplying a fluorescent source, amplifying the fluorescent signals from transgenic daphnia, and reading the outcome signals, respectively. The feasibility of the kit is studied through measuring the outcome fluorescent signal which is originally started from the LED system, then passed through transgenic organisms, amplified in the PMT, and finally read by the fluorometer. Basically, the toxicity of toxic materials inhibits the pD18s promoter activity which leads to the reduction of the green fluorescent protein in the exposed transgenic organisms compared to the control transgenic daphnia. Some key parameters including exposure time, LED intensity, and toxic chemicals concentrations are being optimized to improve the sensitivity of the portable kit to detect heavy metals.

**MO064 Effects of environmental factors on chronic toxicity of cyanobacterial microcystin toxin to water flea *Daphnia magna*** J. Kim, J. Seo, T. Kim, National Institute of Environmental Research / Risk Assessment Division; A. Jo, School of Public Health, Seoul National University / Department of Environmental Health; P. Kim, National Institute of Environmental Research; K. Choi, National Institute of Environmental Research / Risk Assessment Division. In water, environmental parameters such as temperature, precipitation, pH, salinity, and UV light irradiation are being altered as consequences of global climate change. These environmental parameters are important in that they might interact with toxicants and could affect the concentration-response relationship of the toxicants in the water environment. Microcystin produced by algal blooming is natural toxin that has reproductive as well as acute toxicity to mammal. Despite its hazardous potential, little is known about reproductive toxicity of microcystin to aquatic organisms. In the present study, we evaluated the effect of two environmental factors, water pH and temperature on chronic toxicity of cyanobacterial microcystin-MR, using freshwater invertebrate *Daphnia magna*. The 21-d *Daphnia* chronic toxicity tests were conducted under the various conditions of water temperature (15, 21, and 25°C) and pH (7.4, 8.3, and 9.2). Since chronic reproduction *Daphnia* toxicity test requires longer exposure and greater cost we evaluated the mRNA expression patterns of double sex and vitellogenin genes in *D. magna* to investigate whether mRNA expression of reproduction-related genes could be used rapid biomarker indicating reproductive effect of *D. magna*. With the preliminary data, we found that changing environmental conditions could affect exposure and concentration-response profile of microcystin and therefore, such conditions should be identified and evaluated in order to better manage ecosystem health under changing global environment.

**MO065 Gene expression of the soil worm *Enchytraeus albidus* when exposed to Zn, Cu, Cd and Ni: mechanisms of response** S.L. Gomes, University of Aveiro / department of Biology & CESAM; S.C. Novais, University of Aveiro / CESAM & Dept. of Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; M.J. Amorim, Universidade de Aveiro / Department of Biology and CESAM. *Enchytraeus albidus* has been used in soil ecotoxicology (ISO, 2004; OECD2, 2004) for many years. More recently, the development of a gene library and an oligonucleotide microarray for *E. albidus* allowed gene expression studies. This has potentiated the means to study further in depth the mechanisms of response to chemicals. In the present study,

the test substances included both essential and non-essential metals. Concentrations tested represented the reproduction effect concentration for 50 and 90% (EC<sub>50</sub> and EC<sub>90</sub>). Organisms were exposed to the reproduction EC<sub>50</sub> and EC<sub>90</sub> of <sup>60</sup>Cu, Zn, Cd and Ni for 4 days. The gene expression analysis was performed through hybridization of fluorescently labelled probes from exposed and control organisms following a microarray experiment. Data was normalized together. Hierarchical clustering analyses showed a separation of Cd from all other metals; this separation occurred for both Cd concentrations. This could be due to the fact that for Cd the EC50?LC40, i.e., organisms survival was affected at similar concentrations as reproduction. In regard to the other metals, Zn EC50 and EC90 clustered separate from Cu and Ni. In regard to Cu and Ni, clustering was EC specific, which could suggest more similar mechanisms. Biological processes affected by each metal were identified and compared, e.g. translation impairment is one of the mechanisms commonly affected by Zn, Cu and Ni, while Cd causes impairment in transcription. This study indicated that the response of *E. albidus* to metals was not separated between essential and non-essential (Cd and Ni were distinct and Ni was more closely related to Cu). The identified mechanisms seem to be metal and dose specific despite similarities. The actual concentration differences (not the ECs), which are *per se* already an indication of differences in modes of response, could be a good starting point for grouping. Further, the chemical kinetics in the organism seems to be relevant in this context.

**Key words:** Oligochaeta, essential metals, non essential metals, transcription

**MO066 Silver response in *Chlamydomonas reinhardtii*: a metabolomic approach** N. Lamari, Eawag / Environmental Toxicology; R. Behra, M. Suter, Eawag. Xenobiotics are accumulating in the environment as a result of anthropogenic activity. Among the numerous data dealing with the effects of metals in algae, the molecular response of the unicellular alga *C. reinhardtii* to silver exposure still remains mostly unclear. Recent evidence suggests that reactive oxygen species induction by heavy metals negatively affects the physiological homeostasis of algae, ultimately leading to inhibition of photosynthesis and growth. Although the silver ion (Ag<sup>+</sup>) has long been known to display toxicity to a broad spectrum of bacteria, it is also among the most toxic metals for various other aquatic organisms. For instance, an on-going transcriptomic and proteomic analysis of *C. reinhardtii* exposed to Ag<sup>+</sup>, shows that transcripts and proteins involved in redox and metal homeostasis are strongly regulated (Pillai et al., paper in preparation). While these approaches are widely used to investigate stress response to metal toxicity, environmental metabolomic studies have to date only rarely been carried out with algae. As the metabolome represents the ultimate response of an organism to genetic and environmental changes, the aim of this work is to assess silver toxicity at the metabolic level of the photosynthetic alga *C. reinhardtii* using high-resolution mass spectrometry. For this, cells are exposed to 500 nM AgNO<sub>3</sub> over a period of 5h. To evaluate the intracellular metabolites, a biphasic extraction method compatible with high-throughput mass spectrometry based on the procedure reported by Taylor and coworkers<sup>1</sup> was optimized for the algal cultures. Preliminary results show that the metabolite profiles of biological replicates are reproducible and that the analytical procedure is adequate for examining the response of algae to silver. Principal component analysis of the LC-MS data revealed that the AgNO<sub>3</sub> exposed algae compared to control cells can be clustered in clearly<sup>3</sup> distinguishable groups based on *m/z*, retention time and chromatographic alignment. On the other hand, no growth inhibition was observed at the physiological level. These results suggest that changes in the metabolome reflect the microalgae capability to activate the defence mechanisms against silver. **References** Taylor NS, Weber RJM, Southam AD, Payne TG, Hrydziuszko O, Arvanitis TN, Viant MR. A new approach to toxicity testing in *Daphnia magna*: application of high throughput FT-ICR mass spectrometry metabolomics. 2009. Metabolomics 5: 44-58.

**MO067 Characterisation of multi xenobiotic resistance (MXR) transporters in *Daphnia* spp.** S. Fischer, C. Drieschner, R. Beer,

Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; P. Spaak, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Aquatic Ecology; K. Schirmer, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology. To sustain chemical stress, organisms must possess mechanisms which confer a certain level of resistance. One such mechanism is active chemical efflux by membrane-bound multi xenobiotic resistance (MXR) transporter proteins. These proteins form an active environment-tissue barrier in a range of aquatic organisms by antagonizing uptake of a wide range of toxic compounds by cells and excreting metabolites out of cells. *Daphnia* species are widely used as *in-vivo* models for ecotoxicity testing of chemicals and environmental samples. Thus, it is crucial to understand the active cellular processes in these models that determine the fate and effect of the chemicals to which *Daphnia* are exposed. However, there is hardly any published work on physiological functions of MXR transporters in the *Daphnia* species. Therefore, the aim of our study is to provide a basic characterization of putative MXR transporters in *D. pulex*, *D. magna*, *D. pulicaria* and *D. galeata*. We obtained full length and partial cDNA sequences of two *abcb1* isoforms, one *abcc1*, one *abcc4*, three *abcc5* and one *abcg2* in all four *Daphnia* species using RT-PCR and rapid amplification of cDNA ends. Identity and phylogenetic analysis based on the obtained sequences revealed that the transporters of the different *Daphnia* species are closely related to other invertebrate orthologs and that they overall fit into eukaryotic MXR transporter system. Furthermore, we examined constitutive expression levels of putative MXR transporters of *Daphnia* by quantitative real-time PCR (qPCR). Transcripts of all MXR genes were identified in total RNA extracts of all four *Daphnia* species from three developmental stages, between 1–10 days after hatching. Expression levels vary depending on stage and transporter type. Experiments with fluorescent transporter substrates (rhodamine B, calcein-am) combined with pharmacological transporter inhibitors (cyclosporine A, MK571) are underway to confirm the protein activity and verify the physiological role of the transporters in antagonizing accumulation of exogenous compounds in the *Daphnids*. Our data thus far indicate that MXR transporters could mediate certain resistance against chemicals in the *Daphnia* species.

**MO068 Mechanisms of PCB Tolerance: Comparing the Oxidative Stress Response in Two Invasive Dreissenid Mussels** C.J. Nowicki, Wayne State University / Biological Sciences; D.R. Kashian, Wayne State University / Biology department. Polychlorinated biphenyls (PCBs) are a significant environmental concern due to their adverse health effects in humans and wildlife. As PCBs are metabolized, reactive oxygen species are formed that can damage the cell lipid membrane and DNA, leading to cell death. Ultimately, oxidative stress can impact an organism's ability to tolerate chemical contaminants and survive disturbance events. In addition, oxidative stress is of particular interest because it is not extensively studied in freshwater macroinvertebrates, and quantifying biomarkers of oxidative stress is a relatively novel approach for assessing environmental stress. The present study provides further understanding into the mechanisms of the oxidative stress response and its potential role in aquatic invertebrate tolerance to PCBs. To examine the role of oxidative stress in chemical tolerance, we used the genetically similar bivalves *Dreissena polymorpha* and *D. bugensis* that are invasive to North America. We exposed mussels of each species to PCB-laden sediments (2200 ?g/g) or water (1.3 ?g/l) collected from a PCB-contaminated site in St. Clair Shores, Michigan, USA. During a 14 day toxicity assay, 30 mussels of each species were exposed to one of four treatments: PCB water, PCB sediment, control water (dechlorinated tap water), or control sediment (clean sand). Mussels were evaluated over time for oxidative stress by removing five mussels from each treatment on days 1, 2, 7, 10, and 14, and analyzing the soft tissue for biomarkers of lipid peroxidation and antioxidants (superoxide dismutase and catalase). Preliminary results indicate that *D. bugensis* have higher levels of antioxidants (p< 0.05) but lower levels of lipid peroxidation (p< 0.05), indicating a stronger oxidative stress response than *D. polymorpha*. The above experiments provide a direct correlation between tolerance to chemical stressors and

antioxidant response in aquatic invertebrates. Furthermore, *D. bugensis* is displacing *D. polymorpha* in the Great Lakes, and *D. bugensis* has exhibited greater tolerances to deeper and colder waters which have likely contributed to a competitive edge over *D. polymorpha*. Findings from this study may demonstrate an additional advantage of superior tolerance to contaminants, PCBs specifically, allowing *D. bugensis* to inhabit contaminated sediments.

**MO069 Effects of DNA methylation on insecticide sensitivity and phenotypic plasticity of the mosquito *Aedes albopictus*** A. Oppold, Goethe University Frankfurt am Main / Ecology Evolution and Diversity; A. Kress, Goethe University Frankfurt; U. Kuch, Biodiversity and Climate Research Centre Frankfurt/Main, LOEWE / Medical Biodiversity and Parasitology; J. Oehlmann, Johann Wolfgang Goethe Universität Frankfurt am / Aquatic Ecotoxicology; R. Mueller, LOEWE Biodiversity and Climate Research Centre / Aquatic Organisms and Ecosystems. A range of environmental factors, including chemicals, can affect epigenetic processes in organisms leading to variations in phenotype. Thus epigenetics display an important environmentally responsive element. Although epigenetic alterations in exposed organisms can be long-lasting and are often inheritable with tremendous impacts on populations and communities, chemically induced epigenetic changes in test organisms and wildlife species have only recently found first consideration in ecotoxicological research. In the current approach we investigated the influence of two known methylation agents, the fungicide vinclozolin and the phytoestrogen genistein, on the overall DNA methylation in the Asian tiger mosquito *Aedes albopictus*. The whole experiment stretched over three generations in a *full-life-cycle-design* with an exposed parental generation and two consecutive non-exposed filial generations. In dependence from DNA methylation levels of the mosquito, the sensitivity of the different treatment groups of *A. albopictus* to model insecticides (imidacloprid and DDT) was assessed. Variations in sensitivity revealed alterations of the mosquito's phenotype. Furthermore, metabolic parameters as contents of lipid, glycogen, protein, and glutathione were determined within each generation. In view of the hypothesis that mechanisms of DNA methylation are associated with detoxification processes this approach enabled statements about effects of toxicants on metabolic processes in a direct as well as transgenerational manner. According to different authors homocysteine holds the key position between detoxification and epigenetic mechanisms. It is basis reactant for glutathione within the second phase of detoxification as well as of S-adenosyl-methionin (SAM) the methyl group carrier of the DNA methylation process. This coherence clearly shows that in case of exposure to toxicants homocysteine is exploited for the synthesis of glutathione and is then less available for synthesis of SAM affecting DNA methylation. The evaluation of the invasive mosquito *Aedes albopictus* from an epigenetic perspective gives important information about its high adaptability. This can further be of high importance concerning distribution scenarios and formation of resistance towards mosquito control operations.

**MO070 Are metal adapted *Daphnia pulex* populations better armed against future climate change stressors than non-adapted populations?** D. De Coninck, Ghent University / Laboratory of Environmental Toxicology & Aquatic Ecology; J. Asselman, Ghent University / Laboratory of Environmental Toxicology; S. Glaholt, Indiana University; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; J.R. Shaw, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; K. De Schamphelaere, Ghent University UGent / Environmental Toxicology and Aquatic Ecology. Genetic variability may allow for natural populations to adapt to a stressor. This can be manifested by an increased tolerance to the stressor. As a consequence of this genetic adaptation, populations may also become more (co-tolerance) or less tolerant (cost-of-tolerance) to other stressors. In this research we investigated if populations of the waterflea *Daphnia pulex* that have adapted to cadmium (i.e. more tolerant to sublethal cadmium concentrations) have become more or less

tolerant to exposure to cyanobacteria. Cyanobacteria are a common stressor to most zooplankton species and are predicted to play an increasingly prominent role in aquatic ecosystems as the occurrence of harmful algal blooms is predicted to increase due to climate change. To test the cost-of-tolerance hypothesis and assess their tolerance to cyanobacteria, 12 isolates of cadmium-adapted and 13 isolates of cadmium non-adapted populations were exposed during 21 days to a diet containing 50% of one of six different cyanobacteria species: *Microcystis aeruginosa*, *Aphanizomenon flos-aqua*, *Nodularia harveyana*, *Oscillatoria* sp., *Anabaena lemmermannii* or *Cylindrospermopsis raciborskii*, respectively. Control animals were fed 100% *Ankistrodesmus falcatus*. Tolerance was determined as control normalized total reproduction. We found that animals originating from cadmium-adapted populations performed better under *Microcystis aeruginosa* and *Oscillatoria* sp. stress than animals originating from reference (non-adapted) populations. This indicates that co-tolerance rather than a cost-of-tolerance occurred. We found no differences between cadmium-adapted populations and reference populations for the other four cyanobacteria species. These findings suggest that cadmium adapted *Daphnia* populations are better suited to face some future climate change stressors than non-cadmium adapted populations.

**MO071 Transgenerational and latent effects of contaminants: developmental and parental environments have strong impacts on subsequent fitness in snails** C.J. Salice, Texas Tech University / Environmental Toxicology; S. Plautz, BASF / The Institute of environmental and human health; D.A. Kimberly, Texas Tech University / Environmental Toxicology. An implicit but important assumption in ecotoxicity testing is that populations are generally static and therefore responses to contaminants are thought to be consistent. While this assumption may facilitate the application of toxicity test results for ecological risk assessments, a growing body of research in ecology and evolution shows that organisms can be highly plastic and that the range of responses is very much dependent on history. Among the most interesting findings are that the environments experienced by parents or developing organisms can have strong impacts on how subsequent offspring or older individuals may respond to the environment, in the absence of selection. For example, cricket mothers in predator-rich environments produce more predator-sensitive offspring. The notion of historical context has not, however, received much attention in ecotoxicology despite the profound implications for understanding how populations respond to toxicants. Research in our lab has focused on better characterizing and understanding the role that transgenerational effects play in the response of freshwater snails, *Biomphalaria glabrata* and *Physa pomelia*, to contaminants such as cadmium. Our results show that the parental environment has a significantly detectable effect on the response of offspring to cadmium. Factors in the parental environment such as dietary differences (lettuce type, fish food present/absent), the presence of predators (crayfish cues) and abiotic stressors (cadmium, temperature, salinity) have strong impacts on the subsequent cadmium tolerance of offspring. For example, parents exposed to predator stress in the form of crayfish and crushed snail cues produce offspring that are more cadmium tolerant than parents in predator-free environments. Similarly, if parents are exposed to sub-lethal concentrations of cadmium, they generally produce offspring that are more cadmium tolerant. Additionally, exposure to cadmium only during embryonic development of F0 *P. pomelia* resulted in detectable effects on the subsequent hatching success of F1 snails. In this example, the effects of cadmium exposure during development manifested a generation and approximately 12 weeks post-exposure. Our results collectively show that effects of contaminants are strongly related to the parental and developmental history. Future research is aimed at exploring potential mechanisms including contaminant transfer and epigenetic effects.

**MO072 Evolved resistance to PCB126 and coal tar induced cardiac teratogenesis in Gulf killifish (*Fundulus grandis*) populations from the Houston Ship Channel** E. Oziolor, Environmental Science; C.W. Matson, Baylor University / Environmental Science. The Houston Ship

Channel, connecting Houston, Texas to Galveston Bay and ultimately the Gulf of Mexico, is heavily industrialized and includes several areas that have historically been identified as containing significant levels of mercury, dioxins, furans, PCBs, and PAHs. Gulf killifish, *Fundulus grandis*, inhabit this entire estuarine system, including the most contaminated areas. *F. grandis* is the sister species of the well-established estuarine model organism *F. heteroclitus*, for which heritable resistance to both PCB and PAH toxicity has been documented in several populations. Populations of *F. grandis* collected from contaminated and non-contaminated sites were collected and manually spawned in the laboratory. The F1 embryos were exposed to various concentrations of PCB126 and coal tar and then screened for cardiac deformities. Embryos from populations collected from contaminated sites show a >1000 fold increase in resistance to PCB126 induced teratogenesis and a >10 fold increase in resistance to coal tar. Reciprocal crosses between reference and contaminated populations exhibit an intermediate level of resistance, which suggests that observed protection is genetic and biparentally inherited. EROD data confirm a reduction in CYP1A induction in resistant populations of *F. grandis*, consistent with responses previously described for resistant populations of *F. heteroclitus*, specifically a recalcitrant AHR pathway. The decreased levels of cardiovascular teratogenesis, and decrease in CYP1A inducibility in response to PCB126 and a PAH mixture suggest that *F. grandis* populations in the Houston Ship channel have adapted to chronic contaminants exposures via a similar mechanism previously described for *F. heteroclitus*. To the best of our knowledge, this is the first documentation of a pollution tolerant population of *F. grandis*. Additionally, the mechanistic similarities between population adaptation observed in this study with previous work in *F. heteroclitus* suggest that genetic variation predating the evolutionary divergence of these two species may be the best explanation of observed similarities and the seemingly independent parallel evolution of pollution tolerance in genetically distinct populations.

**MO073 Trans-generational versus evolutionary impact of pollutants: an experimental test in the freshwater snail *Lymnaea stagnalis*** M. Coutellec, INRA / Aquatic Ecotoxicology, UMR ESE; M. Collinet, INRA; A. Besnard, T. Caquet, INRA / UMR ESE 0985. Pollutants may induce rapid evolutionary processes, which in turn can affect population responsiveness to other environmental stressors. We used quantitative genetics and population genetics to address these questions in a freshwater snail. Family level reaction norms were measured under a four-level gradient of biotic stress (combination of food shortage and competition) applied to laboratory-born snails whose parents were previously exposed to pesticides in outdoor microcosms. Chemical treatment design was based on inputs modeled from two annual crop protection programs (conventional, low pesticide input) vs untreated parcels. The study showed that several life history traits were directly affected by pesticides and biotic stress. First, parental snails exposed to conventional treatments grew more (glm,  $P < 0.001$ ; Tukey's post-hoc test,  $\alpha = 0.05$ ) and had higher fecundity (glm,  $P = 0.023$ ). Low pesticide input also increased fecundity (glm,  $P = 0.23$ ). Second, in the F1 generation, individual growth parameters (Gompertz model) were negatively and gradually affected by the gradient of biotic stress applied (glm,  $P < 0.001$ ). Conversely, parental exposure to pesticides had no effect on progeny reaction norm to the applied biotic stress, that is, no non-genetic trans-generational effect was reflected under the present design. Both generations showed significant *genotype*  $\times$  *environment* interaction, reflecting differential performances among families faced with pesticides and biotic stress. This result indicates potential adaptive evolution in the studied population of *Lymnaea stagnalis* in response to the stressors used. In parallel, the genetic structure was compared across mesocosm populations established from the same initial pool of 40 parental lines, and subjected to two years of the abovementioned protection programs. Population structure was described at 10 polymorphic microsatellites, which reflected significant decrease in genetic diversity (mean allelic richness,  $P = 0.006$ ; expected heterozygosity,  $P = 0.013$ ) within populations exposed to conventional treatments. Moreover, the level of differentiation was significantly

higher among these populations than among control or low-input ones ( $F$  index;  $P = 0.049$ ). The whole results will be discussed in the light of theoretical expectations under random genetic drift and selection, respectively, and in terms of relevance for ecological risk assessment.

**MO074 Impacts of an industrial source of perfluorooctanoic acid (PFOA) on river macrobenthic community: a genetic study** M. Rusconi, Water Research Institute Italian National Research Council / Water Research Institute; R. Bettinetti, Università dell'Insubria; L. Marziali, M. Mazzoni, Water Research Institute - IRSA-CNR / Water Research Institute; S. Polesello, Water Research Institute CNR / Water Research Institute; F. Rosignoli, Water Research Institute - IRSA-CNR; S. Valsecchi, Water Research Institute Italian National Research Council IRSACNR / Water Research Institute; F. Stefani, National Research Council Water Research Institute. The main source of perfluorooctanoic acid (PFOA) in the river Po, which is the major Italian river which flows into the Adriatic Sea, is a fluoropolymer factory, which discharges in the Bormida river (Piedmont, Northern Italy). In order to deep into the impacts on the river ecosystem, macrobenthic communities have been monthly sampled by artificial substrates upstream and downstream the industrial discharge in order to compare the community structure in both sites. To evaluate if the PFOA source has a selective pressure on the macrobenthic community, genetic variation was analyzed with the aim of identifying genetic erosion or selection. With this goal, organisms of specific species, collected in significant abundances up- and downstream the pollution source, have been processed by Amplified Fragment Length Polymorphisms (AFLP), a molecular marker technique that allows us to genotype several *loci* along the genome of every individual. The genetic approach has been applied to two species which differ for their life traits: one characterized by a completely aquatic life cycle (*Echinogammarus veneris*) and the other by both an aquatic larval and an aerial adult stadium (*Hydropsyche modesta*). An outlier *loci* research was performed to identify *loci* candidate for selection, and the differences between the investigated populations have been interpreted in reason of the potential effects of contamination respect to gene drift or overall genetic erosion.

**MO075 Acclimation of early-life stages of zebrafish *Danio rerio* to triclosan: a new workflow from the phenotype to epigenetic regulations** E. Falisse, A. Voisin, University of Namur; F. Silvestre, . Triclosan (TCS) is a bactericide widely used in personal care products. The aim of the present study was to characterize the acclimation response of ELS zebrafish *Danio rerio* to TCS. First, newly hatched fish were exposed to increasing concentrations of the pollutant (0, 2, 20, 50, 100 µg/L) till 7 days post fertilisation (7dpf). Time-to-death challenge was performed after this exposure to establish an acclimation window. It came out that fish exposed to 50 µg/L were significantly more sensitive than the controls while the ones exposed to 100 µg/L have recovered, suggesting that acclimation mechanisms were triggered at this concentration. Secondly, the biochemical and molecular responses of ELS fish exposed to 50 and 100 µg/L were explored. The activity of acetylcholinesterase (AChE) was significantly higher ( $p < 0.05$ ) in organisms exposed to 50 and 100 µg/L (from  $383 \pm 92$  in controls to  $1006 \pm 71$  and  $1180 \pm 390$  U/mg prot. in 50 and 100 µg/L, respectively). Moreover, while glutathione peroxidase activity (GPx) significantly increased from  $10.5 \pm 1.5$  in controls to  $22.4 \pm 7.1$  and  $17.7 \pm 4.0$  U/mg prot. in 50 and 100 µg/L, respectively, glutathione reductase activity (GR) was significantly decreased in fish exposed to 100 µg/L ( $13.4 \pm 4.0$  U/mg prot.) and was not significantly affected in 50 µg/L ( $17.4 \pm 3.6$ ) compared to controls ( $21.9 \pm 3.9$  U/mg prot.). In parallel, a proteomic analysis was performed on whole 7dpf fish exposed to 50 and 100 µg/L and the protein expression profile was compared to controls using 2D-DIGE through a pH range from 5.3 to 6.5. Twenty-three spots of proteins were found to be differentially expressed ( $p < 0.05$ ) by TCS treatments. Among them, beta-enolase, vitellogenin 1 and ATP2a1 were identified using nano-LC-ESI-MS/MS. Lastly, we aimed to investigate a possible link between protein expression level and epigenetics regulation. The methylation level of the CpG sites of the 5' flanking region of the genes coding for these three proteins were quantified by pyrosequencing using

a PyroMark Q24 (Qiagen). This study is the first to link an organismal phenotypic level to biochemical effects, protein expression profile changes and DNA methylation regulation in organisms exposed to a common pollutant such as TCS. This workflow will help to predict the long term effects of pollutants on aquatic organisms.

**MO076 Saltwater-driven genetic erosion of cladoceran populations: multiple stressors, phenotypic plasticity and tolerance to lethal vs sublethal levels**

C. Venancio, University of Aveiro / Biology & CESAM; R. Ribeiro, Universidade de Coimbra / IMAR-CMA, Dept. of Life Sciences; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; I. Lopes, University of Aveiro / CESAM. Natural population exposed to chemical contamination may have their genetic diversity reduced by the elimination of the most sensitive genotypes. This contaminant-driven genetic erosion may theoretically lead to adverse consequences on the populations' viability if exposed to future environmental stressors. The present study aimed at predicting possible transgenerational effects due to sequential inputs of long-term nearly, medium-term moderately and short-term intensely toxic exposures to saltwater, using six clonal lineages of the freshwater cladoceran *Daphnia longispina* differing in their sensitivity to lethal levels of copper. Sodium chloride (NaCl) was found to elicit lethal effects at lower concentrations than Atlantic seawater, allowing, thus, the use of the former as a surrogate of the latter. A significant and differential variation in lethal sensitivity, reproductive output and feeding rate was found among the clonal lineages, after a multigenerational acclimation to a nearly toxic concentration of NaCl, confirming the differential phenotypic plasticity hypothesis. No association was found between the lethal and sublethal endpoints. Some clonal lineages were found to present an inverse sensitivity to lethal levels of NaCl and copper. These negative linkages indicated a potential for further loss of genotypes within an already eroded population, under a sequential exposure scenario.

**MO077 Seawater in low-lying coastal ecosystems: sensitivity before and after acclimation among freshwater primary producers, consumers and decomposers**

C. Venancio, University of Aveiro / Biology & CESAM; B. Castro, Universidade de Aveiro / Biology & CESAM; R. Ribeiro, Universidade de Coimbra / IMAR-CMA, Dept. of Life Sciences; S. Antunes, University of Oporto / Biology; N. Abrantes, University of Aveiro / Biology & CESAM; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; I. Lopes, University of Aveiro / CESAM. Recent projections point to a gradual intrusion of seawater in freshwater coastal ecosystems from vulnerable areas and diversity losses are expected. However, populations are able to cope with environmental stress within certain thresholds, either by avoidance, acclimation or adaptation. Bearing in mind the vulnerability of freshwaters to saline intrusion, three objectives were addressed in the present study: (i) to evaluate the suitability of sodium chloride as a seawater proxy in sensitivity assessments; (ii) to determine the freshwater ecological receptors at most risk due to exposure to increased salinity, within a battery of producer, consumer and detritivore species; and (iii) to evaluate the ability of freshwater species to acclimate to low sublethal levels of NaCl. For this purpose, standard monospecific bioassays were integrated using several species representative of different trophic and functional levels and known to exist in Portuguese freshwaters systems, including microalgae, floating macrophytes, primary (zooplankters) and secondary (amphibians and fish) consumers, and benthic detritivores. Lethal and sublethal (feeding, growth, emergency, reproduction) endpoints were evaluated. Overall, tested species were similarly sensitive to increased salinity induced by NaCl comparatively to that induced by the natural seawater, as differences never exceeded 2.5 times, thus, validating NaCl as a proxy to evaluate the effects of seawater intrusion in low-lying aquatic ecosystems. A differential sensitivity among trophic levels/functional groups was observed; macrophytes and fish being the most tolerant to salinity with microalgae and rotifers in the other end. Finally, evidences of acclimation were gathered for none species. The diversity of responses observed in this

study highlights the importance of integrating information from different trophic levels/functional groups and from different endpoints to gain insights on both direct and indirect potential effects at the ecosystem level.

**MO078 Effects of perfluorinated compounds on life history and genetic traits of *Chironomus riparius* (Diptera, Chironomidae): a multi-generational test**

F. Stefani, National Research Council Water Research Institute; L. Marziali, Water Research Institute - IRSA-CNR / Water Research Institute; M. Rusconi, Water Research Institute Italian National Research Council / Water Research Institute; F. Rosignoli, Water Research Institute - IRSA-CNR; S. Valsecchi, Water Research Institute Italian National Research Council IRSA-CNR / Water Research Institute; R. Bettinetti, Università dell'Insubria; S. Polesello, Water Research Institute CNR / Water Research Institute. Perfluorinated alkylated compounds (PFASs) represent an emerging group of contaminants with peculiar physico-chemical and toxicological properties. PFASs are generally persistent in the environment and have been detected extensively in most parts of the aquatic and terrestrial ecosystems. In this study, we investigated the long term sublethal effects of PFOS, PFOA and PFBS on the genetic and life traits parameters of *Chironomus riparius* (Diptera, Chironomidae) populations under a multigenerational assay. Starting from a common wild stock, 8 different subpopulations of *C. riparius* were bred in separate vessels, each containing a 10 µg/l solution of PFOS, PFOA and PFBS or reconstituted water. A total of 300 midges were bred for each treatment for multiple generations. For each generation, the following life traits were estimated: survival, growth, development, reproduction. Heterozygosity, allele diversity, deviation from Hardy-Weinberg equilibrium, linkage disequilibrium, effective genetic dimension, population size contraction and presence of selection were estimated basing on five microsatellites loci amplified by PCR. Most replicates fulfilled the validity criteria for mortality and emergence according to OECD guideline 219 protocol and were considered valid. No effects on survival were found, while sub-lethal effects were shown for PFOS and PFOA treatments in terms of development rate and EmT50 and growth. Reproduction traits showed no significant differences between treatments. All populations, included the controls, showed adaptation to the breeding conditions in the first generations, determining a partial genetic erosion simply by genetic drift. Nevertheless, a divergence of PFOA and PFOS exposed populations respect to control and PFBS exposed ones was evident from the following generations. In particular, pattern of demographic contraction was indicated, but also selective forces may have acted differentially on the studied loci in these populations. Both genetic and life history approaches indicated more relevant effects on a long term scale of PFOS and PFOA than PFBS, suggesting that these substances may produce effects on the evolutionary and adaptive potential of natural chronically exposed populations.

**MO079 Tox-Box: Developing a Test Battery for Toxicological Assessment of Anthropogenic Micropollutants in Drinking-water**

A. Eckhardt, Umweltbundesamt / Toxicology of Drinking Water and Swimming Pool Water; T. Grummt, R. Heinze, Federal Environment Agency / Toxicology of Drinking Water and Swimming Pool Water. Almost any country in the world has regulations for "classical" pollutants like arsenic or lead, based on sound toxicological data which can be easily found in WHO publications like "Chemical Facts". But the field of pollutants is getting more and more complex due to an increasing number of anthropogenic (micro-)pollutants such as pesticides, pharmaceuticals and other manmade compounds. Evaluating these substances is often difficult, because only limited, if any, toxicological data is available. Therefore the German Ministry of Education and Research (BMBF) is funding a joint research project called "Hazard based risk management of anthropogenic micro pollutants to assure drinking water provision (Tox-Box\*)". Ten partners from the public and private sector, including universities, a water supplier, a private company as well as Germany's Federal Environment Agency (UBA) joined for the realisation of this task. The aim of the project is to develop

a test strategy to assess possible toxicological effects of any compound that might be found in drinking water. Based on the set of data and the (assumed) toxicological mechanism the UBA developed a hierarchical system for evaluating these substances. Endpoints that will be studied are cancerogenesis, neurotoxicity and endocrine disruption. In each field different tests will be evaluated, thus establishing the set of tests that is necessary to obtain reliable data for deriving guideline values for drinking water. The theoretical concept of the "Health related indicator value (HRIV)" developed by the UBA, provides the basis for evaluating the substance in question. At the end of the project a set of guidelines for decision makers will be established to enable the people in charge of drinking-water to take the required action once a micropollutant is found and evaluated. \* project ID: BMBF 02WRS1282A

**MO080 An alternative testing strategy for neurotoxicity using in vitro and zebrafish assays** J. Legradi, Institute for Environmental Studies; P. Cenijn, J. Kamstra, VU University / Institute for Environmental Studies; M. Brouns, VU University; R. van Kesteren, VU University / Center for Neurogenetics and Cognitive Research (CNCR); T. Hamers, P. Leonards, J. Legler, VU University / Institute for Environmental Studies. Various recent epidemiological studies have indicated that exposure to low doses of environmental biologically active contaminants during human development can have deleterious effects on cognitive development in childhood. The EU project DENAMIC aims to develop better and sophisticated tools, procedures and testing methods to screen compounds for (developmental) neurotoxicity and to improve assessment of exposures and effects (more information on [www.denamic.org](http://www.denamic.org)). As part of the project, a new alternative assessment strategy based on a combination of in vitro and in vivo assays is under development in order to prioritize compounds for further in vivo testing. To this end, hazard characterisation of pesticides and environmental pollutants at the molecular and cellular level is carried out, with emphasis on adverse effects during neuronal development. Test chemicals are selected based on their: environmental relevance, known neurotoxicity and different modes of action. Experiments are performed with acute or subchronic exposures. An array of in vitro assays is used to investigate (developmental) neurotoxic effects, including neuron differentiation in the SH-SY5Y human neuroblastoma cell line, acetylcholinesterase (AChE) inhibition and transthyretin (TTR) binding. Zebrafish larvae are used as an alternative animal model to study neurotoxicity in vivo. Thereby developmental toxicity as well as altered behaviour is monitored. Compounds inducing behavioural effects are then studied in more depth using biomolecular techniques like antibody staining. The results of the zebrafish and in vitro studies will be combined and a limited subset of the investigated chemicals is selected for in vivo testing in mice to validate the alternative assays. Behaviour tests in mice and rats are the standard test used for neurotoxicity testing. This study will ultimately demonstrate the usefulness of in vitro and zebrafish embryo assays as an alternative testing strategy for developmental neurotoxicity testing.

**MO081 Anaerobic BTEXN biodegradation? The answer is in the genes** M.H. Wagelmans, J. Wittebol, M. Van Bommel, Bioclear. The contamination of groundwaters with aromatic hydrocarbons BTEXN (benzene, toluene, ethylbenzene, xylenes and naphthalenes) is an issue in many (European) countries. Knowledge on aerobic biological BTEXN degradation is extensive however degradation under anaerobic conditions knowledge is fairly limited. Last couple of years genetically oriented research unraveled parts of the anaerobic degradation pathways that microorganisms possess. Detection of these genes in environmental samples represents directly the degradation potential on site. We have developed an extra line of evidence to directly and accurately detect (active) genes involved in anaerobic BTEXN degradation. Using this tool we are able to analyse presence and activity of the enzymes involved. With the applied Q-PCR method quantitative measurements are possible. This direct measurement of the biological activity is a quick and effective tool to give insight into the degradation potency of the contaminated location. Based on this tool on-site remediation

options can be developed and implemented. The compounds benzene, toluene, ethylbenzene, xylenes and naphthalene can be degraded under anaerobic circumstances. For the specific degradation pathways several key enzymes are responsible. Information of these enzymes is located on the genetic material of the microorganism on specific genes. We are able to identify the genes for the key enzymes. In the presentation results of the screening of several locations with different redoxconditions in the Netherlands will be presented. In addition a correlation of the present (active) genes and redox, location within the contamination plume and decrease in contamination will be drawn. Using this molecular tool remediation of anaerobic BTEXN contamination can be performed more cost effective and more precise.

**MO082 Impact of Glyphosate and Cadmium on Pericardial Cells of *Bombus morio* (Apidae, Bombini)** E. Abdalla, UFSCar / Biology; D. Almeida de Camargo, G. Sampaio, UFSCar; M.J. Costa, Federal University of Sao Carlos UFSCar Soroca / Department of Biology; E. Mathias Silva-Zacarin, UFSCar. The pericardial cells play an intermediary function in metabolism and excretion, filtering the hemolymph before it way into the dorsal tube. In *Drosophila* four types or activity stages were described for pericardial cells. All of them, are related to the cycle of formation, coalescence, growth, resorption, and regeneration of intracellular central vacuoles. Adult workers of *Bombus morio* collected from in a Seasonal Semideciduous Florest fragment (23°34'53.1"S 47°31'29.5') of the Federal University of São Carlos (Campus Sorocaba, São Paulo, Brazil) were kept in laboratory into B.O.D., 26°C in the dark, for 27 days and exposed to CdCl<sub>2</sub> and another for 51 days exposed to glyphosate. The exposition to the xenobiotics was made by feeding the bees *ad libitum* with honey and pollen solution containing 1 ppb and 1 ppm of the trace metal and the herbicide, respectively, every 12 days after laboratory rearing. The control bees were collected in the field and kept in laboratory in the same condition, but fed only on solution of honey and pollen. The dorsal tubes were dissected and analyzed under light microscopy techniques. While the effect of glyphosate causes intense developing of the vacuoles of the pericardial cells, the cadmium causes virtually cell death in all of them. The two distinctive effect of xenobiotic on the pericardial cells are reflected on bees lifespan: those exposed to glyphosate has survived over 51 days, while those exposed by CdCl<sub>2</sub> rarely exceed 22 days. The lifespan difference is due to cadmium quickly induce the pericardial cells apoptosis, perhaps by inhibiting its regulatory functionality, whereas glyphosate causes slower effect on these cells, but also show morphophysiological response of the pericardial cells in relationship with the control, increasing the incidence of cells in type IV of activity. The results indicate that the xenobiotics studied, even at doses considered environmentally safe by the Brazilian National Environmental Council (CONAMA) is deleterious to the specie of bee studied.

**MO083 Cyclo-oxygenase (Cox) expression in fathead minnows (*Pimephales promelas*) following ibuprofen exposure** A. Patel, Brunel University / Biosciences; H. Trollope, Y. Glennon, K. Hurd, G. Panter, AstraZeneca / Brixham Environmental Laboratory; J. Sumpter, Brunel University / Institute for the Environment; M. Rand-Weaver, Brunel University / Biosciences. Ibuprofen is a widely used prescription and over-the-counter medicine, treating pain, inflammation and fever, by inhibiting the biosynthesis of prostaglandins through non-selective inhibition of the enzyme cyclo-oxygenase (Cox). To evaluate whether pharmaceuticals pose a risk to aquatic organisms, we are testing the hypothesis that any potential effects will be related to the Mode-of-Action of the drug and will only be seen at plasma concentrations in non-target organisms similar to human therapeutic concentrations. We have established that fathead minnows exposed, via the water for 72-96 hrs, to ibuprofen concentrations of 270 and 370 µg/L result in plasma concentrations within the human therapeutic range (15-30 mg/L). In order to determine the effects of ibuprofen exposure at human therapeutic concentrations, fathead minnows were exposed for 96 hrs, using a flow-through system, to 270 µg/L of ibuprofen, followed by a 72 hr depuration phase. Control (n=4) and treated fish (n=4) were sampled

at 24, 48, 72 and 96 hrs after exposure (and 24 and 72 hrs after depuration). Gills, liver and brain were collected. RNA isolated from these tissues was reverse transcribed to cDNA and amplified by qPCR using specific primers designed to conserved regions of the Cox genes and  $\beta$ -actin reference gene. Ibuprofen was rapidly taken up into plasma and reached human therapeutic concentrations at 96 hrs. Depuration resulted in a rapid fall in ibuprofen plasma concentrations, reaching non-detectable concentrations by 72 hrs. Interestingly, large individual variation in ibuprofen uptake was observed. Cox enzyme activity, as analysed using commercially available Cox assay kits, was detectable in the liver only, and was generally low in both control and exposed fish. In depurated fish, Cox activity appeared to be increased. We have identified the Cox-1, Cox-2a and Cox-2b genes in the fathead minnow, and an up-regulation of these genes was previously seen at ibuprofen plasma concentrations 12-fold higher than human therapeutic concentrations. Data will be presented on the correlation between Cox gene expression and enzyme activity, and ibuprofen plasma concentrations at human therapeutic concentrations.

**MO084 The effects of illicit drugs on immune functions in zebrafish embryos** K.J. Groh, Eawag / UTOX Environmental Toxicology; R.I. Eggen, Eawag; H. Segner, University of Bern / Centre for Fish and Wildlife Health; K. Schirmer, Eawag. Illicit drugs (IDs) constitute a novel group of aquatic contaminants with scarcely characterized effects. Following consumption, IDs can reach surface waters due to incomplete degradation during wastewater treatment. The concentrations may fluctuate in connection with consumer behavior. Due to high pharmacological potency of IDs and conservation of their targets across vertebrates, fish may be especially endangered by these compounds. While acute ID effects on the brain and behavior have been well documented in both mammals and fish, their effects on other organs received less attention up to now. The immune system may constitute a sensitive ID target due to its tight interaction with neural circuits. Since in fish only the innate immune system is present during early development, any modulation during this period may be particularly critical, as this could result in subsequent malfunctioning of acquired immunity, potentially causing long-term impairment. To begin investigating the ID effects on fish immune system, zebrafish (*Danio rerio*) embryos were exposed to several IDs. The exposure started within less than 1 hour after fertilization (hpf) and was either carried out continuously for up to 5 days post fertilization or terminated at 8 hpf followed by washing and raising in clean medium (peak exposure). The endpoints assessed were (i) respiratory burst response (production of reactive oxygen species by macrophages upon stimulation), a general measure of immune health, and (ii) response to challenge with bacterial lipopolysaccharide (LPS). Cocaine exposure attenuated the strength of respiratory burst response. During the LPS challenge, fish continuously exposed to cocaine or methamphetamine survived longer than controls, indicating the lack of, or delayed onset of, acute inflammatory response. These survival differences were also seen in the fish subjected to peak exposure regimes. Since the metabolic half-life of IDs, at least in mammals, is short, it appears unlikely that these effects were due to the ID residues still remaining in the body. Rather, this could be a first indication of a stable (possibly epigenetically regulated) alteration of the immune system ontogeny in fish embryos caused by a short-term exposure. Ongoing work focuses on further investigation of ID effects on the immune system in fish with the aim to examine the correlation between exposure-caused functional impairment, changes in gene expression, and epigenetic alterations.

**MO085 Advantages and limits of using preclinical/clinical data to predict ecotoxicological effects on wild fish: example of azole pharmaceutical compounds.** O. Cardoso, INERIS / Unité d'écotoxicologie in vitro et in vivo; A. Sandrine, INERIS; S. Paris-Palacios, URCA; J. Porcher, W. Sanchez, INERIS. European regulation related to pharmaceuticals and pharmacovigilance require improving environmental risk assessment of these compounds. In this context, identification of biological pathways that could be disrupted in non-targeted aquatic species using qualitative preclinical/clinical data

associated to toxicological data appears as a promising approach. Azole drugs is a widely used group of pharmaceutical active ingredients (APIs) characterized by a lack of data related to ecotoxicological effects. The aim of this study was to generate hypothesis about the potential ecotoxicological effects of 'azole' drugs on teleost fish. Numerous studies have reported that imidazoles and triazoles, which are used in antifungal and anti-tumoral therapies respectively, are steroidogenesis disruptors due to their adverse or therapeutic mechanism of action based on inhibition of cytochromes enzymatic complexes. However, other azole APIs such as protons pump inhibitors, carbimazole, triptans, sartans, setrons, allopurinol, aciclovir, zolpidem, theophyllin exhibit various biological activities. Pharmacodynamical data on targeted and adverse effects could allow identifying ecotoxicological effects, 'Drugs Interactions' data and 'Contraindications/Precautions for uses' data may also inform about possible mixture effects and interactions with pathological contexts. Preliminary results, qualitative pharmacological data indicate possible deleterious effects of protons pump inhibitors (benzimidazole compounds) on acid excretion and Na<sup>+</sup> uptake in gill via interference with Na<sup>+</sup>/H<sup>+</sup> exchanger localized in gill epithelial pavement cells, especially in hypercapnia/hypoxia contexts. These disruptions may imply deleterious consequences on vital functions as respiration, iono and osmoregulation, excretion. Results obtained for other azole drugs will be presented here and advantages and limits of this approach will be discussed. *This work was supported by the French Ministry for Ecology and Sustainable Development (MEDDE-Program 181).*

**MO086 Influence of diphenhydramine and acetylcholinesterase inhibitor mixtures on *Daphnia magna* and *Danio rerio*.** A. Kristofco, Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; B. Du, Baylor University / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; K. Chambliss, Baylor University; J.P. Berninger, US EPA / Toxic Effects Characterization Research Branch; B.W. Brooks, Baylor University / Dept of Environmental Science. In rapidly urbanizing regions of the southwestern and south central US, instream flows are increasingly dominated by discharges from wastewater treatment plants. Future population growth and climate change projections for Texas indicate that base flows of many river systems will depend on these effluents, which contain a complex mixture of contaminants. Interactive effects of pesticides and pharmaceuticals, however, are poorly understood. The objective of this study was to examine the influence of mixtures of the antihistamine, diphenhydramine (DPH), and acetylcholinesterase inhibiting (AChEI) insecticides to daphnia and zebrafish. Though DPH inhibits the H1 receptor, eliciting antihistamine responses, in mammals DPH also inhibits serotonin uptake at the serotonin reuptake transporter and functions as a competitive antagonist for the acetylcholine receptor (AChR). In fact, due to AChR inhibition DPH has been suggested as an emergency medicine treatment for mammalian AChE poisoning. Based on evolutionary conservation of cholinergic neurotransmissions among animals, we hypothesized that the mammalian pharmacology and toxicology data for AChEI and DPH mixtures will predict the nature of chemical mixture toxicity to *Daphnia magna* and *Danio rerio*. We employed standardized methods for cladocerans and the Fish Embryo Toxicity (FET) test method, which represents an alternative to traditional acute fish toxicity testing, and supports the European Union's (EU's) REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) requirement to reduce the number of vertebrate organisms utilized in chemical safety assessments. We evaluated the acute toxicity and developmental malformations of zebrafish embryos at various ages to single compounds and AChEI/DPH mixtures to define interactive effects over development. Developmental timing of exposure was found to influence the magnitude of toxicity observed in zebrafish. We further examined the single compound and DPH/AChEI mixture toxicity in *Daphnia magna*. DPH did not confer a protective effect for cladoceran mortality or reproduction responses to AChEI toxicity; rather, additive toxicity was observed.

**MO087 Comparison of a systems biology based classification versus**



**chemically oriented classification** M. Penninck, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology; L. Vergauwen, UNiveristy of Antwerp; D. Knapen, University of Antwerp / Biology department; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology. In order to perform environmental risk assessment (ERA) in an appropriate manner, sufficient toxicological information on different biological levels is required. Yet, the large number of existing and newly produced chemicals does not allow an in depth ERA for each of these substances. Nowadays, general guidelines are implemented to group substances with similar physicochemical and/or toxicological properties. Nevertheless, the existing guidelines to classify chemicals are not entirely straightforward and may not be implemented in a simple manner. An additional major disadvantage is the fact that the delineation of these categories is mainly based on chemical reactivity principles, while there is no or little attention for the biological aspects. In the present study, a systems biology approach was applied to move towards an integrated understanding of the toxicological mode of action of chemical compounds. Different levels of biological organization were taken into account to characterize but also to fully comprehend the toxicological effects of different compounds. Within this perspective, 8 toxicants (isobutanol, 3-chloropropanol, 2,4-dichlorophenol, aniline, 4-chloroaniline, 3,5-dichloroaniline, 2,3,4-trichloroaniline and cadmium chloride) were selected for short-term EC toxicity experiments using the aquatic test model organism *Daphnia magna*. Different physicochemically based grouping methods that are publicly available from the OECD (Q)SAR Application Toolbox (i.e. the EcoSAR, Cramer, Verhaar and OASIS Acute Toxicity MOA classification) divided the eight compounds into different classes. The effects of the differentially grouped chemicals were investigated on both the molecular (gene transcript levels) and (sub)organismal level (immobilization, growth and energy status). The main objective of this study was to compare the classification of chemicals based on their biological response in *D. magna* to the chemically oriented classification. Our results show discrepancies between these two approaches. In order to increase our understanding of the underlying biological mechanisms we statistically linked the effects at the gene expression level to the response at higher (sub)organismal levels. This allows us to evaluate the impact of the observed discrepancies on regulatory applications and the potential of biology based classification tools to improve the current strategy.

**MO088 Improving the acid volatile sulfide metal bioavailability model by accounting for oxic sediments** D. Costello, A. Harrison, University of Michigan / School of Natural Resources & Environment; R. Mendonca, Universidade Federal de Pernambuco / Depto. de Zoologia, Centro de Ciências Biológicas; D. Marsh, C.R. Hammerschmidt, Wright State University / Department of Earth & Environmental Sciences; G. Burton, University of Michigan / School of Natural Resources Environment. Sediment metals may be toxic to aquatic biota, but toxicity is highly dependent on bioavailability. Metal partitioning and bioavailability in sediments has been primarily linked to sulfides and organic carbon, yet metals can also sorb to Fe oxides in aerobic sediments. Our research aims to improve metal bioavailability models for stream ecosystems by incorporating an oxic partitioning component. Specifically, the development of an empirical model that includes binding by Fe oxides, which appear to be important in binding of metals under oxic conditions. Two reference sediments, with differing binding capacities (i.e., AVS, Fe oxides, organic C), were spiked with five concentrations each of Cu and aged under flow-through conditions in the lab while concurrently exposing *Hyaella azteca* to those sediments to measure changes in toxicity as the sediment ages. During sediment aging (170 d), frequent temporal sampling produced a fine scale understanding of geochemical and toxicological dynamics in the sediment. Metal release from spiked sediments rapidly decreased through time when exposed to flowing waters. For the low binding sediment, toxicity to *H. azteca* declined as sediments aged. Concurrent formation of Fe oxides and Cu extracted in the Fe oxide fraction (ascorbate extracted metals) suggest that binding by Fe oxides are

responsible for reduced toxicity. However, for the high binding sediment, toxicity remained stable through the course of the aging. We suggest that increased oxygen penetration into the sediment through time may have oxidized CuS, releasing Cu<sup>2+</sup> to surface waters or more crystalline Fe oxides may have been less sorptive to Cu. This experiment will set the basis for bioavailability models that more accurately represent lotic sediments.

**MO089 Trace elements in the white-chinned-petrel (*Procellaria aequinoctialis*) from the Kerguelen Islands, Southern Indian Ocean** C.V. Zecchin Cipro, Université de La Rochelle / LIENSs; Y. Cherel, Centre d'Etudes Biologiques de Chizé, UPR 1934 du Centre National de la Recherche Scientifique; P. Bocher, F. Caurant, P. Miramand, Université de La Rochelle / LIENSs; P. Bustamante, Université de La Rochelle / LIENSs. The use of seabirds to monitor marine contamination by trace elements in areas remote from emission points has already been done at various latitudes. However, very limited information is available concerning the Southern Indian Ocean. This study highlighted that the diet was a key factor driving the exposure and trace element levels in the tissue of these birds. However, no information on birds foraging on fish and cephalopods is available from the Kerguelen Islands. Monitoring the contaminants in the Southern Indian Ocean appears necessary as previous studies have highlighted elevated concentrations of non essential Cd and Hg in mollusks, crustaceans and fish. Within this context, the white-chinned-petrel is a fully suitable species due to its lifespan, diet and trophic position. Thirty three accidentally killed (collision with lights/bycatch in longline vessels) individuals were collected in Kerguelen from October/1998 to March/2000 and had their tissues (liver, kidney, pectoral muscle, feathers and for mature males, testis) analysed for Cd, Cu, Hg, Se and Zn. Elevated Hg concentrations (average of 58.4 mg g<sup>-1</sup> dw in liver) are likely due to the presence of mesopelagic prey in the diet of *P. aequinoctialis*. Indeed; the trophic status is not the principal factor in determining Hg concentration: the presence of mesopelagic prey in the diet was likely to contribute more to the patterns of Hg burdens. Cephalopods can also be considered an important source of Hg, especially because its Hg is mainly present under organic form, highly bioavailable. Cd concentrations (average of 65.7 mg g<sup>-1</sup> dw in kidney) can be attributed to a high level of squid consumption, as well as some amphipods' (notably *Themisto gaudichaudii*). This study presents new and more detailed trace elements data on a species of high conservational concern. There is evidence of accumulation of Hg and Cd especially in liver and kidney (main storage organs, respectively) tissues and interaction of elements, specially the detoxifying activity of Se, and in a lesser degree, of Zn (which acts indirectly: due to competition with Cd for binding to metallothioneins, Zn is displaced and induces new metallothionein synthesis, increasing the overall number of binding sites). This work also indirectly confirms ecological data from the wintering period of the species, which is rather scarce. Seasonal diet change and moulting accounted more for the obtained results than sex of the birds.

**MO090 Presence of toxic and genotoxic compounds in sediments of Metztitlan Lake, Hidalgo, Mexico** A.S. Sobrino-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia, Laboratorio Alejandro Villalobos; A. Perez-Rojas, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia lab. Geologia. Sediments are an important component of aquatic ecosystems because they play an important role in the exchange of chemicals. Physical analysis of these is insufficient to have an assessment of their toxicity, as they must assess adverse effects on organisms caused by exposure to sediments and obtain the information necessary to establish criteria that can be used as guides to locate risk zones. This study was evaluated the toxicity and genotoxicity of sediments from the Metztitlan lake in two contrasting seasons of the year (dry and rainy 2007), in order to define areas of risk. Sediment samples were collected at 8-12 stations in the lake, in the samples were evaluated your toxicity by means of a battery of bioassays (*Selenastrum capricornatum*, *Daphnia magna*, *D. exilis* and inhibition of germination of *Lens esculenta*), and genotoxic effect by the

SOS-Chromotets technique. The information generated was integrated in a multivariate analysis to establish environmental quality. Higher levels of toxicity and genotoxicity were observed in the dry season. The degree of contamination of the systems varied from moderate (south) to heavily polluted (north). The northern area is an area where activities such as cultivation of organisms, fishing or recreation involves health risks.

**MO091 Behaviour of personal care products in the soil-water system after biosolid soil application** B. Eulalia M., INIA / Environmental; M. Porcel, J. Pro, M. Torrijos, M. Garcia, C. Fernandez, G. Carbonell, INIA. Personal care products (PCPs) comprise a diverse collection of thousands of chemical substances, including fragrances and cosmetics. PCPs have been recently recognized as emerging pollutants of particular concern. These compounds, found in many commercial applications, are inefficiently removed during conventional wastewater treatment and/or municipal solid waste (MSW) composting, and remain in biosolids. Thus, irrigation water or soil amendments may be considered an *input* of PCPs into agricultural soils. This work aims to identify the behaviour of PCPs (galaxolide and parabens) in amended agricultural soils, and their leaching and runoff waters, after a single application of biosolids to a typical Spanish agricultural soil. To fulfil this objective, a semi-field study was carried out with four biosolids (MSW composts, and sewage sludge: compost and thermal drying sludge) obtained from different Spanish urban waste treatment plants. Biosolid application rates were calculated by considering plant N requirements. The system consisted in 16 trays (2 x 2.5 m<sup>2</sup>), containing a 5-cm-thick soil layer, supported by a metal frame with a 10% slope. Two individual systems were connected to each trial for collecting rainwater: leachate and runoff. During the experiment, from October 2011 to July 2012, there were three rainfall events. At the beginning and the end of the experiment, galaxolide and parabens were analysed in all the samples (soil, leachate and runoff waters) to know the transfer ability of PCPs: biosolid-soil-water. To assess the toxicity of leachate and runoff water, ecotoxicological tests on algae (*Chlorella vulgaris*) and aquatic invertebrates (*Daphnia magna*) were conducted. This study has been funded by Spanish projects CTM2010-19779-C02-01/02 and RTA2010-00004.

**MO092 Survey of POPs and PAHs in the Orange-Senqu River Basin, Southern Africa** H. Bouwman, R. Pieters, North West University; C. Mor, UNOPS. Lesotho, South Africa, Botswana, and Namibia share 1 million km<sup>2</sup> of the catchments of the Orange-Senqu Rivers. The Orange-Senqu River Commission (ORASECOM) promotes the equitable and sustainable development of the water resources. This project supported a Transboundary Diagnostic Analysis of the basin to identify and prioritise issues and areas related to POPs and PAHs. Sediment (61 sites), fish (4 sites), and aquatic bird eggs (4 sites) were analysed for the 22 POPs, and PAHs. Dioxin-like TEQ levels were higher in sediments from North West and Gauteng, Free State, and Lesotho; and became less further west. PCBs in sediment had the same pattern as for dioxin TEQs. In biota it seems as if fish and birds had higher levels towards the west. Appreciable levels were found in some bird eggs, especially at Bloemhof Dam. PAHs shared the same pattern as for dioxin TEQ and PCBs. The highest level was 862 µg/kg. The main sources of PAHs were mainly pyrogenic. PFOS was not detectable in sediments. The most surprising finding was the very high levels of PFOS in bird eggs. The sources of PFOS are unknown. The high PFOS levels could indicate high-concern to biota and human health. This project has shown that a catchment-wide POPs and PAH assessment is possible, that it will identify organic pollutants of management concern, that regions of concern can be identified, and that it can inform and guide international catchment management.

**MO093 Relationships of macrozoobenthos indexes to contamination and land use - nation-wide biomonitoring from the Czech Republic** S. Némethová, Faculty of Science, RECETOX; K. Brabec, L. Blaha, Masaryk University / Faculty of Science, RECETOX. Aquatic ecosystems are exposed to enormous stress from side of the human

population, which has resulted in negative changes in water quality in many cases. Macroinvertebrates are well established bioindicators in waters, and various indices have been developed to characterize pollution-induced changes in communities. For example, the SPEAR index (SPECies at Risk) combines information about physiological sensitivity with the information on life history and discriminate individual taxa as "sensitive to a stressor" (species at risk) or tolerant taxa (species not at risk). The objective of our present study was to compare outcomes of various indices (including the SPEARpesticides index) for biomonitoring data from the Czech Republic. It was found that the SPEARpesticides index correlated with other indices used in Europe such as biotic index, Saprobic index and ASPT indices. Additional analyses based on the land use patterns showed correlations of most indices with the proportion of arable land, discontinuous urban fabric and forest in the basin for  $P < 0.01$ . Index SPEARpesticides was most significantly correlated with discontinuous urban fabric. The relationships of indices with concentrations of DDT in soil and environmental risks and runoff potentials were also investigated but simple. Correlations were less apparent most probably due to insufficient amount of data). In conclusion, correlations among different biotic indices was observed. SPEAR index responded to the risks associated with agricultural land use but the influence of the urbanization in river corridors was also reflected.

**MO094 The SPEAR trait based biomonitoring index; can it detect impacts of pesticides in the wet and monsoonal tropical north-east Australia?** B.J. Kefford, University of Technology Sydney / Department of Environmental Science; R.J. Wood, University of Technology Sydney; J. Dunlop, S.C. Choy, Queensland Government. The SPEARpesticides (SPECiesAt Risk from pesticides) biomonitoring has been shown to decline in response to pesticides but not with other stressors in temperate regions of Europe and Australia. SPEARpesticides uses traits of macroinvertebrate taxa indicating their physiological sensitivity to pesticides and the ability of their populations to rapidly recover following population declines (resilience). Tropical rivers typically have large floods in the wet season and the traits that have been used successfully in temperate regions to indicate taxa resilience (generation time, migration ability & period of life-cycle spent out of water) may not be as relevant in tropical rivers. Here we calculate SPEARpesticides at 11 pesticide contaminated sites and 3 reference sites in catchment of the Great Barrier Reef in north-east Australia. The climate of this region covers both the wet and monsoon tropics, the latter experience more pronounced wet and dry seasons than the former. The major agriculture crops in the region include sugarcane, bananas and other horticulture. At all contaminated sites, the concentrations of > 100 pesticide and metabolites were measured in water samples during both base flow and during floods using event triggered samplers. We will present results showing the relationship between pesticide toxicity (in terms of toxic units to *Daphnia magna*) and the SPEARpesticides. We will discuss the usefulness of SPEARpesticides and other potential trait based biomonitoring indexes for tropical regions.

**MO095 Realistic assessment of the effects of bisphenol A on a fish population in ecosystemic conditions.** G. de Kermoyan, Z. Akrou, M. Lonjaret, F. Lestremay, C. Chatellier, S. Joachim, INERIS; A.R. Pery, INERIS / Unit METO; J. Porcher, R. Beaudouin, INERIS. There is a clear lack of information on the impact of bisphenol A (BPA) on ecologically relevant endpoints, at both population and community scale. The pattern of effects is complex and ranges from behavioral disorder to reproductive impairments, therefore the resulting effects on a population can only properly be assessed through long term exposure in an ecosystemic context. Mesocosms studies provide such information for small fish like *Gasterosteus aculeatus*, a known suitable fish species to study endocrine disruption. INERIS mesocosms are designed for long term study of communities, in an ecosystemic context characterized by spatial heterogeneity, species heterogeneity and density-dependent effects. The mesocosms were set up in November with the selected non-fish species, introduced in quantities and at locations defined in a uniform protocol for each treatment. In the beginning of March, initial

tagged populations were introduced in each mesocosm. 15 females and 10 males per mesocosm were selected. Based on literature data, three nominal concentrations of BPA were selected: 1, 10 and 100 µg/L. Treatment began in mid-April and ended by the end of September. Five groups of fish were identified: juvenile (< 25 mm), non-founder fish (? 25 mm without tag) male or female, founder fish male or female (? 25 mm with tag). The populations were compared based on the abundance of these different groups of fish, on the sex-ratio, on the condition index, and on the length frequency distribution of the fish population. The value of pH was significantly lower in 100 µg/L of BPA than in the other treatments. This observation could be explained by the more important number of invertebrates such as gastropods and/or the lower biomass of macrophyte. Concentrations of BPA measured were close to the nominal concentrations at 0 m from the inlet of the water in mesocosm then, concentrations decreased and were stable 5m after the inlet. Populations exposed to 100 µg/L of BPA showed a number of non-founder females and males and a juvenile length significantly higher than in the other populations. In our study, BPA perturbed the structure of the population at the maximum exposure concentration. Other trophic levels observed during the same experiments are being analysed to complete these results. We believe that the outcomes of the whole study would be a right basis for ecologically relevant risk assessment of bisphenol A.

**MO096 Effects of a long term exposure of bisphenol-A on macrophyte and invertebrate populations and communities in lotic mesocosms** s. joachim, INERIS / ECOT; R. Beaudouin, INERIS / METO; D. Heintz, Institut de Botanique / CNRS UPR2357; F. Lesaulnier, P. Baudoin, INERIS / ECOT; d. Goulwen, A. Pery, J. Porcher, INERIS / METO. Bisphenol-A is manufactured for the plastics industry as an intermediate in the production of polycarbonate and epoxy resins. Around 3 million tons of this substance is produced each year worldwide. There is evidence from numerous *in vitro* and *in vivo* studies that BPA exhibits an estrogen-mimetic action at concentrations as low as 2 µg L<sup>-1</sup>. As high levels of BPA have been reported in the aquatic environment, potential effects on aquatic ecosystems are suspected. Nevertheless, to current knowledge, few studies have been performed to assess the long term effects of BPA at the population and community levels. A lotic mesocosm study was thus carried out, in twelve 20 meter long channels, under continuous, environmentally realistic concentrations of bisphenol A (1, 10 and 100 µg L<sup>-1</sup> in triplicates). The mesocosms were set up with artificial sediments, macrophytes, periphyton, benthic and pelagic invertebrates, decomposers and one fish species (*Gasterosteus aculeatus*). Care was taken to ensure equal stocking densities in each mesocosm. Treatment began on the 15<sup>th</sup> of April 2012 and followed on for 6 months until the 29<sup>th</sup> of September 2012. Periphyton biomass, macrophyte biovolume, zooplankton and invertebrate abundance and diversity and fish population dynamics were the measured biological endpoints. The biovolume of watercress, *Nasturtium officinale*, was significantly and negatively affected at the highest tested concentration. In order to account for the observed effects, concentrations of BPA in plant tissue and roots are currently being measured. Community structure of macroinvertebrate was also affected at 100 µg L<sup>-1</sup> with Oligochaeta and Planariidae being the most sensitive taxa. Gastropods and Chironomes on the contrary increased in abundance. More specifically, fecundity of *Radix peregra* was significantly increased resulting in a modification of the population structure. **The extent to which the alterations in population and community structure of lower trophic levels affected the responses of higher trophic levels such as fish is discussed. The authors highlight the importance of indirect effects which results mainly from competition and predation in structuring the overall toxic response.**

**MO097 LSPEAR index : from mesocosms to field application** M. Roucaute, INRA / UMR ESE; T. Caquet, INRA / UMR ESE 0985; L.L. Lagadic, INRA / UMR INRAAgrocampus Ouest Ecology and Ecosystem Health. LSPEAR (Lentic SPEcies At Risk) is an adapted version for lentic ecosystems of the original SPEAR index developed by Liess and von der Ohe for the assessment of the exposure of stream

invertebrate communities to pesticides. LSPEAR has been developed and tested in experimental conditions using the outdoor pond mesocosms of the INRA experimental facilities of Rennes, France. It could successfully keep track of community exposure during a two-year long experiment where mesocosms were submitted to repeated applications of pesticides based on simulated, realistic, exposure scenarios derived from actual crop protection programs. Our mesocosm facilities are characterized by neighbouring non connected ponds. Aerial dispersal of insect adult stages from control ponds is therefore a key factor for mesocosm recovery after toxicant exposure. LSPEAR index has the potential to be deployed for field survey of natural pond exposure to pesticides. To adapt LSPEAR to natural environment, we should consider some landscape features, especially the connectivity and degree of isolation between hydrosystems. Here, we present an ongoing study to test the role of pond isolation on LSPEAR results in ecosystems exposed to different intensities of agricultural practices. The corresponding survey takes place in the vicinity of Rennes, where our initial pond mesocosm experiments were ran and for which data about insect emergence phenology are available. A set of 12 natural ponds was selected to simultaneously encompass two degrees of exposure to agricultural pressures (low vs. high exposure) and of isolation (isolated vs. connected). Each modality comprised three replicates. Exposure to pesticides was assessed using multi-residue analysis applied to water samples collected in April, a critical time for pesticide application. Nutrient concentration and additional classical physico-chemical parameters were also measured. Macroinvertebrate communities were sampled using artificial substrates and sorted to the lowest practical taxonomic level. Results of the LSPEAR index are presented and discussed with respect to previous mesocosm results.

**MO098 Integrating chemical fate dynamics and population-level effect models for pesticides on a landscape scale** A. Focks, Wageningen UR / Mathematics/Computer Science; M. Ter Horst, E. van den Berg, Alterra; h. haveco; P. van den Brink, Alterra Wageningen UR / (a) Aquatic Ecology and Water Quality Management Group; (b) Alterra. Aquatic species in agricultural landscapes are regularly exposed to pesticides. The fate dynamics of pesticide compounds determine together with the hydrological regime of the water courses critical exposure concentrations of pesticides in the water and how far those reach on a landscape level. Population life-history traits such as generation time or dispersal abilities drive the recovery of populations when they were reduced in abundances by exposition to pesticides. The combination of both chemical and population processes on a landscape level may result in higher or lower risks as compared to typical edge-of-field scenarios. To shade more light on the question of environmental risk assessment on a landscape scale, we used spatially explicit pesticide exposure patterns as calculated with the CASCADE-TOXSWA model as input for population effect simulations with a landscape-scale version of the MASTEP model for the water louse *Asellus aquaticus* (Galic et al. 2012) in a realistic water course network setting. None-mitigated potato crop treatment in a Dutch agricultural area of approximately 10 km<sup>2</sup> size was assumed to result in spray drift input of 5% of the applied dose of the pyrethroid ?-cyhalothrin into parts of the water course network at 15 application days. This scenario resulted in exposure patterns highly variable both in space and time. Downstream transport of the pesticide led to exposure of water courses that did not directly receive spray drift input, despite the dissipation of ?-cyhalothrin from water was assumed to proceed fast (DT50=1 day). Effect simulations using a logistic dose-response function with an LC50 of 24 ng/L for *Asellus aquaticus* (Schroer et al, 2004) showed drastic effects in the water courses that received direct spray-drift input. In addition, also adjacent regions of the water network were considerably affected by downstream transport. Simulated recoveries of the populations were dependent on the connectivity of the respective water courses to unpolluted tributaries and took partially more than 2 years. Our results indicate remote effects of pesticide inputs in the simulated water network. For the used compound ?-cyhalothrin the fast dissipation limited the spatial scale of such remote effects. For some parts of the water course networks that receive spray drift, the FOCUS ditch scenario that assumes pesticide entry on 100m of

a 1000m long water course are likely to underestimate the risk in the landscape.

**MO099 ChimERA: Coupling exposure and effects into a predictive integrated framework for risk assessment** F. De Laender, Université de Namur ASBL / Lab of Env.Tox&Appl.Ecol.; h. baveco; A. Di Guardo, University of Insubria / Department of Science and High Technology; P. van den Brink, AlterraWageningen UR / (a) Aquatic Ecology and Water Quality Management Group; (b) Alterra. The environmental realism, ecological relevance, and methodological accuracy of the currently used exposure and effect assessment approaches have been questioned for the last 25 years. Bearing in mind the ecological and environmental complexity inherent to natural ecosystems, risk assessors increasingly realize that ecological risk cannot be adequately assessed using the existing procedures that disregard most, if not all, of this complexity. ChimERA project wants to address this issue by coupling separate exposure and effect models into an integrated exposure and effect ecosystem model for ecological risk assessment for the aquatic environment. The project will integrate ERA's building blocks into one predictive tool that will be subject to extensive testing using data from dedicated experimental work and from existing experiments performed with model-ecosystems and using uncertainty and sensitivity analyses. In addition, this tool will be used to evaluate the risk of (mixtures of) model chemicals in a spatial network of lentic aquatic ecosystems.

**MO100 Development of Landscape Scenarios for Population Modelling** M. Wang, WSC Scientific GmbH / Dept. Efate & Modelling; R. Luttik. Population models are considered as a powerful tool for risk assessment of long-term effects of pesticides on the population level. While more and more models have recently been developed, some of which were particularly designed for use in risk assessment, a critical question for the application of such models is the decision on which scenario shall be used for such assessments. While in environmental fate analysis scenarios have been especially developed for the risk assessment of pesticides in that past by the FOCUS group, no agreed landscape scenarios exist at present for population modeling. For regulators scenarios need to cover most or a given percentile of the agricultural area under consideration and they need to represent a realistic worst-case. At the same time, due to practical reasons, selected landscapes need not be too large. In the present work we present a method for the development of such landscape scenarios for population level risk assessment. Based on GIS landscape data and using a habitat quality criterion we select a set of potentially usable worst-case landscape. This process is repeated for different landscape size in order to reveal a landscape size which can maintain a population on the long term. The presented procedure can potentially be used for various terrestrial species and model types.

**MO101 Automated Landscape Analysis for Exposure and Ecological Risk Assessment** M. Wang, WSC Scientific GmbH / Dept. Efate & Modelling; B. Kind, WSC Scientific GmbH. Higher tier risk assessments for plant protection products are often based on a landscape analysis using Geographical Information Systems (GIS). These approaches either consider spatial exposure patterns, e.g. the co-occurrence of target crop and water bodies or habitats of non-target organisms, or the spatial distribution of animals, e.g. when considering ecological models, such as population models. Single features (e.g., ponds, streams, fields or hedges) are usually digitized and stored in a geodatabase, which is then converted to be used in special exposure or risk assessment software (e.g. Schad, 2009; Wang and Rautmann, 2008). However, the digitizing process is often very time consuming and the quality of the results strongly depends on the individuals involved. An automation of the digitizing process can increase the speed and helps standardizing the procedure. Hence, we evaluate the applicability of image recognition algorithms to automatically detect and classify landscape features. We show that combined with exposure models the new method offers an efficient approach for landscape level risk assessment.

**MO102 Refined risk characterisation for Non-Target-Terrestrial Plants at Landscape-scales using the Xplicit model framework** T. Schad, EnSA; A. Solga, Ecology; M. Dollinger, Bayer CropScience; S. Bub, BCS. Natural or semi-natural plant communities occurring in cultivated landscapes (Non-Target-Terrestrial Plants, NTP) are often limited to e.g., herbaceous stripes, hedges, riparian vegetation, groves, or wood margins. Agriculturally managed grassland provides a further type of plant community. This study was intended to take initial steps towards introducing more realism in NTP risk assessment (RA). In the first step, variability of ecotoxicological effect data on individual-level and refined exposure was considered in the risk characterisation. The second step will propagate individual-level effects to plant populations and communities (EFSA 2010a, 2010b), by means of Individual-Based-Modelling. Current Tier-1 RA of NTPs is designed to be protective, and so, does not allow for detailed risk quantification. The first step started at the definitions and data of the Tier-1 RA level for spray-drift, which was not passed by the test herbicide. A Xplicit-NTP-Model was derived from the Xplicit-Framework approach and was used to refine risk characterisation by taking into account variability of exposure (spray drift) and variability of individual-level effects (in the present case, dose-responses of 10 species, each of them represented with 7 Assessment Endpoints). Quantification of effect extent was done for the three risk dimensions *species*, *Assessment Endpoint* and *space*, at edge-of-the-field- and regional-scale. Exposure calculation considered variability of wind directions and of spray-drift depositions. At the edge-of-the-field-scale, a small NTP community of 3 m width occurring downwind from the field and represented by 70 endpoints (10 *species*, 7 *Assessment Endpoints*) was assessed. In this community only a small fraction of the 70 endpoints showed pronounced effects, among which sublethal were dominating. At regional scale, a landscape of dense arable cultivation was investigated. Herbaceous NTP communities were assessed, which frequently occur in small patches and in close vicinity to fields (again represented by the 70 endpoints). The calculation revealed that only a small fraction of the plant community showed pronounced effects. The results encouraged the question whether sublethal effects on individual-level, which are limited in *effect magnitude*, *number of species* showing effects, *Assessment Endpoint*, and *spatial extent* can cause unacceptable effects at community level under real-world conditions.

**MO103 Consequences of model simplification for assessing risks of chemicals for populations: A case study with soil invertebrates** M. Meli, A. Palmqvist, Roskilde University / Department of Environmental Social and Spatial Change; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; V. Grimm, Helmholtz Centre for Environmental Research UFZ / Department of Ecological Modeling. For Environmental Risk Assessment (ERA) of chemicals several assumptions are made: the general idea is that application of assessment factors covers uncertainty related to using simplified, short-term test, and extrapolation from laboratory to field. In addition, effects are measured at the level of individuals, whereas for most species protection goals are at the population level. Unless it is known to what degree simplification results in imprecise estimates of risk, it is not possible to know whether current procedures lead to over- or under-protection. One assumption in ERA is that chemical exposure is well represented by a homogeneous exposure scenario, when in reality chemicals have a patchy distribution in the environment. Furthermore, chemicals are often present in different mixtures, and the stress they cause on natural populations is combined with natural factors such as food scarcity. Individual-based population models (IBMs) are suitable tools to extrapolate risks from individual to population levels; nevertheless they are often seen as too complex and somewhat obscure, and questions are raised about how much and which complexities need to be incorporated to get a robust estimate of risk. In contrast, matrix population models may offer a simpler approach and have a long history of use in applied ecology. To understand whether individual behaviours (which are not taken into account in traditional ERA or with simpler modelling tools) are necessary for a realistic prediction of risk at the population level, we

built a metapopulation matrix model of the collembolan *Folsomia candida* as an aggregation of a complex spatially-explicit IBM, which we previously developed. By this we obtained a simplified model which is easier to understand, run, and parameterize, but which also does not capture some key aspects of the real population. Simulation results using a model contaminant show that the metapopulation model is less sensitive than the IBM to changes in spatial distribution of the toxicant. Whereas, the metapopulation model predicted higher population-level effects when compared to IBM simulations with avoidance behaviour it predicted lower effects when compared to simulations without avoidance. This suggests that individual-level behaviours such as foraging and avoidance can have an important influence on outcomes at the population level and may need to be included in population models used for ERA in order to answer specific regulatory questions.

**MO104 A spatially explicit model for the effects of plant protection products on a representative vulnerable fish species in edge-of-field water bodies** I. Ibrahim; A. Focks, Wageningen UR /

Mathematics/Computer Science; P.J. van den Brink, Alterra and Wageningen University; C.R. Hazlerigg, Imperial College / Division of Biology; P. Thorbek, Syngenta / Environmental Safety; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research; U. Hommen, Fraunhofer IME. We propose the use of population modelling for vulnerable representatives (focal species) of fish in edge-of-field water bodies as a tool to refine the effect assessment of plant protection products. A focal species should be a vulnerable species which is a) likely exposed to plant protection products in edge of field water bodies, b) ecologically sensitive with respect to its life cycle and dispersal potential and c) sensitive to the toxicant. In terms of risk of exposure, we compiled a list of freshwater fish species which inhabit edge-of-field water bodies in agricultural landscapes in the EU. These species are, thus, considered to run a high risk of being exposed to pesticides. The population resilience, and hence the ecological sensitivity of these species, was compared by means of elasticity analysis on age-based matrix models. That allowed the identification of 2 species, one that was most sensitive to effects on fecundity and another one that was most sensitive to effects on juvenile survival. Adult survival was not considered relevant for the analysis since any visible mortality of fish should be avoided. The third pillar of vulnerability, intrinsic sensitivity, was not used to define focal species because sensitivity is substance-dependent. Therefore, the sensitivity of the surrogate test species will be extrapolated to the representative vulnerable species by the use of assessment factors or other more refined tools. To allow extrapolation of effects from the organism level to the level of populations in more detail (in particular spatial and temporal aspects) than the matrix models allowed, an individual based model of fish was built. This model was parameterized for the representative vulnerable species. The MASTEP approach was followed in simulating small water bodies (streams, ditches and ponds) and surface water concentrations as simulated with FOCUS step 3 were used as input. For the simulation of population dynamics for the fish species of interest an already existing individual based model for zebrafish was adapted to the representative vulnerable European species. The model included mobility, growth, and development, as well as survival during different life stages, mating and reproduction, and mechanisms of density dependence. In this poster we present the model concept, the parameterization for one species, and first results of predicted population dynamics under different scenarios.

**MO105 Effects of food and pesticide exposure on individual movement, recolonization and population recovery of an aquatic macroinvertebrate** J. Augusiak, Wageningen UR / Environmental

Sciences - AEW; N. Galic, AlterraWageningen UR / School of Biological Sciences; h. haveco; P.J. van den Brink, Alterra and Wageningen University. In agro-ecosystems, organisms may be often exposed to anthropogenic stressors such as pesticides. The sensitivity and recovery of affected populations depend on toxicity, life-history, species' dispersal and landscape structure. Different testing strategies are applied for the ecological risk assessment of pesticides to understand

potential environmental side effects of their application. Rarely, however, do standard tests account explicitly for the impact on animal movement and its relevance for recovery of populations from adverse effects. In the case aquatic macroinvertebrates (e.g. *Asellus aquaticus* and *Gammarus pulex*), recovery by immigration of individuals from uncontaminated sites is an important factor for re-establishing population densities after pesticide exposure. Yet, not much information on movement patterns of aquatic macroinvertebrates is available nor which factors increase or decrease their dispersal potential. Hence, we performed video tracking experiments to derive information on movement behaviour of individual *A. aquaticus* under different conditions, such as varying population densities, presence/absence of food and shelter, or sub-lethal pesticide exposure. We found that differences in movement behaviour were exhibited under different testing regimes. Especially, exposure to different concentration levels of the single pesticides showed levelled effects on overall animal activity; resting times increased with increased exposure concentration and directionality of movement paths was found to decrease at the same time. The findings imply that currently applied techniques could lead to unrealistic estimations of recovery timeframes if environmental heterogeneity and/or pesticide residue effects are not accounted for. An individual-based model, MASTEP (Metapopulation model for Assessing Spatial and Temporal Effects of Pesticides), is used to estimate the combined allogenic and autogenic recovery of populations of *Asellus aquaticus* after exposure to an insecticide. We investigate how our experimental findings translate to larger time and spatial scales when extrapolated to the landscape level. We will present exemplary results regarding the implications of our movement studies for population dynamics and recovery.

**MO106 Comparing recovery from pesticide stress in continuous-habitat and metapopulation individual-based models applied at landscape scale** h. haveco; A. Focks, Wageningen UR /

Mathematics/Computer Science; P. van den Brink, AlterraWageningen UR / (a) Aquatic Ecology and Water Quality Management Group; (b) Alterra. For pesticide risk assessment at the landscape level, individual-based population models which are developed to simulate local dynamics, can be scaled up. For a network of ditches in the Netherlands we applied such a model for the waterlouse (*Asellus aquaticus*) exposed to different types of pesticides, using (model-based) spatially- and temporally-explicit exposure data. As the network consists of several kilometres of connected ditches, simulations of individual-based population dynamics for this small and numerous macro-invertebrate are computationally intensive and require a distributed computation approach, with calculation-load for parts of the network divided over different cores or machines. In this study we investigate whether and when it is possible to approximate the results (recovery times at a number of locations in the network) of these large-scale distributed simulations, with simplified models, applying a classical metapopulation concept. Instead of modelling the network as a spatial continuum of connected habitat, in the metapopulation approach it is divided into several local populations with limited exchange of individuals among them. Connected local populations make up a network. The topology of this population network is determined by both the underlying ditch network and its hydrodynamics, and by species-specific movement attributes affecting in particular its connectivity. While in the full model (continuous habitat) individuals may inhabit and crawl through each  $m^2$  of the ditch network, in the metapopulation model we represent one or a few  $m^2$  of each ditch as the site of a local population and define probabilities of exchange with nearby populations for each of its individuals. Note that for simulating dynamics the same individual-based approach can be used in both cases. For the limit case of each ditch containing many well-connected local populations, the results of the metapopulation model should be identical to the results of the full model. The challenge is to find the minimum number of local populations for which we still can approximate full model results. We thus compare local recovery times calculated from the metapopulation model with results obtained in the continuous habitat model, for different pesticide properties (toxicity and persistence) and for different

approaches in deriving and defining the metapopulation network from the physical ditch network.

**MO107 Microcystins gene expression induction influenced by nutrients, environmental factors, and cladoceran infochemicals** R.

Pineda Mendoza, G. Zuniga, Escuela Nacional de Ciencias Biológicas-I.P.N.; E. Martinez-Jeronimo, Escuela Nacional de Ciencias BiológicasIPN / Zoología. Cyanobacterial blooms reduce the water quality, through the production and release of toxic metabolites, which promote toxic effects in different organisms. Microcystins (MCs) are the most frequently synthesized cyanotoxins; this synthesis could be determined by several factors including: temperature, light intensity, pH, nutrients concentration ( $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N,  $\text{PO}_4^{3-}$ ,  $\text{Fe}^{2+}$ ), salinity,  $\text{CO}_2$  concentration, turbidity, and the presence of infochemicals released by the herbivorous zooplankton. In the present study, the expression of the *mcyA*-Cd region (this is part of microcystin synthetase gene cluster), was assessed through RT-qPCR as result of testing different environmental stimuli, nutritional conditions, and indirect exposure to infochemicals released by the *Microcystis*-feeding cladoceran *Daphnia magna* (adults and neonates). Two strains of *M. aeruginosa* were tested: UTEX LB2835 (reference strain) and CH10 (local strain isolated from an urban lake located in Mexico City). Different nutrient concentration ( $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$  and  $\text{Fe}^{2+}$ ), temperature values (20 and 30 °C), light intensities (16 and 50  $\text{mmol m}^{-2} \text{s}^{-1}$ ), and exposure to the synthetic infochemical sodium octyl sulfate (SOS), and natural infochemicals (produced by daphnids consuming *Microcystis*), were tested on both strains. The gene expression of the *mcyA*-Cd, using the *cpcBA* region as reference gene, was assessed in real time in the middle and at the end of the exponential population growth phase, as well as in the stationary phase. The values of relative expression were determined with the  $2^{-\Delta\Delta C_T}$  method ( $2^{-\Delta\Delta C_T}$ ). Population growth was measured as chlorophyll-*a* concentration in the cells of each treatment; microcystins content in the stationary phase was measured with an ELISA kit. *Microcystis* strains showed differential expression in the *mcyA*-Cd, being CH10 the one with the highest expression in most of the assayed treatments; differences in expression were also observed in each strain. In both strains, the highest expression was observed when they were exposed to infochemicals released by neonates, whereas SOS produced no concentration-related effects on the expression. MCs synthesis was detected in early phases in the cultures; this suggests that these metabolites could be essential for the population growth of cyanobacteria. The different expressions found in both strains also suggest diverse ecological strategies in the same species; this could provide dominance and a major permanence during the blooms.

**MO108 Proteomic approach for the evaluation of the effects of polyaromatic hydrocarbons (PAHs) irradiated in the marine fish *Rachycentron canadum*** L.M. Salvo, Cellular and Developmental

Biology; A.M. Lopez, University of Malaga; D. Severino, University of São Paulo IQ/USP / Chemistry Institute; K.H. Fehlauer-Ale, University of São Paulo CEBIMar/USP; J.M. Silva, University of São Paulo CEBIMar-ICB/USP / Cellular and Developmental Biology; S. Arijó, University of Malaga / Department of Microbiology. Proteomics is a recent research field extremely appropriate to address environmental toxicology studies, allowing identification of new biomarkers that are not yet known to commonly used methods. This study utilizes proteomic analysis as a tool to evaluate and compare the sublethal effects of polyaromatic hydrocarbons (PAHs) under irradiation on the juvenile marine fish species *Rachycentron canadum* (Linnaeus, 1766). The PAHs was obtained through ultrasonication of petroleum and analyzed in Horiba OCMA 350 to determine its concentration. *Rachycentron canadum* juveniles were maintained in the laboratory of the Centre for Marine Biology of the University of São Paulo, where they underwent to a period of acclimatization of 20 days under the following controlled abiotic conditions: photoperiod (12h light/ 12h dark), temperature ( $23 \pm 2$  °C), salinity (34 ‰), acidity (pH= 7.4) and dissolved oxygen ( $4 \pm 2$  mg/L). After this period, the fishes were randomly divided into three groups (Control, C1; PAHs, PAHs 1; PAHs under irradiation, PAHs 2) of seven individuals each and transferred to aquariums of 140L capacity. Under

the same above mentioned abiotic conditions, the fishes were waterborne exposed to respective concentration of PAHs, 0.4 ppm for a period of 10 days in semi-static system. The livers were dissected out and placed in RNAlater®. 2-D PAGEs were carried out for the proteomic identification, using liver from five fishes. 2D-spots with over-expression against Control Condition were picked out and identified. Peptides resulting from protein digestion were analyzed by mass spectrometry (MALDI/ TOF TOF). Results were compared in the MASCOT DataBase. 2D- PAGE indicated the existence of a different protein profile between fish exposed with petroleum and petroleum irradiated. Additionally, the activation of several pathways by irradiation was identified. SUPPORT: Malaga University/FAPESP: 2010-50547-8/NP-BioMar (USP) Ethical Committee for use of Animals in Research:124-CEUA

**MO109 Immunotoxicity in Invertebrates - a Review** L. Vorberg,

A. Coors, ECT Oekotoxikologie GmbH. Over the past decades concern was growing about anthropogenic pollutants that may act as immunotoxins and thereby disturb the immune function of both human and wildlife. For various wildlife species, contamination of their habitats was discussed to be linked to an increasing susceptibility to infection. The attention regarding immunotoxicity has focused on vertebrates so far, especially mammals, while little information is available regarding immunotoxicity in invertebrates. One possible reason could be that there is only very little known about chemical-induced immunosuppression in invertebrates at this time. Although invertebrates are playing an important functional role in the ecosystem and are used as key-organisms in ecotoxicology, immunotoxicity is a regulatory endpoint only with regard to toxicological but not to environmental risk assessments. To collect all relevant publications related to contamination-induced immunotoxicity in invertebrates a literature search was performed using the databases *Web of Science* and *Scopus*. Three major topics were defined ("pollution/contamination", "pathogens/parasites" and "invertebrates as hosts") that were supplemented by relevant synonyms. The three major topics were combined in an advanced search by using the "AND" function. From an initial number of about 4,000 publications in peer-reviewed literature all relevant articles were selected manually by the following criteria: (1) only publications about combined effects of pollution and infection with parasites or other pathogens, (2) only effects to invertebrates as hosts, (3) no publications about invertebrates as parasites with vertebrate or plant hosts, and (4) no publications on methods for parasite control. After a critical evaluation of the collected results the review aims to give an overview of the relevance of adversely affected immune function in invertebrates caused by anthropogenic pollution. First compiled results of the literature search will be presented at the SETAC Europe meeting in Glasgow.

**MO110 Effect of acute atrazine exposure on biochemical blood indices of common carp (*Cyprinus carpio*)** J. Blahová; L.

Zelnickova, H. Modra, Z. Svobodova, University of Veterinary and Pharmaceutical Sciences Brno. Atrazine is used as a herbicide for weed control in various field crops and in industrial applications. The widespread use has resulted in the contamination of surface and ground water by atrazine and its metabolites. It has low acute toxicity in mammals but is toxic to aquatic animals. The aim of the present study was to investigate the toxic effects of 96h atrazine exposure on common carp (*Cyprinus carpio*) and assess changes of selected biochemical indices in blood through selected biochemical parameters in blood plasma samples. The acute toxicity test (96 h) was performed on common carp according to OECD No. 203 (Fish, Acute Toxicity Test). Control and four experimental groups (concentration of atrazine – 5, 15, 20 and 30 mg/l) were used in the toxicity test. The experiment was conducted in a semistatic system, and the test solutions were replaced once a day. During the test, the condition of fish was checked and the number of dead fish was recorded for different concentrations. At the end of the test, blood samples were taken by cardiac puncture, stabilized with aqueous solution of heparin, centrifuged and used for biochemical analyses. Biochemical indicators including glucose, total protein, aspartate aminotransferase, alanine

aminotransferase, alkaline phosphatase and lactate dehydrogenase were determined by the biochemical analyzer Konelab 20i using commercial test kits (BioVendor). In the group of fish exposed to 5 mg/l there were no mortalities or behavioural changes compared to the control group. At 15, 20 and 30 mg/l fish exhibited altered behaviour compared to the control group, the fish show various behavioural responses like floating on the side, jerky movement; body pigment was decreased. At 30 mg/l of atrazine, fish began dying on day 3 of exposure; total mortality in this group was 60%. The abnormal behaviour of the fish indicates the toxic effect of atrazine on central nerves and cardiovascular system. Exposure of juvenile common carp to atrazine caused elevation in all blood biochemical indices. The most pronounced changes were obtained in glucose level. In all experimental groups, glucose level showed increase ranging from 261 to 851% in relation to the control. This research was supported by GACR P502/12/P163.

#### **MO111 Sublethal exposure to phenanthrene causes osmo-ionic imbalance and oxidative stress in tropical freshwater fish species.**

M.N. Fernandes, Universidade Federal de São Carlos / Ciências Fisiológicas; L. Martins, J. Ferreira, Universidade Federal de São Carlos / Departamento de Ciências Fisiológicas; A. Bianchini, Universidade Federal do Rio Grande FURG / Instituto de Ciências Biológicas; P. Costa, Fundação Universidade Federal do Rio Grande; G. Fillmann, Universidade Federal do Rio Grande / Institute of Oceanography; J.B. Fernandes, Universidade Federal de São Carlos / Química. Recent studies have shown that since 1990 there was an abrupt and substantial increase in the concentrations of phenanthrene in the aquatic environment, due to the increase of using petroleum derivatives. This study determined the acute toxicity (LC50/96h) of phenanthrene to *Prochilodus lineatus* and investigated the effect of phenanthrene on gills and kidneys of a fish species (*Prochilodus lineatus*) exposed, during 24 and 96 h, to sublethal phenanthrene concentrations (corresponding to 1/100 to 1/50 and 1/5 of 96h:LC50) using biochemical, physiological and morphological biomarkers. After the experimental periods, samples of gills and kidneys were collected and fixed for morphological analysis or frozen for biochemical and physiological analysis. Phenanthrene can be considered moderately toxic to *P. lineatus*; the estimated 96h:LC50 was  $1050 \pm 250 \text{ ?g L}^{-1}$  phenanthrene. Sublethal exposure to phenanthrene ( $200 \text{ ?g L}^{-1}$ ; LC50/5) caused significant changes in morphological, biochemical and osmo-ionic variables. The plasma osmolality and ions changes suggested an osmo-ionic unbalance; which was confirmed by the activity of enzymes involved in osmoregulation carbonic anhydrase in gills and kidneys (increase of Na<sup>+</sup>/K<sup>+</sup>-ATPase in gills and kidneys and increase of carbonic anhydrase in the kidneys), the changes in the number of Na<sup>+</sup>/K<sup>+</sup>-ATPase-rich cells in kidneys. Histopathological changes in the gills and kidneys were distributed throughout the organs. The inhibition of the activities of the antioxidant enzymes catalase and superoxide dismutase in the gills of these animals led to an unbalance between the antioxidants and pro-oxidant levels and then, an increase of lipid peroxidation. In conclusion, the biochemical, physiological and morphological changes in *P. lineatus* exposed to  $200 \text{ ?g L}^{-1}$  affects fish health and increase the energy demand which may affect the growth and reproduction during chronic exposure. Financial support: CNPq/INCT-TA, FAPESP, CAPES

**MO112 Comparison of ecotoxicity of heavy metals against three organisms in different stages in ecosystem** M. Shimabukuro; R. Shoji, Tokyo National Col. of Technology. Recently, heavy metals have been used in the various products, though many of heavy metals have strong toxicity against aquatic ecosystem. Toxicity of heavy metals against mammals have already been elucidated by some toxicity tests such as those using rat and mouse. On the other hand, ecotoxicity of 60 elements against organisms such as fishes, daphnids and algae in fresh water have not been elucidated yet. The objective of this study is to elucidate the ecotoxicity of 10 elements by toxicity tests using three organisms living in different stages in ecosystem, and to improve the reliability of the QSAR (Quantitative Structure Activity Relationship) to predict metal ecotoxicity. There have already been many literatures performing the trials to predict the mammal's toxicity of unknown heavy

metals by properties heavy metals such as ? (softness index) and ion radius. The highest correlation between ? of metals and the toxicity examined by the growth inhibition of a producer, *P. subcapitata* (*Pseudokirchneriella subcapitata*) was found ( $R^2=0.933$ ). The significant correlation the between ? of metals and the toxicity examined by the natation inhibition of a first order consumer, *D. magna* (*Daphnia magna*) was also found ( $R^2=0.741$ ). The worst correlation the between ? of metals and the toxicity examined by the lethality of a second order consumer, *O. latipes* (*Oryzias latipes*) was found among the three ecotoxicity tests ( $R^2=0.548$ ). The order of toxicity strength of heavy metals exposed to the organisms is as follows, EC<sub>50</sub> (Effective Concentration 50%) of *P. subcapitata* < EC<sub>50</sub> of *D. magna* < LC<sub>50</sub> (Lethal Concentration 50%) of *O. latipes*. Among 10 kinds of heavy metals examined in this study, Pb and La do not conform to the order of toxicity strength of heavy metals because they have different their own original speciation, and the dependency of the water solubility on the pH are unique compared to other metal's. On the other hand, metal ion activity is more reliable predictor for the metal bioavailability than the total concentration. So, instead of using EC<sub>50</sub> as the toxicity index, EA<sub>50</sub> (Effective Activity 50%) and LA<sub>50</sub> (Lethal Activity 50%) should be used because they do not depend upon the pH. The order of toxicity strength of heavy metals exposed to the organisms is as follows, EA<sub>50</sub> of *P. subcapitata* < EA<sub>50</sub> of *D. magna* < LA<sub>50</sub> of *O. latipes*. This order enables us to improve the reliability of the QSAR to predict metal ecotoxicity.

#### **MO113 Chronically sequential pulsed copper exposure-associated bioaccumulation and bioenergetics in tilapia** Y. Cheng, National Taiwan University / Department of Bioenvironmental Systems

Engineering; W. Chen, National Taiwan University / Dept Bioenviron Sys Eng; C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering. Aquatic organisms may live in environments with pulsed/fluctuating contaminants. Therefore, it is important to precisely predict metal toxic effects under such exposure patterns. The purpose of this study was to provide information on bioaccumulation and bioenergetics in tilapia *Oreochromis mossambicus* exposed to pulsed copper (Cu) chronically. An integrated approach was used by linking a systems-level approach-based biotic ligand model and a threshold damage model associated with a dynamic energy budget-based bioenergetic model. We carried out 28-day pulsed Cu exposure and growth toxicity experiments to obtain toxicokinetic parameters and dynamic growth biomass data. The estimated uptake and elimination rate constants were  $25.18 \pm 4.61 \text{ mL g}^{-1} \text{ d}^{-1}$  (mean  $\pm$  se) and  $1.09 \pm 0.29 \text{ d}^{-1}$ , respectively. The recovery mechanism was found triggered between the pulsed intervals. The predicted steady-state bioconcentration factor value of  $25.30 \text{ mL g}^{-1}$  was consistent with the experiment derived  $23.10 \text{ mL g}^{-1}$ . The estimated growth cost coefficient of adult tilapia in control was  $0.029 \pm 0.0015 \text{ g}^{1/3} \text{ d}^{-1}$  higher than  $0.019 \pm 0.0017 \text{ g}^{1/3} \text{ d}^{-1}$  in pulsed Cu exposure. This study reveals that the sequential Cu exposure can increase tilapia energy acquisition for overcoming externally fluctuation-driven environments. We suggest that the integrated mechanistic models incorporating with adequate pulsed Cu bioassays may assist the understanding of interactions among toxicokinetic, toxicodynamic, bioenergetic, and recovery mechanisms that reflect the mode of actions in ecophysiological response processes for aquatic organisms exposed to metal stressors. Our results provide a new dimension to understand the chronic sequential pulsed Cu exposure on aquatic organisms.

#### **MO114 Environmental extremes and In vitro-In vivo Scaling of Trifluralin Bioconcentration in Sheepshead Minnows (C. variegatus)** I.R. Schultz, Battelle Northwest Laboratories / Marine Science Laboratory, Ecotoxicology Group; W. Hayton, Ohio State University / College of Pharmacy. The importance of REACH

legislation and growing interest in developing adverse outcome pathways has heightened the need for better QSAR and in vitro based approaches to estimating contaminant bioaccumulation and target organ dosimetry. In previous studies, we have extensively studied the toxicokinetics of the lipophilic herbicide Trifluralin (TF) in fish with a



focus on allometric and interspecies differences. Our approach has been to characterize the uptake, distribution and excretion of TF using a static water exposure system and compartmental toxicokinetic models. This approach allows quantitative estimation of important toxicokinetic parameters such as uptake clearance, apparent volume of distribution and clearance due to biotransformation. In the present study, we exposed sheepshead minnows (a small subtropical estuarine fish common to Eastern N. & S. Americas) to TF under a variety of environmental conditions that included temperature extremes (10°C – 35°C), salinity extremes (60 PPT) and oxygen extremes (25-40% of saturation). The *in vivo* results were then compared to separately measured oxygen consumption rates, total lipid content, plasma protein binding and *in-vitro* biotransformation rates obtained from liver homogenates, to evaluate whether these physiological and biochemical parameters along with published physicochemical properties of TF can be used to predict differences in the *in-vivo* determined toxicokinetic parameters. The strongest correlations were observed between uptake clearance and oxygen consumption and metabolic clearance and *in-vitro* biotransformation estimates. The apparent volume of distribution tended to decrease at warmer temperatures. TF was highly protein bound in plasma with an unbound fraction of approximately 0.05. Model based estimates of the bioconcentration factor (BCF) were relatively unchanged with acclimation temperature but increased at lower oxygen levels. The results from this and past studies with TF can be used to help develop strategies for estimating bioaccumulation based solely on QSAR or limited *in vitro* data.

**MO115 Developing a mechanistic toxicokinetic toxicodynamic (TKTD) model for organism survival based on chemical properties and species characteristics** K. Veltman, Radboud University / Department of Environmental Sciences; M.A. Huijbregts, Department of Environmental Science; J.A. Hendriks, Radboud University Nijmegen / Department of Environmental Sciences. A key challenge facing ecotoxicology is the need to assess the risk of thousands of substances, for a wide range of species, with increased accuracy and ecological relevance. Toxicokinetic-toxicodynamic (TKTD) models are essential to address this challenge, but are currently underutilized, partly because model parameters need to be derived empirically for each toxicant-species combination. TKTD modelling can significantly advance if both TK and TD parameters are quantitatively related to well-known and easy-to-measure chemical properties and species characteristics. At present, such relationships are well-established for toxicokinetics in the form of mechanistic bioaccumulation models. These models accurately predict toxicokinetics of non-polar organic chemicals as a function of chemical properties (e.g. octanol-water partition coefficient), and species characteristics (e.g. size, metabolic type, and fractions of polar and non-polar lipids). Analogous mechanistic models are not yet available for toxicodynamics. The aim of the present study is to develop a mechanistic TKTD model for organism survival, applicable to the Mode of Action “reactive organic chemicals”, by linking both toxicokinetics and toxicodynamics to chemical properties and species characteristics. Reactive organic chemicals elicit their toxic action by reacting non-specifically and irreversibly with nucleophilic biological targets sites, such as enzymes, proteins and DNA. The chemical-specific toxicant-target interaction rate is thus of key importance in explaining differences in observed toxic response between chemicals. We therefore develop species-specific TKTD models by integrating an existing mechanistic model for toxicokinetics with a toxicodynamic model that expresses the chemical reactivity towards the target site. To this end, QSARs for toxicant target-interaction are developed based on literature-collected *in vitro* assay data on target inhibition and a chemical property expressing electrophilic reactivity (e.g.  $E_{LUMO}$ ). The species-specific models are further parameterized and calibrated with literature collected external toxicity data ( $LC_{50}$  and  $LT_{50}$ ). The options to further develop TKTD modelling by quantitatively linking TD parameters (particularly target site density, target site regeneration rate) to species characteristics are discussed.

**MO116 Estimating population-relevant toxicokinetic parameters for**

**body burden modeling** S. Mastitsky, BASF / Crop Protection - Ecotoxicology; N. Kreling, BASF SE / Crop Protection - Ecotoxicology. Body burden modeling (BBM) is considered a potentially useful tool for application in higher-tier assessment of risks posed by chemicals to birds and mammals (EFSA Journal 2009, 7(12): 1438). This approach accounts for the absorption, distribution, metabolism, and excretion processes, allowing for estimation of the actual body burden of a chemical which can be compared with thresholds for mortality (e.g., LD50) or sublethal effects (e.g., food avoidance behavior). Estimation of the population-relevant toxicokinetic parameters required for BBM is often heavily influenced by inter-individual variation. Such a variation can be accounted for when using nonlinear mixed effects models (MEM), a class of statistical models also known as “multilevel” and “hierarchical” models. Although MEMs have been long used in pharmacokinetic analyses, their potential for wildlife risk assessment has thus far been overlooked. In this study we used a simulated dataset to demonstrate how a nonlinear MEM can be employed to describe the kinetics of a hypothetical chemical in a one-compartment open system after a single dose. The analysis was conducted using the functionality of the open source statistical computing environment R (R Development Core Team 2012). In addition to the kinetic parameters (e.g., absorption, elimination, and clearance rates), the model is able to estimate the effects of physiological covariates (e.g., body weight and sex), leading to a greater realism in prediction of the chemical’s body burden. We discuss the possibility of extending the results obtained with MEMs to the probabilistic risk assessment approach by employing Monte Carlo simulations that would account for uncertainty and variability of the estimated kinetic parameters and effects of covariates.

**MO117 Physiologically-based pharmacokinetic (PBPK) modelling of PBDEs in breast milk of women living in an e-waste recycling area** f. fabrega, Universitat Rovira i Virgili / Chemical engineering department; s. kumar; M. Nadal, University Rovira i Virgili; J. Domingo, Universitat Rovira i Virgili; M. Schuhmacher, Rovira i Virgili University. Polybrominated diphenyl ethers (PBDEs) are a large family of compounds used as additives in consumer and industrial products, and especially in electronic compounds. PBDEs are very lipophilic, bioaccumulative in animal and human tissues, and persistent in the environment. These compounds can reach the human body at concentrations that may pose immediate or long-term harmful effects on the human health risk. In order to estimate the theoretical body burdens of chemical pollutants, physiologically-based pharmacokinetic (PBPK) models have been proved to be very useful. PBPK models are mathematical representations of the human body where organs are considered as compartments, where the distribution and accumulation of contaminants is predicted along time. Electronic-waste (e-waste) recycling is an important potential emission source of PBDEs. In Guiyu (China) soils with high concentrations of PBDEs have been detected in an area where e-waste recycling has been carried out for approximately 15 years. As they may easily reach tap water and the food chain, presumably higher levels of PBDEs could be found in biological tissues of the local population. As persistent organic pollutants mainly bioaccumulate in fatty tissues, nursery women and breast-fed infants could be especially affected. In order to determine the content of PBDEs in breast milk, a multi-compartment PBPK model was constructed and validated in a long-term exposure scenario. Blood, liver, kidney, fat, and breast milk were considered as the main target organs, while the rest of the body was modeled as a residual tissue. Special attention was paid on the mammary tissue, to calculate the PBDE concentration, on one hand, and the infant exposure, on the other. The model parameterization took into account the tissue and blood flow modification according to age. For validation purposes, the theoretical results of the whole body model was compared with experimental values of PBDEs in breast milk from women living in the region, and considering available data on food concentrations. Further analysis has been performed for time-related factors and sensitivity analysis of the model. Result is showing that women living in Guiyu (China) have relatively higher concentrations of PBDEs in breast milk compare to non exposed area. In the present study, the developed and validated PBPK model was found to be

capable to predict the levels of PBDEs in the main target organs in general, and in breast milk in particular.

**MO118 Toxicokinetics of cadmium in *Acartia tonsa* under different environmental conditions** M.D. Pavlaki, University of Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; C.A. van Gestel, VU University / Department of Ecological Science, Faculty of Earth and Life Sciences; R. Calado, University of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; S. Loureiro, University of Aveiro / Department of Biology & CESAM - Centre for Environmental and Marine Studies. Brackish ecosystems, such as estuaries and brackish seas or lakes, are simultaneously affected by natural changing conditions (e.g. weathering and erosion or increase of phytoplankton) and anthropogenic activities that introduce contaminants (e.g. metals, fertilizers, pesticides or polycyclic aromatic hydrocarbons) to aquatic systems through river runoffs, as well as industrial, agricultural or domestic wastes discharges. Brackish ecosystems are characterized by complex processes and are considered of great economic value due to their natural productivity. Copepods play an important role in the trophic chains of such systems, as they are a main component of the dietary regimes of several fish and macro-invertebrates. Previous studies have revealed that the accumulation of heavy metals in aquatic invertebrates can differ significantly. The toxicokinetics of cadmium under different pH values, salinities and temperatures were determined in the euryhaline calanoid copepod *Acartia tonsa*. *A. tonsa* was exposed to cadmium for a period of 48 h followed by a 48 h depuration phase (animals were transferred into a clean medium). Three different temperatures (15, 20 and 25 °C), pH values (7.0, 7.5 and 8.0) and salinities (10, 20 and 30‰) were used in combination with one cadmium concentration of 6,875 µg/L. The aim of this study was to assess cadmium uptake and elimination rates in *A. tonsa* under different environmental conditions. Results from this study are expected to show that cadmium concentration in *A. tonsa* tissues will significantly change as natural parameters change.

**MO119 Assessing combination of chemical stressors using a Dynamic Energy Budget (DEB) approach.** E.M. Gudmundsdottir, Plant and Environmental Sciences; N. Cedergreen, Royal Veterinary Agricultural University / Department of Plant and Environmental Sciences; T. Jager, Vrije Universiteit / Dept. of Theoretical Biology. It is important to be able to predict the consequences of mixture toxicity from data of toxicity of individual chemicals. Previous studies have shown, however, that the results of mixture toxicity experiments depend both on the time of assessment as well as the endpoint chosen. Dynamic Energy Budget (DEB) theory allows for an integration of the effect of stressors on different life history traits such as survival, growth and reproduction over time. However, there are currently just a few examples where DEB theory has been applied to multiple stressors. The question is whether it is possible to predict the effect of mixtures of chemicals affecting different life history traits, based on the knowledge on how the individual chemicals affect the DEB-parameters. To address this question, experiments were conducted on the nematode *Caenorhabditis elegans* exposed to cadmium and fluoranthene, alone and in combinations of the two. These chemicals were chosen because they are known to have a clearly distinguishable mode of action of toxic effects on *C. elegans*. The worms were followed during their entire lifespan. Growth was determined on the basis of body length. During the reproduction period, juveniles, fertilized and unfertilized eggs were counted separately, as it is important to include unfertilized eggs in the energy budget also. All data were modeled using DEBtox and the mixture data and model were compared with predictions based on the concepts of Concentration Addition and Independent Action conducted at different endpoints and times.

**MO120 Towards ecotoxicological modelling in a laboratory microcosm: a first step focused on daphnid-algae interaction.** BERNARD, ENTPE/LEHNAIPE / UMR CNRS 5023 LEHNA; U.

HERBACH, Université de Lyon / UMR CNRS 5558 Laboratoire de Biométrie et Biologie Evolutive, équipe Modélisation et Ecotoxicologie Prédictives (LBBE-MEPS); D. LAMONICA, ENTPE; F. ORIAS, Université de Lyon ENTPE / UMR CNRS 5023 Ecologie des Hydrosystèmes Naturels et Anthropisés; C. LOPES, Université de Lyon / UMR CNRS 5558 Laboratoire de Biométrie et Biologie Evolutive, équipe Modélisation et Ecotoxicologie Prédictives (LBBE-MEPS); F. NEZONDET, Université de Lyon ENTPE / Département Ville & Environnement; S. CHARLES, Université de Lyon / UMR CNRS 5558 Laboratoire de Biométrie et Biologie Evolutive, équipe Modélisation et Ecotoxicologie Prédictives (LBBE-MEPS). Some years ago, Clement and coll. worked out a protocol of ecotoxicological bioassay in laboratory microcosms. Effects are assessed on five species based on classical life history trait measurements such as survival, growth or reproduction. This protocol allows taking into account interactions between species, as well as between the biota and medium. In such a complex microcosm, organisms can be exposed through multiple ways (water, sediment, food, suspended particles). Hence it is difficult to relate observed effects to exposure concentration, since concentration may vary in time and/or space for various reasons (degradation, sorption onto particles, etc.). Moreover, conversely to single-species bioassays, in which individual responses can be simply linked to toxicant concentration, observed differences within microcosms may result from indirect as well as direct effects. Facing this complexity, modelling of observed effects within a microcosm appears as a relevant way of improving interpretation. Indeed, taking into account functional interactions within mechanistic effect models provides a more in-depth understanding of biological responses and a proper way of testing various assumptions on the underlying mechanisms. In a first step, we performed two preliminary experiments focusing on daphnid-algae interactions. The first experiment aimed at measuring the system dynamics under control conditions. The second one aimed at quantifying the effects of cadmium. In a second step, a mathematical model based on ordinary differential equations was developed to describe daphnid and algae population dynamics without toxicant. A sensitivity analysis was performed that allowed identifying not only non influent parameters on model predictions (that is parameters that can be arbitrarily fixed to a nominal value), but also, and concomitantly, parameters that need to be estimated from experimental data. These parameters were then estimated by fitting the dynamical model to data from microcosms. Various techniques were used, among which Bayesian inference involving the algorithm of Metropolis-Hastings and a more efficient algorithm based on a particle filtering approach. First results are in good agreement with observed data; the model seems relevant to properly describe the functioning of the algae-daphnia-sediment system. These promising results encourage us to further develop the model, including all trophic interactions as observed in the microcosms.

**MO121 Bioaccumulation of three protozoa *Cryptosporidium parvum*, *Giardia duodenalis* and *Toxoplasma gondii* by zebra mussel *Dreissena polymorpha*** M. Palos Ladeiro, Interactions Animal-Environnement (IAE); A. Bigot, Université de Reims Champagne Ardenne / Interactions Animal-Environnement (IAE); I. Villena, D. Aubert, Hôpital Maison Blanche / Laboratoire de Parasitologie-Mycologie; A. Geffard, Université de Reims Champagne Ardenne / Interactions Animal-Environnement (IAE). *Cryptosporidium* spp, *Giardia* spp and *Toxoplasma gondii* are the main protozoa associated with waterborne outbreaks. Indeed, they have been detected in various watercourses around the world with a high prevalence. Thus, interaction with aquatic organisms is unavoidable. Zebra mussel, *Dreissena polymorpha*, is already used in ecotoxicological studies to reveal watercourse chemical contamination. Nevertheless, ecotoxicological studies must take into account biotic or abiotic environmental fluctuations. In fact, specific parasitism of zebra mussel has already been demonstrated as confounding factor. *C. parvum* and *G. duodenalis* bioaccumulation by zebra mussels has already been demonstrated. This accumulation ability could raise some scientific interogation including (i) aquatic invertebrate role in protozoa lifecycle,

furthermore, in the potential use of aquatic invertebrate as a tool in watercourses sanitary biomonitoring and (ii) protozoa impact on biological responses used as biomarkers. To strengthen knowledge about protozoa interaction with zebra mussel, laboratory *in vivo* exposure has been conducted. Zebra mussel were exposed to various protozoa concentration in simultaneous or single exposure. Results highlight bioaccumulation of each protozoa tested by zebra mussel. Moreover, bioaccumulation is time and dose dependant which suggest zebra mussel potential use in watercourses sanitary biomonitoring. More interestingly, the protozoa which are detected in stronger concentration in zebra mussel tissue are *T.gondii* and no data are available for this time in literature. Then, we attempt to localize *T.gondii* in mussel organs. We focus on three organ which are used in ecotoxicological studies: muscle, gills and digestive gland. Protozoa DNA was detected in all organs after only one day of exposure to 200 oocysts by mussel and by day. However, dominating localization are pointed out in muscle after 7 days of exposure. Future investigations will confirm this localization in muscle or in hemolymph. Since hemolymphatic cells play an important role in invertebrate immunity and defense system, *ex vivo* exposure will permit to improve understanding of non specific parasite and cells and *in vivo* exposure will point out parasite impact in whole organism. *In fine*, both biological and chemical stress will be tested on mussel biological responses used as biomarkers to determine if non specific pathogen can be considered as confounding factor.

**MO122 Life-cycle toxicity assessment of *Caenorhabditis elegans* exposed to selenium** Y. Liao, W. Li, Y. Ju, National Taiwan University. Selenium (Se) is a growing problem of global concern. Se is an essential element required for the health of humans, other animals, and some plants. However, the behavior of Se is unusual in that it also acts to cause adverse effects on reproductive success, which have been linked to declines in animal population. The soil nematode *Caenorhabditis elegans* (*C. elegans*) is a ubiquitous soil organism that plays a primary role in decomposition and nutrient recycling in the soil ecosystem. *C. elegans* is increasingly being utilized as the model organism in aquatic and soil toxicology. In the present study, the experimental data of individual body length, survival rate, brood size, and hatching rate were used to evaluate possible effects of Se on *C. elegans*. A stage-classified matrix model was applied to the experimental data to provide information on the population dynamics of *C. elegans* and to assess Se-affected asymptotic population growth rate. Estimates of survival probability showed that significant decreases in survival at all stages when *C. elegans* exposed to Se. The growth probability of *C. elegans* for L1 stage has the most significant decline from  $0.11 \text{ h}^{-1}$  (for control) to  $0.04 \text{ h}^{-1}$  (for exposure to 3 mM Se). The results showed that Se has a rapid impact on *C. elegans* population dynamics; the asymptotic population growth rate ( $\lambda$ ) was found from 1.0041 to  $0.6416 \text{ h}^{-1}$  for increasing Se concentrations, implying that a potential risk of population decreasing for *C. elegans* exposure to Se contaminated environment. Our present works showed how a mechanistic view based on the Se effects on the *C. elegans* physiology which can promote life-cycle toxicity assessment. An important implication of the analysis is that mathematical models can be used to give a population stage structure and clarity to the analysis of the key population-level endpoint (the asymptotic population growth rate) of population dynamics and to evaluate the influences for other species response to environmental Se. Sequentially, provide a candidate for environmental criteria contemplating the population impacts on organisms.

**MO123 POSTEXPOSURE FEEDING OF THE TROPICAL CLADOCERA *Ceriodaphnia silvestrii* AS ENDPOINT TO EXPOSURE TO THE FUNGICIDE PYRIMETHANIL IN A MESOCOSM SYSTEM** C. Araujo, University of Coimbra / IMAR - Instituto do ar; C. Shinn, University of Coimbra / IMAR - Instituto do Mar; L. Costa, L.B. Mendes, A.L. Sanchez, D. Delello-Schneider, C. Botta, A. Nogueira, University of São Paulo / Hydraulics and Sanitation. Pyrimethanil is an anilinopyrimidine fungicide that has been developed to protect fruit and vegetable cultures against fungal diseases. The

efficiency of pyrimethanil to inhibit the growth of resistant fungus strains has led to an increase in its use. In Brazil, pyrimethanil has been indiscriminately applied in various cultures such as: apple, banana, carrot, citrus, grape, melon, onion, potato, strawberry, and tomato. The spray-drift generated upon its application can be a risk factor to non-target organisms if it reaches adjacent aquatic ecosystems. Therefore, the effect of the pyrimethanil on the feeding rate of tropical cladocera *Ceriodaphnia silvestrii* was studied. Pyrimethanil is the active ingredient of the commercial formulation Mythos, which was applied in a mesocosm system at a concentration of  $1 \text{ mg L}^{-1}$  of the fungicide. Two treatments were considered: three mesocosms treated with Mythos and three reference mesocosms (uncontaminated). In order to guarantee a complete homogenization of the application within the treated mesocosms, organisms were introduced 24 h later. Neonates (72 h old) were selected and introduced in the 250 ml, partially-meshed ( $44 \mu\text{m}$ ) chambers, which were introduced in the six mesocosms: three chambers with 10 organisms per chamber (30 organisms per mesocosm). All chambers were removed after 24 h in-situ exposure and the organisms were counted and recorded for mortality. Recovery rate was 100% and no mortality was recorded in both treatments. Ten organisms were randomly selected from each mesocosm and individually fed for 4 h in darkness with algal suspension of *Pseudokirchneriella subcapitata* ( $4.6 \times 10^4 \text{ cells mL}^{-1}$ ). Feeding rates (expressed as cell number individual $^{-1} \text{ h}^{-1}$ ) for the organisms exposed to reference mesocosm were  $0.50 (\pm 0.05)$ ,  $0.42 (\pm 0.05)$  and  $0.42 (\pm 0.11)$ ; those exposed to pyrimethanil-treated mesocosm were  $0.35 (\pm 0.07)$ ,  $0.32 (\pm 0.10)$  and  $0.25 (\pm 0.09)$ . Considering the mean feeding rate of the organisms exposed to reference mesocosm as the maximum rate (100%), the feeding inhibition was calculated for the three other mesocosms, where organisms were exposed to pyrimethanil. Feeding inhibition obtained was 21, 29 and 44%. These results indicate that pyrimethanil at a sublethal concentration as low as  $1 \text{ mg L}^{-1}$  has a detrimental effect on the feeding rate of *C. silvestrii*.

#### **MO124 The effect of Dissolved Organic Carbon exported from riparian wetlands on the toxicity of heavy metals to *Daphnia magna*.**

E. Geropanagioti, University of Reading / Department of Environmental Science; A. Callaghan, University of Reading; S. Robinson, University of Reading / Department of Environmental Science. Dissolved Organic Carbon (DOC) has been shown to affect the speciation, transport, and toxicity of metals in aquatic environments. Free metal ions can bind to humic substances, becoming less toxic to aquatic biota. Wetlands are able to trap but also release most contaminants, including metals. They are ecosystems usually rich in organic content, consisting of high molecular weight humic and fulvic acids. The dissolved organic carbon that is exported from wetlands to surface waters can affect the toxicity of metals. To date, research has mainly focused in metals cycling within wetlands and their subsequent release to adjacent aquatic systems. The effects of DOC exported from wetlands on contaminants already present in the receiving water body have received little attention. Also, the majority of studies on the DOC-metal interaction have been conducted using commercial humic acids that are significantly different from those in nature. In this study acute immobilisation tests were performed using *Daphnia Magna* to study the effect of natural water DOC on metal toxicity. Natural waters from two contrasting sites in terms of DOC composition were selected; the first having high molecular weight and coloured classes of DOC, and the second being a chalk catchment, with non coloured water and low molecular weight DOC. DOC composition was characterised using UV and fluorescence spectroscopy, to indicate molecular weight. Metal concentrations at the beginning and end of the exposure experiments were estimated using inductively coupled plasma mass spectrometry, thereby providing an assessment of the role of DOC chemistry and concentration on metal toxicity.

**MO125 Ecological and ecotoxicological responses to water pollution: a case-study of the temporary river Brejo do Carragão (South of Portugal)** P. Palma, C. Matos, Instituto Politécnico de Beja; M. Kuster, Institute of Environmental Assessment and Water

Research (IDAEA), Spanish Council for Scientific Research (CSIC); L. Ledo, I. Simoes, Instituto Politécnico de Beja; M. Lopez de Alda, D. Barcelo, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Council for Scientific Research (CSIC). Temporary rivers occur throughout the arid and semiarid regions that cover approximately a third of the world's surface. They are defined as rivers that are normally dry, at least during an extended part of the year. They expand during wet period, through floods events, and contract and fragment during dry periods. These events connected to several environmental factors of the drainage basin (climatic, geological, and topographical) and anthropogenic actions induce the disruption of the dynamics of the river, with effects on the physical and chemical composition of the water, on the aquatic communities, and on the amount of material transferred along the basin. The temporary river of Brejodo Carragão is located in Mira River Basin (South of Portugal), in a semi-arid region where the agricultural is one of the main activities. Water samples were analysed considering: the chemical profile (pH, temperature, electrical conductivity, chloride, phosphorus, kjeldahl nitrogen, ammonia, nitrite, nitrate, alkalinity, hardness, biochemical oxygen demand, chemical oxygen demand, iron, manganese and arsenic); the occurrence of medium to polar pesticides; the evaluation of the benthic macroinvertebrates communities; and the ecotoxicity assessment through bioindicators of different trophic levels (luminescence inhibition of *Vibrio fischeri*, 24-h mortality test with *Thamnocephalus platyurus*, 48-h mortality and reproduction assays with *Daphnia magna*). Considering the physical-chemical analysis the results showed high levels of organic matter and nutrients, mainly in the dry period. The main pesticides detected were terbutylazine, 2-methyl-chlorophenoxyacetic (MCPA), bentazone, mecoprop and metolachlor. The benthic macroinvertebrates analysis showed low levels of communities' diversity, with populations dominated by groups resistant to the organic pollution, mainly the Chironomidae family. On the other hand, the results from the ecotoxicological assessment showed that there was a marked decreased of the *D. magna* reproduction when exposed to water samples; this fact may be correlated to the amounts of pesticides quantified. Thus, this integrative approach highlights the importance of a biological evaluation that covers the interactions between the ecosystem's species, as well as the detection of different types of pollution.

**MO126 Reconstructing environmental trends in potable lakes and reservoirs: The paleoecotoxicology toolbox** B. Lucas, University of Saskatchewan; T. Tse, K. Liber, University of Saskatchewan / Toxicology Centre; P.D. Jones, University of Saskatchewan / School of Environment and Sustainability; J.P. Giesy, University of Saskatchewan / Toxicology Centre; H.S. Wheeler, University of Saskatchewan; L.E. Doig, University of Saskatchewan / Toxicology Centre. Natural lakes and man-made reservoirs often serve as sources of potable water. Although these bodies of water are important to local and regional economies, and critical to future economic development, without a real or perceived threat to water quality long-term monitoring programs are often lacking. As such, changes in ecological structure or function can go unnoticed until such changes manifest in water quality problems (i.e., algal blooms with or without toxin production, taste and odour issues). Once impaired, it can be difficult, costly and take considerable time to reverse undesirable changes to a lake or reservoir. This is made all the more challenging by a lack of information regarding system changes over time and possible tipping points or thresholds prior to undesirable consequences. Aquatic sediments contain a tremendous amount of information that, once interpreted, can help fill these knowledge gaps and help inform the choice of mitigation strategies. Stratigraphic analysis of depositional sediments is commonly used to study temporal changes in the distribution of taxa, to reconstruct historical ecosystems, and to infer past environmental conditions in inland lakes. Depositional sediments can also be used in paleoecotoxicological investigations to reconstruct the timeline of contamination and toxicant-induced changes in aquatic ecosystems. Using Lake Diefenbaker (Saskatchewan, Canada) as a test case, we discuss various tools useful in reconstructing the ecological history of this northern Great Plains reservoir.

Physicochemical variables (e.g., phosphorus, trace metals, stable isotopes) and subfossil (diatom, chironomid) and chemical remains (faecal sterols) will be discussed as lines of evidence.

**MO127 Bacteria as a key-community to track variations on karstic aquifers** N. Abrantes, University of Aveiro / CESAMDAO; D. Figueiredo, University of Aveiro / CESAM & Biology Department, University of Aveiro; I. Rosa, University of Aveiro / CESAM & Department of Environment; P. Saraiva, University of Aveiro / CESAM & Biology Department; A. Goncalves, IMAR - Institute of Marine Research / Department of Life Sciences; M. Bessa, University of Aveiro / CESAM & Biology Department; A. Reboleira, Universidad de La Laguna / Departamento de Biología Animal; F. Goncalves, University of Aveiro / CESAM & Biology Department. Surface habitats have received considerable attention and are currently managed for conservation through a number of relevant regulations. In contrast, groundwater ecosystems appear as a "forgotten realm", despite their high contribution to global biodiversity and relevance as freshwater reservoirs. In particular, karst systems are highly vulnerable compared to other groundwater systems, since potential contaminants produced by anthropogenic activities that occurred at the surface can easily reach the karst aquifers. The Estremenho karst massif, the case study of the present work, is one of the greatest aquifer in Iberian Peninsula with great importance as water reservoir for populations. Chemical and microbiological analyses were performed in groundwater and sediment samples from 5 different caves in this system in different times of year. Toxicity tests with standard organisms (*Vibrio fischeri*, *Pseudokirchneriella subcapitata*, and *Daphnia magna*) were also performed. Although results revealed relevant fecal contamination, chemical analysis showed values below the threshold levels leading to low toxicity effects on the tested organisms. Additionally, preliminary results using molecular methodologies such as PCR-DGGE revealed potential connection in groundwater bacterial diversity among samples, which suggests a hydrological connection among caves. From this, and considering that Estremenho karst massif is severely subjected to anthropogenic pressures (e.g. agriculture, tanning industries, automobile repair garages, and olive oil mills, urban areas), it is important to highlight that a local source of pollution can be spread throughout the karst system. The present work gives exploratory insights on the use of the bacteria as a key-community to track environmental variations on groundwater.

**MO128 Laboratory Cultivation of Qatari Acropora: Studying Dynamic Factors that Influence Coral Growth and Photosynthetic Efficiency** N.M. Al-Naema, ExxonMobil Research Qatar / Environmental Program; C. Richard, CREOCEAN; S. Saeed, E. Febbo, ExxonMobil Research Qatar. Coral ecosystems are very important as they provide a foundation habitat for many aquatic species. An extensive two year field study was conducted to evaluate the effectiveness of Pulse Amplitude Modulation (PAM) fluorometry in monitoring the health of sensitive ecosystems such as coral reefs along the coast of Qatar. The study demonstrated that PAM fluorometry can provide reliable and objective information on coral health in advance of visual signs of stress. The scope of this study has now been expanded to include laboratory based research. The objectives for this research are: a) to establish a viable laboratory based coral (*Acropora sp.*) culture system and b) to utilize laboratory based Imaging PAM fluorometry to compile baseline data, and gain an understanding of environmental parameters that affect the health of the coral. Laboratory studies were initiated in December 2011; *Acropora* samples were collected from mother colonies and the "nubbins" were cultured in pre-acclimatized laboratory aquaria. Imaging PAM fluorometry was utilized to measure photosynthetic processes that were correlated to laboratory culture conditions. A wide range of water quality parameters have been measured, including; temperature, salinity, ammonia, nitrate, nitrite, phosphate, calcium and pH. This research showed that it is possible to successfully culture *Acropora* coral; the initial colonies have grown to the point that several subsequent colonies have been produced to initiate laboratory assay development. The results of the Imaging PAM also show good

correlation with the data obtained using the instrument used in the field. This study demonstrated for the first time the successful culture of Qatari *Acropora* in a laboratory setting. The Imaging PAM fluorometry was also used to obtain detailed visual images of photosynthesis processes. Future studies include *Acropora* eco-toxicological experiments to study contaminants that could affect coral health.

**MO129 The influence of food dependent eco-physiological processes on the response of *Mesocyclops leuckarti* to triphenyltin exposure** D. Kulkarni, RWTH Aachen University / Institute for Environmental Research. Population models have been recommended for extrapolating ecotoxicological test results to the relevant ecological effects in the field. To simulate effects on populations as realistically as possible, detailed information on life-history traits of species and their dependence on environmental factors needs to be incorporated into these models. In this study, we investigated the influence of food dependent eco-physiological processes i.e. development, reproduction and survival on population dynamics under exposure to triphenyltin. The cyclopoid copepod *Mesocyclops leuckarti* was used as a representative species for freshwater copepods. This species is known to exhibit herbivory during early copepodite stages, while the late copepodite stages and adults switch to omnivory. It has also been speculated that early naupliar stages do not feed but rather survive on fat reserves obtained from the egg. An individual-based model (IBM) was developed for *M. leuckarti* using data from laboratory experiments under different food regimes (pure algal diet of *Cryptomonas obovoidea* or a mixed diet of the algae and the rotifer *Brachionus calyciflorus*). This IBM was coupled with the General Unified Threshold model for Survival (GUTS), which was calibrated to 96 h laboratory acute toxicity data, to describe the toxicokinetics and toxicodynamics of TPT. We simulated a single-peak exposure scenario at 20°C for the two different food regimes and calculated extinction probabilities. We found that under a pure algal diet, the late copepodites and adults showed slower development and survival parameters were underestimated leading to a faster extinction of the population. However, assuming food switching from the late copepodite stage onwards and non-feeding nauplii, population resilience was higher and extinction was comparatively slower. We conclude that population models developed on copepod species in particular, and zooplankton in general, should consider the respective complex feeding behaviours in order to simulate realistic population dynamics under toxicant exposure.

**MO130 Organism-level mechanistic effect models: assessing combined effects of chemicals and environmental stressors for *Folsomia candida*** N. Hamda, . With the current focus on predicting population-level effects of chemicals, a tendency has developed to integrate population dynamics in risk assessment process. The intent of these models is to extrapolate organism-level effects observed in laboratory to population-level impacts. Currently, individual-level effects are assessed using dose-response relationships derived from standard ecotoxicological tests. However, extrapolating these effects to population is flawed because typical bioassays do not take into account the factor of time and other environmental stressors that can influence effects of chemical at individual-level and its consequence to the population. Formulating mechanistic effect models to interpret organism-level effects of chemicals helps to understand the interactions between individuals and the environment. It can also help to make an educated extrapolation to the population-level. Such mechanistic effect models can be derived from a general and comprehensive energetic theory: the Dynamic Energy Budget (DEB) model. DEB is an energy/mass balance based metabolic theory which provides a mechanistic interpretation of how organisms acquire and use energy. The development and implementation of this model framework for particular species require, however, reasonable model parameters for the organism of interest. As it is not possible to study and parameterize all species, this should be done at least for organisms used in standard ecotoxicological bioassays. One such organism is the springtail *Folsomia candida*. In this study we parameterized a simplified DEB model ("DEBkiss") for *F. candida* and we implemented the model to explore the combined effect of cadmium,

food limitation and temperature on individual life history and population dynamics of the organism.

**MO131 Elevated temperature prolongs long-term effects of a pesticide on *Daphnia* spp. due to altered competition in zooplankton communities** M.A. Beketov, UFZ - Helmholtz Centre for Environmental Research / Department of System Ecotoxicology. Considerable research efforts have been made to predict the influences of climate change on species composition in biological communities. However, little is known about how changing environmental conditions and anthropogenic pollution will affect aquatic communities in combination. We investigated the influence of three warming periods in combination with a pulse exposure to the insecticide esfenvalerate (0.03, 0.3, and 3 µg/L) in 55 outdoor pond microcosms. Warming periods increased the cumulative water temperature, but did not exceed the maximum temperature measured under ambient conditions. Under warming conditions alone the abundance of some zooplankton taxa increased selectively compared to ambient conditions. This resulted in a shift in the community composition that had not recovered by the end of the experiment, eight weeks after the last warming period. Short-term effects of the pesticide on the community structure and the sensitive taxa *Daphnia* spp. did not differ between the two temperature regimes. In contrast, the time until recovery of sensitive taxa under warming conditions was twice as long as than at ambient temperature. Under both temperature regimes, we identified interspecific competition as an underlying mechanism that determined the time until recovery. However, interspecific competition under warming conditions was prolonged and thus delayed recovery of *Daphnia* spp. from esfenvalerate. These results show that, for realistic prediction of the combined effects of temperature and toxicants on sensitive species, the impact of the two stressors on the competitive interactions within the community needs to be considered. For details see the upcoming publication: Knillmann S., Stampfli N.C., Noskov Yu.A., Beketov M.A., Liess M., 2012. Elevated temperature prolongs long-term effects of a pesticide on *Daphnia* spp. due to altered competition in zooplankton communities. Global Change Biology, accepted.

**MO132 Effects of chemical stressors on predator-induced defenses in snails: subtle effects with strong ecological implications** C.J. Salice, Texas Tech University / Environmental Toxicology. There has been a long-recognized need to incorporate more ecology into ecotoxicology and ecological risk assessment. The mainstay of ecotoxicology has been to evaluate the effects of chemical contaminants on key life cycle traits related to survival, growth and reproduction. Ecological systems are, however, far more complex and species interactions are key drivers of community structure and function. Predator-prey interactions can have especially strong impacts at the community level. Because predator represent a significant stressor, many prey have evolved a suite of phenotypic response collectively referred to as predator induced defenses that reduce the risk of predation. In freshwater snails, for example, predator induced defenses manifest as alterations in behavior, shell shape and shell thickness. In the presence of predator threat, snails generally change habitat use to reduce risk, generate lower profile and thicker shells. Given the ability of prey to achieve predator defended phenotype is critical to reducing the risk of being consumed, we were interested in whether chemical contaminants might alter the ability of freshwater snails to achieve a defended phenotype. We evaluated the effects of environmentally relevant concentrations of malathion and a common road de-icing salt (NaCl) on the ability of *Physa pomelia* and *Helisoma trivolvis* to achieve defended phenotypes in the presence of cues from predator crayfish fed conspecifics. *P. pomelia* exposed to a single pulse of 0.25 ppm malathion showed a significant reduction in predator avoidance behavior two days after the cessation of exposure. In subsequent predator trials, snails exposed to 0.25 ppm malathion were significantly more susceptible to crayfish predators than control snails. Similarly, *H. trivolvis* snails in the presence of predator threat and exposed to 4000 micro S/cm NaCl produced thinner and taller shells, especially at higher temperatures, compared to control snails. Importantly, in both examples, effects on

predator induced defenses occurred at stressor levels below what would be considered a significant effect on life cycle traits. We argue that these types of ecological interactions have potentially profound impacts on community structure and function and would likely be missed with traditional toxicity testing and especially molecular or model-based approaches to toxicity and risk.

**MO133 The regulatory framework of marine litter and its associated risks** A. stoefen, Faculty of Law, Institute of Environmental and Technological Law. Marine litter is seen as one of the most important threats to the marine environment of our times. During the Rio+20 Conference in 2012, the States have committed themselves to take measures addressing marine litter by 2025. It is argued that this intention to act does not reflect the urgency in which marine litter has to be addressed. Since no single agreement integrates the different sources of marine litter, an overview on existing international, regional and EU law is given that theoretically relate to its regulation. Particular challenges in this context are the integration of the manifold pathways and consequences of marine litter and the differing jurisdictions that apply to its regulation. The paper aims to contextualise the different instruments in order to systematically understand the challenges that a marine litter regulatory framework has to integrate. To set the background, legal parameters are defined that are indispensable for an effective regulatory framework of marine litter. These parameters represent the need of prevention at the source and the integration of scientific knowledge on the impacts of marine litter. In a second step, these predefined parameters are applied to existing applicable agreements such as the MARPOL Convention 73/78, the OSPAR Convention and also the Marine Strategy Framework Directive (MSFD) that was adopted in the framework of the EU. The analysis demonstrates that along the cascade of international public law, a differentiated regulatory approach can be discerned. The selection of potentially applicable agreements results in a “patchwork regime” which does not automatically cater to the comprehensive requirements that a marine litter mitigation approach necessitates. Globally applicable agreements usually apply to a sectoral source of pollution, for example vessel-based pollution. A somewhat different approach can be seen on a regional level. Regional Seas Agreements such as the OSPAR Convention are more specifically tailored to the specific vulnerabilities and anthropogenic activities occurring in the region. The material scope of these agreements includes both land- and ocean-based sources of marine pollution and therefore an unnatural fragmentation between both compartments is avoided. The MSFD is seen as an important instrument in this context. It creates a legally binding framework in which a structured assessment and monitoring approach regarding marine litter is established.

**MO134 Introducing FP7 CLEANSEA: Towards a Clean, Litter-Free European Marine Environment through Scientific Evidence, Innovative Tools and Good Governance** H.A. Leslie, Institute for Environmental Studies VU Amsterdam; D. Vethaak, DELTARES; T.S. Galloway, University of Exeter / Biosciences Department; C. Perez, EUEC Mediterranean; R. Brouwer, Institute for Environmental Studies; B. van Bavel, MTM Orebro University; S. Altwater, Ecologic Institute; A. Boon, DELTARES; J. Veiga, EUEC; M.F. Ferreira, University of Brasilia; P. Fernandez, EUEC Mediterranean; H. Nilsson, MTM Orebro University; A. Kalfagianni, N. van der Grijp, Institute for Environmental Studies; M. Cristea, National Institute for Marine Research and Development “Grigore Antipa”; O. Sheremet, E. Papyrakis, Institute for Environmental Studies; E. Anton, G. Tiganov, S. Nicolaev, National Institute for Marine Research and Development “Grigore Antipa”; C. Lewis, University of Exeter; C.R. Tyler, The University of Exeter; M. Depledge, University of Exeter / European Centre for Environment & Human Health; M. Skourtos, A. Kontogianni, University of the Aegean; J. Gerritse, F. Kleissen, G. ElSerfay, DELTARES; J. Robbens, L. Devriese, B. De Witte, K. Bekaert, Institute for Agriculture and Fisheries Research; H. Grahn, P. Geladi, G. Nordstrom, Corpus Data Mining; E. Papathanassiou, E. Kaberi, Hellenic Centre for Marine Research; Y. Wolthuis, Investments in Sustainable Innovations; B.

Veerman, W. Westerman, KIMO Netherlands and Belgium; D. Hadzhiyska, K. Rasheva, B. Rashev, denkestatt Bulgaria; K. Christiansen, L. Christiansen, P. Stephensen, KC Denmark; D. Herzke, N. Warner, Norwegian Institute for Air Research; T. McInnes, Callisto Productions; J. de Boer, VU University / IVM; R. Rashid, denkestatt Bulgaria. Marine litter is widely recognized as a threat to Europe’s marine ecosystems. It is a major societal challenge because it impacts the vast natural marine capital that supports economies, societies and individual well being. Marine litter, of which plastic is a main component, is explicitly identified as a descriptor for determining Good Environmental Status (GES) under the Marine Strategy Framework Directive (MSFD). Europe aims to achieve GES by 2020 and CLEANSEA – the first European framework program research project dedicated to the marine litter issue – is providing key scientific knowledge and tools for marine litter monitoring and action plans. The CLEANSEA project will i) provide comprehensive characterization and analysis of the marine litter problem (biological, chemical, social, economic, legislative and policy-oriented) in the EU’s four main marine regions, ii) propose innovative monitoring tools and standard protocols to facilitate monitoring marine litter in a harmonized way, and iii) present cost-effective management measures and policy options to meet MSFD and other international objectives regarding marine litter. CLEANSEA aims to break down interdisciplinary barriers by synthesizing data and knowledge generated across its 5 RTD work packages, and uses an integrated framework to construct a Road Map for European marine litter reduction. Advanced techniques in the fields of (eco)toxicology, analytical chemistry, satellite imaging, oceanographic modeling and materials biodegradation testing will be used to assess the distribution, fate and impacts of marine litter. Economic, institutional and policy analysis research methods, tools and participatory approaches will be applied to inform trade-offs in policy and decision-making, identify economic, social and governance barriers to GES, and recommend effective policy options and management measures to remove these barriers and incentivize sustainable use of marine resources. CLEANSEA contributes to an adaptive ecosystem approach to the management of human activities in EU marine regions. Project outcomes will benefit not only the MSFD but also various EU directives and strategies including the Europe 2020 Strategy. Measures, strategies and policies that promote upstream sustainable production and use of plastics or recycling of waste that will be highlighted in the CLEANSEA Road Map can also contribute to the EU’s policies and initiatives for a Resource Efficient Europe.

**MO135 Spatial patterns of microplastic distributions along shorelines influenced by riverine discharges** W. Shim, Korea Institute of Ocean Science and Technology / Oil and POP Research Group; N. Heo, Korea Institute of Ocean Science and Technology; S. A. Goeje-shi; S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; G. Han, M. Jang, Korea Institute of Ocean Science and Technology; S. Hong, J. Lee, Our Sea of East Asia Network. EPS spherule (98%) exceptionally predominated along shorelines, on the other hand microplastics more or less evenly distributed. None of correlation in cross-sectional and inter-beach spatial distribution of both micro- and mesoplastics input from offshore and fragmentation on the beach

**MO137 Combined effects of microplastics and other environmental contaminants on predation behaviour of juvenile *Pomatoschistus microps*** L. Guilhermino, CIIMAR University of Porto. In this study, the combined effects of microplastics and other environmental contaminants on the predation behaviour of juveniles (0+) of the common goby (*Pomatoschistus microps*) were investigated. Fish from wild populations were collected in estuaries of the NW Iberian Peninsula. After a period of acclimatization to laboratory conditions, they were exposed for 96h to sub-lethal concentrations of different environmental contaminants (metals, polycyclic aromatic hydrocarbons, and pharmaceuticals) in the presence and absence of microplastics. At the end of the bioassays, the post-exposure fish predation behaviour was individually assessed using *Artemia* and microplastics (of different sizes

and colours) as preys. The results suggest that fish may confound some microplastics with preys, and that the presence of microplastics is able to modify the toxicity of some of the other tested substances highlighting the need of more research on the subject.

**Acknowledgements:** This work done in the scope of the project *SIGNAL – Effects of pollution on estuarine ecological interactions zooplankton-zooplanktivorous fish in relation to climate changes*” (PTDC/AAC-AMB/110331/2009), funded by the COMPETE programme (FCOMP-01-24-FEDER-01387601) and the Portuguese Foundation for the Science and Technology (FCT) through the OE component. M. Oliveira had a post-doc grant of FCT and L. G. Luís had a grant in the scope of the project.

**MO138 Microplastics observations in wastewater treatment effluents – much ado about something** H. Leslie, Vrije Universiteit Amsterdam. A new type of pollution is surfacing in municipal wastewater treatment plants (WWTPs): they're known as microplastics. Tiny particles of synthetic polymers often invisible to the naked eye, these microplastics are finding their way into the world's oceans and have been recognized as part of the global marine litter problem – or as it is sometimes called, 'plastic soup'. Versatile, high-performance yet very cheap, plastics make up a relatively high percentage of litter overall. Plastics particularly dominate the floating fraction of freshwater and marine litter. Wastewater effluents and rivers are a source of land-based marine litter and are just beginning to be investigated as such. We examined wastewater influent, sewage sludge and effluent from local wastewater plants in the Netherlands and have detected a variety of synthetic plastic particles. Wastewater from household washing machines contain synthetic textile fibers which become concentrated with other microplastics in the sewage sludge, but which cannot be fully degraded and removed in the bioreactors. What makes microplastics peculiar compared to other pollutants entering wastewater treatment plants is their degradation half-lives are on the order of hundreds of years – many times longer than even the persistent organic pollutants (POPs). The emergence of microplastic pollution in municipal wastewater is a reflection of the city's metabolism. The main drivers are our massive consumption of plastic products, from clothing to single-use items such as plastic bags and microplastic particles in cosmetics (e.g. shower gel, toothpaste etc.) on the one hand, and our current limitations regarding resource efficiency on the other hand. Microplastics are incompatible with and detrimental to biological cycles. Therefore we suggest ways to close the plastics cycle as opposed to releasing the materials as waste to municipal waterways and ultimately the marine environment.

**MO139 Toxicity of micro polystyrene particle for marine copepod *Tigriopus japonicus*** K. Lee, Korea Institute of Ocean Science and Technology KIOST. We investigated the influence of three polystyrene (PS) micro-beads (0.05, 0.5 and 6 µm diameter) which were selected to measure the sized effect of microplastic for survival, development and fecundity of the copepod *Tigriopus japonicus* using acute and chronic toxicity test. In this study, *T. japonicus* ingested and excreted all PS beads used in the study even at the condition with livefeed without food selection. The copepods (nauplius and adult female) could survive at all sized-beads and its various concentrations tested in the acute toxicity test for 96 h. In the two-generation chronic toxicity test, 0.05 µm PS beads with over 12.5 µg/mL concentration caused the mortality of nauplius or copepodite in the *F*<sub>1</sub> generation even at 1.25 µg/mL concentration in the next generation. In 0.5 µm PS beads treatment, although there was no significant effect in the *F*<sub>1</sub> generation, the highest concentration (25 µg/mL) induced a significant decrease in the survival compare with the control in the *F*<sub>1</sub> generation. 6 µm PS beads did not affect the survival of *T. japonicus* over two generations. The length of nauplius phase and generation time of the copepod were similar pattern with the result of survival. Sex ratio showed no significant difference at all treatments over two generations. The smallest sized-beads (0.05 µm) did not affect the fecundity of the copepod. However, 0.5 and 6 µm PS beads caused a significant decrease of the fecundity at all concentrations. These results suggest that the microplastic such as micro

sized-PS bead may lead to negative effect to marine filter feeder.

**MO140 Microplastic ingestion by zooplankton** M. Cole, . Small plastic detritus, or microplastics, are a widespread and ubiquitous contaminant of marine ecosystems. The ingestion of microplastics by a wide range of marine biota, including mussels, worms, fish and seabirds, has now been documented. However, despite the vital ecological role of zooplankton in the marine food web, the impact of microplastics on zooplankton has remained under-researched. My work explores the uptake and biological effects of microplastics on a range of zooplankton species. We have used an integrated approach, combining feeding rate studies and novel bio-imaging techniques to document ingestion and egestion for a range of plankton displaying different feeding behaviours. Our initial experiments, using fluorescence- and coherent anti-Stokes Raman scattering (CARS) microscopy, have established that many zooplankton species, including copepods and decapod larvae, can ingest a range of polystyrene microplastics (0.4 – 31 µm diameter). We have further demonstrated that the presence of 7 µm microplastics significantly decreases algal feeding in the copepod *Centropages typicus*. Suppressed feeding may have repercussions on zooplankton health (e.g. reduced egg production and growth), which is the focus of our on-going work. In our most recent study we consider how the ingestion of microplastics by zooplankton may have adverse effects on a range of biological scales.

**MO141 Time controlled Cryogenic Zone Compression (timed CZC) GC-HRMS – a Novel Tool for Target Compound Analysis at Ultra Trace Levels?** D. Krumwiede, Organic Mass Spectrometry Scientific Instruments; H. Mehlmann, ThermoFisher Scientific; K. D'Silva, Thermo Fisher Scientific / Organic Mass Spectrometry; I. de Dobbeleer, Thermo Fisher Scientific. **Introduction:** The analysis of dioxins and furans with low limits of detection (LODs) is often challenging and requires highest selectivity and sensitivity in GC-MS analysis. Combined with increased sample size preparation techniques these low detection limits can be achieved routinely for many sample types, such as feed and food or also environmental samples with low contamination levels. However, small sample sizes with low residue levels present a unique analytical challenge, such as bio-monitoring of environmental contaminants in infant dried blood spots with sample volumes as small as 20-100 µL. Current routine analysis techniques do not achieve the required instrument sensitivity. However, large archives of new born dried blood spot samples exist in hospitals globally and their study would be of highest toxicological relevance. In this study a novel GC signal enhancement tool was investigated for lowest level Dioxin analysis in human serum samples for application areas as described above: time controlled cryogenic zone compression (CZC) coupled to high resolution GC-MS. In CZC analytes eluting from the first column dimension are trapped completely in one event maximizing the signal enhancement effect as known from GCxGC. Time controlled CZC basically consists in timed controlled switching of a single cryogenic modulator jet. **Results and discussion:** CZC relevant parameters where investigated and optimized in order to achieve maximum sensitivity and targeted refocusing of selected analytes within a GC analysis run. In all experiments, for standards and for real sample extracts, the CZC signal enhancement effect could be seen unambiguously with peak heights increasing inversely in function of the peak width, e.g. 2378-TCDD in standard analysis with peaks of 9-10 sec baseline peak width vers. 600-700 ms and thus more than 10 fold increased peak height in CZC. A number of real serum samples with different target analyte concentrations was analyzed and compared to standard analysis conditions. Down to 500 ag/ul 2378-TCDD could be detected in standards under optimized conditions. Although further optimisation is required; combining time controlled CZC with the sensitive and selective detection of high resolution mass spectrometry seems to be a promising approach to push absolute instrumental detection limits to the very low femtogram range and potentially further down into the attogram range for specific POPs including 2378-TCDD.

**MO144 Chemical and sensory analysis of chlorine dioxide in drinking water** R. Devesa, Aigües de Barcelona / Chemistry



Laboratory; V. Garcia, I. Fonseca, Aigues de Barcelona; E. Rullan, CETAQUA. Drinking water supply companies have traditionally focused their efforts on providing a product with health guarantees, a safe and clean water. Disinfection has been the main purpose of the water treatment. A broad range of disinfectants and technologies are available. Chlorine dioxide has been increasingly used because of its ability to avoid the formation of trihalometans (THMs), the most common and well known disinfection by-products. The origin of the present work was a taste and odour episode which occurred in the water supply system city of Barcelona and was attributed to the use of chlorine dioxide for final disinfection in one of the sources. The study had two main objectives: first, develop an analytical method to analyse low levels of chlorine dioxide in presence of chlorine; and second, estimate the organoleptic properties of chlorine dioxide in order to know if they were in accordance with the effects reported by consumers (odour complaints). The chemical study showed that the classical colorimetric methods indicated in the literature for the analysis of chlorine dioxide are not selective enough to obtain reliable results when free chlorine is present. The addition of glycine as masking agent in the DPD (N,N'-diethyl-p-phenylenediamine) method inhibits the response of free chlorine but also decreases the signal of chlorine dioxide. On the other hand, the loss of colour of Lissamine Green B in presence of chlorine dioxide is significantly interfered by residual chlorine depending on the relative concentrations between the two agents. As far as the sensory analysis, three types of experiments were performed on chlorine dioxide odour: determination of the threshold concentration (OTC); discrimination test between chlorinated vs dioxichlorinated waters; and preference test, between chlorinated and dioxichlorinated waters. The results obtained permitted the odour event to be explained: the change of compound for final disinfection was detected by sensitive consumers.

**MO145 Optimisation and validation of an on-line SPE/UHPLC-MS/MS method for the analysis of perfluoroalkyl acids in drinking and surface waters** M. Rusconi, Water Research Institute Italian National Research Council / Water Research Institute; C.P. Martins, Thermo Fisher Scientific; L. Marziali, M. Mazzoni, Water Research Institute - IRSA-CNR / Water Research Institute; S. Polesello, Water Research Institute CNR / Water Research Institute; F. Rosignoli, Water Research Institute - IRSA-CNR; F. Stefani, Water Research Institute - IRSA-CNR / Water Research Institute; S. Valsecchi, Water Research Institute Italian National Research Council IRSA-CNR / Water Research Institute. An UHPLC-MS/MS multi-residue method based on an on-line SPE procedure was developed for the simultaneous determination of 9 perfluorinated carboxylates (from 4 to 12 carbon atoms) and 3 perfluorinated sulphonates (from 4 to 8 carbon atoms) in drinking and surface waters. Analytical elution gradient and matrix modification of the aqueous sample were optimised in order to achieve the best sensitivity for the mixture of analytes with a broad range of polarity and acidity. The transition from a chromatographic elution gradient separation, commonly used by an analysis performed by direct injection, to an on-line SPE elution gradient, including chromatographic separation, is proposed. Manual sample preparation was reduced to sample centrifugation and acidification, thus eliminating several procedural errors and significantly reducing time consuming and costs. Ionization suppression between target perfluorinated analytes and their co-eluting SIL-IS were detected for homologues with number of carbon atoms less than 9 but quantitation was not affected. Total matrix effect corrected by SIL-IS, inclusive of extraction efficacy and of ionization efficiency, ranged between -34 and + 39 %. The results of the in house validation, for 5 mL injection, showed good linearity ranges spanning two orders of magnitude between 1 and 100 ng/L for PFUnDA and PFDoDA and 2 and 200 ng/L for the other compounds. The percentage of recoveries, between 76 and 134%, calculated in different matrices (tap water and rivers impacted by different pollutions) were generally satisfactory. LODs and LOQs of this on-line SPE method, which also incorporate recovery losses, ranged from 0.2 to 5.0 ng/L and from 1 to 20 ng/L respectively.

**MO146 Multi-residue method for PAH, PCB and OCP - Validation**

**for trace analysis in forest soil** P. Lehnik-Habrink, Analytical Chemistry; S. Hein, B. Aichner, K. Kaminski, C. Piechotta, Federal Institute for Materials Research and Testing. Analysing organic pollutants in forest soil is challenging because they are strongly bound to soil organic matter (SOM) by chemical and physical interactions. Within the framework of a forest soil inventory an analytical protocol for the determination of polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and organochlorine pesticides (OCP) using one and the same extraction- and cleanup procedure was established and validated [1]. Different soil samples representative for the humic layer from typical mixed and coniferous soil had been used for the analysis. Three extraction solvents of different polarity and six standard extraction techniques like pressurized liquid extraction (PLE), Soxhlet extraction, fluidized bed extraction (fexIKA), sonication, shaking and one-step extraction recommended for analyzing agricultural soil in Germany [2] were compared concerning their extraction efficiency. For additional matrix separation cleanup procedures such as gel permeation chromatography (GPC) and solid phase extraction (SPE) with different sorbent materials were tested. Quantification was carried out using gas chromatography combined with mass spectrometry (GC-MS). Labeled internal standards, added prior to extraction, were used for method evaluation. For the simultaneous extraction of PAH, PCB and OCP from organic forest soil PLE with acetone/cyclohexane provided the highest extraction efficiency. A two step cleanup procedure consisting of gel permeation chromatography (GPC) followed by solid phase extraction (SPE) with silica gel was entirely sufficient for the separation of humic substances. For sample injection best results were achieved using an optimized programmable temperature vaporizer (PTV) injection system as it highly reduced the breakdown of thermolabile pesticides. The described method allows the determination of PAH, PCB and OCP in the trace level range ( $1 - 2 \mu\text{g kg}^{-1}$ ). The protocol is applicable for field laboratories with a high level of automation and for analyzing high sample amounts in line with monitoring programs. [1] P. Lehnik-Habrink, S. Hein, T. Win, W. Bremser, I. Nehls „Multi-residue analysis of PAH, PCB and OCP optimized for organic matter of forest soil“, J Soils Sediments (2010) 10:1487-1498 [2] Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik; Bd. VII, 3 rd Edition, VDLUFA Verlag (2008)

**MO147 PAHs and NSO-Heterocycles in River, Coastal and Marine Sediments of Northern Germany and the German Bight** Siemers, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry; J.S. Maenz, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry; D. Steffen, Lower Saxony Water Management, Coastal Defence and Nature Conservation Agency; W. Palm, W.K. Ruck, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry. Polycyclic aromatic hydrocarbons (PAHs) are regulated in all environmental compartments. Besides the priority compounds naphthalene and fluoranthene, the European Water Framework Directive (WFD) classifies anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene as priority hazardous substances. Due to their toxicological relevance, the structural related class of NSO-heterocycles (NSO-HET) has attracted considerable attention in recent years. However, only little information is available concerning sources, concentrations, reactions and distribution in the environment. To investigate the occurrence of NSO-HET in surface waters, more than 60 river-, coastal- and marine-sediments of Northern Germany and the German Bight were analysed. Campaigns carried out address the following questions: (a) Which concentration levels are found for NSO-HET, especially in comparison to PAHs? (b) Do correlations exist between NSO-HET and PAHs and is it possible to identify main point or diffuse sources of NSO-HET? (c) Is it necessary to include NSO-HET in the regulation process or is the regulation of 8 PAHs in the WFD sufficient to assess the quality of surface waters? For the analysis of sediment samples, two extraction methods (ultrasonic and accelerated solvent extraction) in the low  $\mu\text{g/kg}$  range and subsequent fractionation by pH variation on Lichrolut EN cartridges were developed. GC-MS and LC-MS/MS were used in the

simultaneous analysis of more than 85 NSO-HET, phenols, EPA-PAHs and alkylated PAHs. Extraction efficiencies for the EPA-PAHs were investigated by extracting standard reference material. Unfortunately, reference materials for NSO-HET in sediments are still unavailable. Main heterocycles found in river-sediment samples were dibenzofuran (up to 39 µg/g C), dibenzothiophene (up to 11 µg/g C), carbazole and acridine (in the range of 4-8 µg/g C). However, homocyclic PAHs prevailed in river-sediment samples. As already known and confirmed in our studies, very good correlations (with  $R > 0.99$ ) were found for EPA-PAHs as for pyrene with fluoranthene. Moreover, numerous correlations (with  $R > 0.97$ ) of NSO-HET with PAHs were found, such as for 1-benzothiophene (with naphthalene), dibenzofuran (with fluorene) or dibenzothiophene (with phenanthrene). Concentration levels, compound patterns and additional investigations of coastal and marine sediments, still under way, will be discussed on the poster.

#### **MO148 History of PAH Contamination in Clyde Sediment Cores**

A. Smith, Marine Scotland Science; I. Hussy, Marine Scotland; L. Webster, M. Russell, C.D. Robinson, Marine Scotland Science. The Firth of Clyde is a partially enclosed inland sea in the most industrialised and urbanised area of Scotland. Contamination in this area stems from shipping and industrial activity both in the Firth itself and from the river Clyde, as well as its proximity to Glasgow, Scotland's largest urban centre. Organic contaminants in the Clyde have been the subject of a long term monitoring programme by Marine Scotland Science (MSS). In order to gain better understanding of the history of PAH contamination prior to the beginning of regular monitoring, sediment cores were taken from three sites which are near point sources and are known to have elevated contaminant concentration: the former sewage sludge dumping ground of Garroch Head (GH), the former naval base at Holy Loch (HL) and the major coal loading terminal at Hunterston (HU). In addition a core was taken from a reference site in Kilbrannan Sound (KS), which is remote from any point source, west of the Isle of Arran. The cores were dated using estimates of the sedimentation rates at the sites provided by  $\text{Am}^{241}$  and  $\text{Cs}^{137}$  radioisotope profiles. The periods of sediment deposition in the cores ranged from the date of collection in November 2009 to the 1950s in the HU core, the 1920s in the GH core, the 1900s in the KS core and the 1800s in the HL core. The cores were divided into 4 cm sections, and the hydrocarbons extracted by ultrasonication in polar solvent, prior to the determination of PAH using gas chromatography - mass spectrometry (GC-MS). Total PAH concentrations in the core sections ranged from 3322.2 – 11933 µg/kg dry weight (DW) at HU; from 1254.5 – 6017.3 µg/kg DW at GH; from 85.1 – 2034.8 µg/kg DW at KS and from 703.1 – 18546 µg/kg DW at HL. Comparison of the concentrations of ten individual parent PAH to OSPAR background assessment concentrations (BAC) revealed that with the exception of KS core sections deposited before the 1980s and the bottom section of the HL core (early 1800s), more than half of the assessed compounds – particularly the heavier PAHs – were above their BAC. Application of diagnostic PAH ratios indicated that the majority of PAH at all of the sites were of pyrolytic origin except for those in the deepest 4 sections of the KS core (early 1900s).

#### **MO149 The EU Water Framework directive and the GCMS amenable compounds**

L. de Dobbeleer, Thermo Fisher Scientific; C.P. Martins; P. Silcock, J. Gummersbach, Thermo Fisher Scientific. The European Water Framework directive (2008/105/EC) commits EU member states to actively monitor all types of water bodies against an extensive list of environmental contaminants. The types of contaminants with their desired levels are based on their toxicity, establishing set limits in the following types of water: ground, surface, marine, potable water sources and bathing water. In addition, surface water sediments and biota are also included for monitoring. The compound levels are expressed as Environmental Quality Standard (EQS), with the annual average (AA) and the Maximum Allowable Concentrations (MAC) being set. The actual required limits of quantitation per compound will be expressed by dividing the EQS by a factor of three. This factor was obtained following discussions with various environmental institutes in

Europe. In addition, the proposal (COM(2011)876) for newly established limits for contaminants along with newly added analytes will be discussed; this will amend the present 2008/105/EC directive. This new proposal establishes even lower limits for some of those contaminants that present significant challenges for testing laboratories. In this presentation we are following the workflow in terms of extraction and the various compound classes of the environmental lab and showing the results of initial validations such as method detection limits; repeatability and linearity using a GCMS in SIM mode and a GC-triple quad in MS/MS mode.

#### **MO150 Target, suspect and non-target screening of lake sediments using HR-MS/MS**

A.C. Chiaia-Hernandez, Eawag / Environmental Chemistry; E. Schymanski, Eawag Swiss Federal Institute of Aquatic Science; H. Singer, Eawag - aquatic research; J. Hollender, Eawag / Dept Environmental Chemistry. Organic contaminants can enter natural waters via waste water treatment plant effluents, urban and industrial sewage, erosional runoff, and leaching from agricultural areas. Once in natural waters these compounds may sorb to sediments depending on their physical chemical properties. Sediments are excellent archives of environmental contaminants if the chemicals persist over time since they can act as integrators of many inputs within a catchment. One lake from the north, one from the south and one from the northwest of the Alps in Switzerland were characterized to obtain a coarse survey of the spatial and temporal contamination of the lakes and their different patterns of organic contaminants with time. The whole extraction and purification procedure was optimized to yield suitable recoveries for a wide range of organic pollutants with log  $K_{ow}$  values above 2 (approx. 160 personal care products, biocides, pesticides, pharmaceuticals, industrial chemicals and known transformation products). The screening of compounds was carried out by liquid chromatography followed by electrospray ionization (ESI) and atmospheric pressure photoionization (APPI) coupled to high resolution Orbitrap mass spectrometry (HRMS/MS). The results show that biocides, musk fragrances and other personal care products were the most frequently detected compounds with concentrations ranging from pg/g<sub>dw</sub> to ng/g<sub>dw</sub>, whereas none of the targeted pharmaceuticals were found. The concentrations of many urban contaminants originating from wastewater correlate with the highest phosphorus input into the lake as a proxy for treatment efficiency. HRMS enabled a retrospective analysis of the full-scan data acquisition allowing the detection of suspected compounds like quaternary ammonium surfactants, the biocide triclocarban, and the tentative identification of further compounds without reference standards, among others transformation products of triclosan and triclocarban. Time series analysis was performed between annual laminations within a core, showing input patterns of non-target compounds with a high correlation to the total phosphorus concentration in the lake. Automated exact mass filtering and peak detection in combination with *in silico* fragmentation for computer assisted identification of mass spectra was used to identify non-target contaminants in the lake sediment.

#### **MO151 Stereoselective separation of MDMA enantiomers in sewage water**

E. Emke, KWR Watercycle Research Institute; S.E. Evans, University of Bath; B. Kasprzyk-Hordern, University of Bath / Chemical and Biological Sciences; P. de Voogt, University of Amsterdam / IBED. In 2011 a European campaign was executed to estimate the daily loads of drugs of abuse into sewage treatment plants (STP) of 19 European cities. During one week every day a representative 24h composite sample of the influents was taken. For one particular case, the city of Utrecht, an abnormally high load of MDMA was encountered. The levels of MDMA found did not correspond with those observed in the previous year 2010. The samples were measured by HPLC-LTQ-Orbitrap-MS, and calculated loads varied from 350 g per d for the first day to 75 g/d for day seven. Compared to the preceding year the levels were 35x higher on average. The results raised suspicions about the origin of these loads. The Netherlands and Belgium are known for being the major producers of MDMA in the world, according to information of the EMCDDA. After viewing police reports on the internet it became clear that a possible cause for the abnormally high levels could be a

police raid into a private home two days before the sampling started. The location raided appeared to be equipped for producing MDMA tablets, but only additives, empty tablet bags and a few tablets were found. MDMA is produced as a racemate. When swallowed, it is transformed during passage through the human body. However, the rates of transformation of the two enantiomers of MDMA are different in the human body. The S-enantiomer is transformed at a slightly higher rate than the R-enantiomer, resulting in a shift in the enantiomer fraction from racemic (0.5) to  $\approx 0.65$ . The enantiomer fractions for all samples from the campaigns in 2010 and 2011 were measured using chiral chromatography on CBH columns and triple-quadrupole MS detection. The results showed that the samples from 2011 invariably had enantiomer ratios fractions close to 0.5, demonstrating that the observed MDMA in these samples is racemic and therefore could not be subjected to human metabolism. It can be therefore concluded that MDMA originated from direct disposal into the sewer. The total amount of MDMA that was disposed in the sewage system was assumed to be 30 kg equivalent to approximately 500,000 tablets. As MDMA is not removed at all in the STP plant of Utrecht the amount discharged is directly transferred to the environment. The implications of these direct discharges for ecosystem health remain largely unknown. The present study demonstrates the potential of sewage analysis by LC-MS methods and that of chiral separations for forensic purposes.

**MO152 Simultaneous Direct Determination of Paraquat and Diquat in Water Samples by HPLC-MS/MS** L.J. Wang, X. Liu, M. Godula, G. Jiang, Thermo Fisher Scientific. Paraquat and diquat are widely used herbicides for both terrestrial and aquatic plants and their application poses concern for aquatic life and human health. Diquat is currently regulated by US EPA at 20  $\mu\text{g/L}$  in drinking water. Analytical determination of both compounds has been challenging due to their high hydrophilicity. This study describes a high performance liquid chromatography tandem mass spectrometric method for high throughput, sensitive and selective quantitation of both compounds in environmental waters. Retention and separation were achieved on a specially designed prototype mixed mode column. Without using ion pairing reagent, sensitivity was significantly improved and detection limit was extended to sub-ppb levels. Water samples were injected directly for online SPE cleanup using a weak anion exchange cartridge. Sufficient retention and improved chromatographic resolution were achieved with isocratic elution on a mixed mode column featuring reverse phase, anion/cation exchange retention mechanisms. Analytical time was within 5 minutes per sample ensuring routine high throughput. Selected reaction monitoring was used for quantitation with isotope labeled internal standard to achieve quantitation accuracy. Lower limit of quantitation (LLOQ) was determined at 100 ng/L for both analytes. Precisions were within 10% at 500 ng/L and 4% at 50  $\mu\text{g/L}$ . A good calibration range was achieved from 100 ng/L to 100  $\mu\text{g/L}$  with coefficient of determination ( $r^2$ ) greater than 0.999 for both analytes using  $1/x$  weighting factor. Other performance parameters such as specificity, carry over, accuracy, matrix effect, and real sample analysis were also evaluated and will be presented.

**MO153 Development of acrylamide residue analysis in waters : in situ studies** a. togola, BRGM / Laboratory Division; C. Coureau, S. Touze, A. Guezennec, BRGM. Polyacrylamide-based-flocculants are used in many fields such as agriculture, mineral processing and wastewater treatment and in particular for the suspended solids decantation processes. Flocculants are essentially formed by ultra-high molecular weight polymer, stemming from a polymerization process between acrylic acid and acrylamide. During the production of polyacrylamide, 0.025% to 0.05% of residual acrylamide is left in the polyacrylamide molecule. This monomer is known to be a human neurotoxin, an animal carcinogen and neurotoxin, and is believed to be a human carcinogen. In France, the washing water used in Aggregates industry is recycled by flocculation / decantation in thickeners. The aim of this research is to examine the fate and the transport of acrylamide in Aggregates industry and for that analytical development is needed to study acrylamide in the industrial process and in the aquatic

environment nearby the site. Extraction and analysis of acrylamide from several kinds of waters (process, surface water and groundwater) have been optimized. A detection limit of 20 ng/L has been reached and the performances of the method have been validated according to standard ISO 17025. The repeatability of the method is constant, efficient and accurate by the use of  $\text{C}^{13}$  tracer. Effect of suspended particular matter and stability of acrylamide both in waters and organic extracts has been evaluated. In field application has been undertaken showing the occurrence of acrylamide in the industrial process and in aquatic compartments from few nanograms g per liter two several micrograms per liter, highlighting the need of further experiments to investigate the fate and behavior of this compound.

**MO154 RAPID AND SENSITIVE ANALYSIS OF ARTIFICIAL SWEETENERS IN ENVIRONMENTAL WATER** D.P. Roberts, Waters; M.J. Scotter, FERA. Artificial sweeteners are permitted for use as sugar substitutes in foods, animal feeds, beverages, and sanitary products worldwide. Their advantage to the food industry is that once ingested they provide negligible calorie intake or glycemic effect in the body. However, there are still some ongoing discussions around the potential adverse health effects of these compounds in humans. Moreover, very little data on their environmental distribution and ecotoxicological impact is available. Some of these compounds are not readily metabolised by mammals allowing them to be excreted unchanged in urine and faeces. After decades of use, their occurrence is of particular concern since they are known to pass through wastewater treatment plants unchanged, allowing them to bio-accumulate within the environment. While some artificial sweeteners are permitted in foods, others are banned by various regulatory bodies. For example, neotame is banned within the European Union, while both cyclamate and neohesperidin dihydrochloride are banned by the US-FDA. Hence, there is a requirement for a sensitive multi-analyte quantitative method to be able to detect this new group of emerging contaminants in a range of environmental matrices at levels much lower than those used for sweetening foods and beverages. Most of the existing analytical methods focus on their determination in food and beverage products, which cannot be directly applied to environmental waters because of their very low levels in these matrices. Here we present an adequately sensitive and robust method for the determination of acesulfame-K, aspartame, cyclamate, neohesperidin dihydrochloride, neotame, saccharin, stevioside and sucralose in environmental waters. Water samples were collected from various locations in the United Kingdom and acidified immediately prior to storage at 4 °C. Standards, controls and samples were isolated and concentrated using solid phase extraction (Oasis HLB) and elution solvents were optimised in this study, which was particularly challenging due to the different chemical classes of the compounds. Separation of the sweeteners was achieved using a Waters HSS T3 100 x 2.1, 1.8  $\mu\text{m}$  column and measurement was performed on a Waters Xevo TQ-S LC-MS-MS system using electrospray ionisation. The method was validated in house and showed excellent limits of detection, accuracy and precision. A variety of samples were analysed blank and fortified to demonstrate the method performance.

**MO155 Environmental screening of pesticides: a new scientific information system to enable routine accurate mass non-targeted screening** G. Bondoux, EHQ marketing; J. Joumier, Waters; A. Gledhil, Waters Corp.. Tandem quadrupole instruments are widely employed for the routine quantitative LC/MS analysis of pesticides in environmental samples. However, the number of compounds that can be determined in a single injection is limited by the acquisition speed and compounds which are not in the MRM method will be definitely missed. Consequently, tandem quadrupoles have limitations when screening for a large list of compounds or when investigating the presence of unknown or unexpected molecules. Linked to major gains in sensitivity and linearity, it's now possible to use high resolution MS analysers, like TOF instruments, for environmental analyses. TOF-MS provides accurate mass full spectrum data. Compound detection is based on their exact mass, together with confirmatory information like the isotopic pattern, fragmentation and retention time. Recently a new scientific

information system was introduced to acquire and process these complex datasets. This new system streamlines the workflow and utilises all the available data to ensure that all residues are detected whilst minimising false positive results. The LC/MS method was developed on an UPLC/Qtof from Waters. All data were acquired in MS<sup>E</sup> mode. In this mode, the collision cell switches very quickly from low collision energy for obtaining the molecular weight information, to high collision energy, for the fragmentation information. Data were searched against a library containing molecular formulae, fragment ions and retention times. The list of the compounds found based on the exact mass information was rationalized automatically by using the isotopic pattern, fragments and retention time. Identified compounds were then quantified. Further investigation for compounds that were not present in the library was undertaken using structural elucidation tools including elemental composition, isotopic pattern matching and halogen filtering. Results obtained on surface water samples will be presented and discussed

**MO156 Detection and Occurrence of Purine-Containing Drugs in the Aquatic Environment** J. Funke, Federal Institute of Hydrology, Jan Funke, Henning Wesely, Carsten Prasse, Thomas A. Ternes Federal Institute of Hydrology, Am Mainzer Tor 1, 56068 Koblenz, Germany funke@bafg.de **Session:** Environmental and analytical chemistry - Innovations in environmental analytical chemistry: the quest for pollutants at trace levels **Keywords:** purine, LC/MS, pharmaceuticals, water cycle **Presentation preference:** poster Purine is a structural element of many different pharmaceuticals, such as antivirals, cytostatics, and immunosuppressive drugs. The high chemical reactivity of cytostatic and antiviral drugs might cause possible environmental effects. The first purine derivatives detected in the aquatic environment was caffeine nearly a decade ago.<sup>[1]</sup> After its detection several human metabolites of caffeine were found in a variety of aqueous samples.<sup>[2]</sup> Recently purine-containing antiviral drugs, such as acyclovir and abacavir, were detected in WWTP effluents, surface water and even in ground water.<sup>[3]</sup> However, many other purine based antiviral drugs such as tenofovir and azathioprine are known to be administered as human medicine, appropriate analytical methods are missing for environmental waters. Therefore, new analytical methods were developed to measure purine derivatives in wastewater, surface water and ground water. First a solid phase extraction method using Isolute ENV+ cartridges was developed to enrich the polar purine-based drugs. For detection, a LC Tandem MS system (Agilent 1200 HPLC with AB Sciex API 4000 QTrap MS) was used. The separation was performed with a Phenomenex Synergi Hydro RP as HPLC-column. As alternative, a direct-injection method using the same HPLC-column was developed, but applying a more sensitive mass spectrometer (AB Sciex QTrap 5500). Nevertheless, both analytical methods enabled the detection of more than 50 purine derivatives. Ganciclovir and tenofovir are the first emerging contaminants, which were detected at low ng/L concentrations in wastewaters and hospital effluents. **Literature:** [1] T. Ternes, M. Bonerz, T. Schmidt, *J. Chromatogr. A*, **2001**, 938, 175-178. [2] K. Nödler, T. Licha, K. Bester, M. Sauter, *J. Chromatogr. A*, **2010**, 1217, 6511-6521. [3] C. Prasse, M. P. Schlüsener, R. Schulz, T. A. Ternes, *Environ. Sci. Technol.*, **2010**, 44, 1728-1735. **Acknowledgement:** Financial support by the Federal Ministry of Education and Research, Germany for the project "TransRisk" is gratefully acknowledged.

**MO157 Analytical sensitivity of direct injection LC-MS methods for the compounds of EU Water Frame Work directive** L. Hollosi, S. Westrup, C. Martins, M. Godula, Thermo Fisher Scientific. Chemical pollution of inland or other surface water is an acute threat not just to the aquatic environment and the whole ecosystem but also to the human health. European Commission recognised the importance of the pollution and puts effort in identification of economically and environmentally effective pollution treatment methods for which reliable analytical methods are needed. The types of contaminants with their desired levels are based on their toxicity, establishing set limits in different types of water samples. The compound levels are expressed as Environmental Quality Standard (EQS), with the annual average (AA)

and the Maximum Allowable Concentrations (MAC) and together with a priority compound set list is recently defined in the 2008/105/EC directive. Since these levels are typically lying in the low ppb concentration range, the identification and quantitation of these compounds is achievable only with high sensitive analytical methods. Most sensitive current applications are carried out by triple quadrupole mass spectrometers coupled either to GC or LC. Further improvement in sensitivity can be achieved by application of different sample preparation methods resulting in pre-concentration and enrichment of the target compounds. Since most of these sample manipulation steps are long, laborious and expensive, the appearance of more and more sensitive equipments facilitated to turn analytical chemists' interest to easier and faster direct injection methods. In this work, high volume direct injection method has been applied for identification and quantitation of the priority water pollutant compounds. The main focus was to demonstrate analytical method parameters for LC amenable compounds which cannot be analysed by GC-MS/MS. In addition, other compounds still under consideration by the member states were included since they may become priority compounds in the near future. The fast and effective chromatographic separation occurred on a pore-shell HPLC column while detection was performed by a triple quadrupole mass analyser working in selected reaction monitoring mode. Method sensitivity, repeatability and linearity were established as method performance parameters according to the European Directive 2002/657/EC.

**MO158 Analysis of glyphosate and ampa without the need for derivitization by ion chromatography tandem mass spectrometry in environmental water samples** F. Schoutsen; C. Bruggink, ThermoFisher Scientific. Glyphosate and AMPA are compounds widely used in weed prevention. As a consequence they occur in many parts of the environment. Several methods have been published. In order to reach the low detection and quantitation limits mostly labourious fmoc derivatization combined with LC-MS (triple quadrupole) has been used. Water samples of Friesland, a part of the Netherlands, were analyzed for the nonselective herbicide glyphosate (*N*-(phosphonomethyl)glycine) and its breakdown product aminomethylphosphonic acid (AMPA). The maximum contaminant level (MCL) for glyphosate is set at 0.7 mg/l [1]. In contrast to HPLC methods that needs derivatization [2] with ion chromatography these components can be natively separated preventing frequent cleaning (maintenance) of the MS. Ion chromatography is hyphenated with a tandem MS for analyzing water samples, such as surface and ground waters. The presentation (poster) will discuss the method setup, analytical characteristics, and the obtained results in these water sample matrices. References [1] [http://www.epa.gov/safewater/contaminants/dw\\_contamfs/glyphosa.html](http://www.epa.gov/safewater/contaminants/dw_contamfs/glyphosa.html) [2] Stalikas CD, Konidari CN 2001, Analytical methods to determine phosphonic and amino acid groupcontaining pesticides. *Journal of Chromatography A* 907: 1-19.

**MO159 A novel approach for the analysis of phthalates in environmental waters** J. Thomas, Sepa / Trace analysis; S. Bowers, S. Brown, Scottish Environment Protection Agency. James Thomas<sup>1</sup>, Sue Bowers<sup>1</sup> and Sarah Brown<sup>1</sup> <sup>1</sup>Scottish Environment Protection Agency, 5 Redwood Crescent, Peel Park, East Kilbride G74 5PP \n E-mail contact: james.thomas@sepa.org.uk Phthalates (benzene-1,2-dicarboxylic acid esters) are used as plasticisers in a wide range of industrial and domestic products (e.g. toys, food packaging, lubricants and adhesives). Some of the phthalates have been identified as substances of concern under the Water Framework Directive: diethylhexyl phthalate is a "Priority Substance", benzylbutyl phthalate is a proposed "Priority Substance", and dibutyl phthalate is a "Specific Pollutant". Phthalates are not chemically bound within the material to which they have been added. They therefore have a tendency to be released to the environment when the material deteriorates or flexes during use. This leads to their widespread presence in the laboratory environment and subsequent interference with the quantitative analysis of environmental samples. Environmental samples have traditionally been analysed for phthalates using liquid/ liquid sample extraction and

quantification by GC-MS. This analysis has suffered from inconsistent laboratory process blanks, many of which are significant with regard to the required limit of detection. Solid phase extraction (SPE) can be used but this also leads to similar issues with elevated background levels. An alternative approach has been developed to address this problem. Solid phase extraction (SPE) of samples, using a less aggressive polar solvent for elution/ extraction than normal, has been used. Furthermore, to maintain compatibility with the extracts, quantitative analysis was changed to LC-MS-MS. By adding SPE powder directly to the sample within the sample bottle, background levels of phthalates are now fully minimised and, more importantly, are controllable. This technique for sample extraction can also be used for other determinands and has been demonstrated to be highly successful for other suites of analysis such as alkyl phenols and ethoxylates, and polychlorinated biphenyls. This extraction technique also provides the following advantages: extension of sample stability; improved recoveries and precision; less waste; cost savings; capability for larger sample volumes. This new method provides a major step forward in the analysis of phthalates in the water environment. Details of the method and its performance characteristics will be presented.

**MO160 Fast and easy method using QuEChERS extraction and LC-Orbitrap-MS for analysis of two anticonvulsants and their metabolites in marine mussels**

**M. Martinez Bueno**, Hydrosiences Montpellier UMR 5569,; C. Boillot, D. Rosain, H. Fenet, S. Chiron, C. Casellas, E. Gomez, University of Montpellier 1. Environmental field studies have shown that carbamazepine (Cbz) is one of the most frequently detected human drugs in the different aquatic compartments. It has been regarded as a potential tracer in surface water due to its persistency to biodegradation and poor elimination during wastewater treatment, which makes to Cbz a pharmaceutical of high environmental relevance [1]. However, little information on the detection of such substance in marine organisms and no data about its metabolites or degradation products, is available in literature. In this way, the main objective of this study was to optimize and validate a simple and sensitive analytical methodology for the detection and quantitation of two anticonvulsants (carbamazepine and oxcarbazepine) and their main metabolites in marine mussel (*Mytilus galloprovincialis*). Most of the methods commonly employed until now, for analysis of pharmaceuticals from aquatic organisms are based on isolation of lipid, which often involve complicated extraction and clean up steps. These techniques are long and tedious, time-consuming and require large volumes of organic solvents. However, QuEChERS methodology presents a large number of advantages since it constitutes a fast and inexpensive procedure, simplifies and minimizes the time taken to extraction and clean-up processes. In view of this, a modified QuEChERS extraction method followed by analysis with liquid chromatography coupled with high resolution and high mass accuracy mass spectrometry was the procedure developed. The more demanding requirements regarding mass spectrometric confirmation currently set by EU regulations (Commission Decision 2002/657/EC) were taken into account [2]. Analyses were performed in MS and MS/MS which allowed revealing the different fragmentation pathways highly useful for the identification and quantitation of the isomeric compounds. Quantification limits were below 1 ng/g. Mean recovery ranged from 66-122%. Relative standard deviation was lower than 25% for intra and inter-day analysis. Finally, the method was applied to marine mussel samples collected from Mediterranean Sea in the southeast of France. Residues of the psychiatric drug carbamazepine were frequently found at levels up to 0.32 ng/g dried weight. Therefore, these organisms have proven to be a useful tool for monitoring micro-pollutants in marine water, facilitating detection of trace environmental contaminants, despite their low concentration in the medium.

**MO161 Is it possible to reveal interaction of melanin-containing micromycetes and humus substances in nutrient media using spectral techniques?** **V. Terekhova**, Institute of Ecology and Evolution RAS; M. Gladkova, Moscow State University / Soil Science Department; D. Khundzhua, A. Belik, S. Patsaeva, Moscow State

University. Dominance of melanin-containing forms in micromycete communities is a good marker of unfavorable conditions in biotopes. On the other hand, according to the theory of humus formation through melanoides, fungal melanins can be involved in the synthesis of humus substances. Many species of micromycetes produce dark brown polymers, and thereby might contribute directly to the pool of humus substances creating a buffer organic matrix. It is known that communities in biotopes rich in humus substances demonstrate increased resistance to toxic anthropogenic stress. However, mechanisms of interaction of humus substances and fungal metabolites were little investigated. One of the advantageous tools to analyze the interaction of fungal metabolites and humus substances can serve spectral analysis, allowing using absorbance values and fluorescence characteristics to record the results of the mutual influence of extracellular fungal metabolic products and dissolved humus substances. The work objective was to study spectral features of chromophoric organic substances released by soil fungi in Chapek-medium without and in presence of humus substances. Spectral properties of fungal metabolites in liquid medium were measured and compared with that for natural humus substances and commercial humate preparations in water. Chromophoric organic matter released by various fungi strains showed absorption and fluorescence spectra similar in main spectral features for the samples with different pigmentation of mycelium: decreasing absorbance values towards longer wavelengths with a shoulder at 280-290 nm and fluorescence emission spectra with two overlapping bands (UV fluorescence of phenolics and proteins within 300-350 nm and blue fluorescence of fungal polymers). After two-three weeks of cultures growing in the aqueous humate solution its fluorescence characteristics became more similar to humic-type fluorescence of natural water. Wavelength of emission maximum and quantum yield of humic-type fluorescence were found to be excitation wavelength dependent because of increased heterogeneity of humus substances in culture medium compared to initial commercial humate solution. Transformations in humus substances composition caused by microscopic fungi have been monitored and characterized using spectral measurements.

**MO162 Transformation of three human metabolites of carbamazepine during sand filtration**

**E. Kaiser**; C. Prasse, T. Ternes, Federal Institute of Hydrology. The usage of pharmaceuticals has led to their ubiquitous presence in the aquatic ecosystem, with so far widely unknown consequences for the environment and human health. Although a lot of publications are dealing with pharmaceuticals, only little is known about the fate of their human metabolites. Carbamazepine (CBZ) is one of the most frequently detected pharmaceuticals in the aquatic environment. It has been detected in treated wastewater, surface water and even in drinking water with concentrations up to 6.3 µg L<sup>-1</sup>, 1.1 µg L<sup>-1</sup> and 0.03 µg L<sup>-1</sup>, respectively<sup>[1]</sup>. However, in the human body CBZ is metabolised to approx. 70%, leading to the formation of several metabolites such as 2-hydroxy-CBZ (2OHCBZ), 3-hydroxy-CBZ (3OHCBZ) and 10-hydroxy-CBZ (10OHCBZ) which are mainly excreted via urine. Studies conducted so far revealed a similar persistence of these metabolites during wastewater treatment as described for CBZ, with elimination rates of less than 30%<sup>[2]</sup>. Hence, it is most likely that also the human metabolites of CBZ are discharged into surface water and therefore also drinking water resources. To investigate the presence of CBZ metabolites in the drinking water process, samples from a drinking water treatment plant (DWTP) were investigated. For this, a highly sensitive analytical method for the simultaneous detection of CBZ and its metabolites was developed using SPE followed by LC tandem MS detection. In the feeding water of the DWTP, 2OHCBZ, 3OHCBZ and 10OHCBZ were detected with concentrations up to 30 ng L<sup>-1</sup>. However, during drinking water treatment utilizing mainly sand filtration, a significant decrease of the concentrations of the analytes was observed. To investigate this in more detail, batch experiments with sand filter material were performed in the laboratory. Transformation kinetics were determined and transformation products (TPs) were identified using high resolution MS (Orbitrap and TripleTOF) and NMR. Although 2OHCBZ, 3OHCBZ and 10OHCBZ are closely structurally related, significant differences in the degradation

kinetics were observed. In addition, also chemical structures of formed TP's differed significantly. First results indicate that several reactions such as hydroxylation or the loss of the carbamoyl group are occurring during the biodegradation of 2OHCBZ, 3OHCBZ and 10OHCBZ. [1] Kosjek, T., (2009), *Environmental Science & Technology* **43**(16): 6256-6261. [2] Bahlmann, A., (2012), contribution abstract, Jahrestagung der SETAC GLB und der GDCh. S.139.

**MO163 Analysis of anticoagulant rodenticides in liver using solid supported liquid extraction and LC-ESI/MS/MS.** Schenke, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection; A. Broll, A. Esther, J. Jacob, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants / Institute for Plant Protection in Horticulture and Forests, Vertebrate Research; E. Schmolz, Federal Environment Agency / Section IV 1.4 Health Pests and their Control. Systematic data about the fate and behaviour of anticoagulant rodenticides (ARs) are not available for the specific conditions in Germany. Therefore the aim of a current study is the quantification of rodenticide residues along the exposure pathway bait/prey/predator and a large-scale estimation of residues in non-target predators. For this purposes an analytical method was developed for the 8 ARs included in Annex I of the Biocidal Products Directive 98/8/EC. Liver samples were defrosted, spiked with a surrogate mixture and homogenized in a polypropylene tube by an Ultra Turrax in two steps with methanol for 3 min and after addition of water (2:1/v/v) a second time for 1 min. After centrifugation, 5 ml of NaCl - solution (20%) were added to 15 ml of the supernatant, shaken by vortex mixer and transferred on a diatomaceous earth column (Chem Elut, 20 ml, Agilent). The solid supported liquid extraction was done with 100 ml dichloromethane. Two ml of this solution were evaporated and the residue was refilled with the internal standard solution. Chlorophacinone-d4 was used for the 1,3-indandione and warfarin-d5 for the 4-hydroxycoumarine substances as internal standard. Quantification of ARs was performed by LC-MS/MS (QTRAP 5500, AB SCIEX) in electrospray ionization negative mode using the most prominent deprotonated molecular ion-product ion-transition. Spectra comparison between sample and references was done additionally. Mean recovery values were achieved with a spike level of 100 ng g<sup>-1</sup> (turkey liver): chlorophacinone 83%, warfarin 118%, coumatetralyl 100%, difenacoum 78%, bromadiolone 77%, brodifacoum 58%, flocoumafen 65% and difethialone 41%. Before analysis, all study samples were spiked with surrogate solution (acenocoumarol, coumachlor, diphacinone-d4, phenprocoumon) for ongoing validation of the analytical performance. Storage tests showed that the stability of ARs in liver could be guaranteed over 33 weeks at -20°C and also with one freeze-thaw-cycle in light for a half hour each week. Over this whole period the ARs are also stable in acetonitrile at -20°C, +4°C and at room temperature but only in the dark. However, in light and at room temperature bromadiolone and brodifacoum were degraded below the threshold of 70% very quickly within one week. Both rodenticides were only stable for four weeks storage at -20°C with one freeze-thaw-cycle each week. This study is funded by the German Federal Environment Agency (3710 63 401).

**MO164 Water-Sediment Biodegradation: Challenges in Screening for Pharmaceutical Transformation Products.** F. Ericson, Pfizer Inc / Environmental Sciences / PDM. The current OECD 308 test is a water-sediment simulation test that is typically conducted over a 100 – 200 day period. It is intended to estimate the parent half life in the water phase, sediment phase and collectively in the total water-sediment system; determine the distribution and mass balance of the residues in the test system; as well as characterize the potential transformation products of the parent substance over the study period. Given the length of the test period, the analytical expertise and costs associated with conducting the test, it is often desired that a more rapid screen for assessing the potential transformation of pharmaceuticals would be available. Such information would be helpful in developing the ERA testing strategy especially when the identification of a key transformation product would be helpful early on in the risk assessment; as well as optimizing sampling intervals and conditions for the OECD

308. This presentation looks at the observed transformation products identified in the OECD 308 study and reviews preliminary data obtained in a 2- 4 week water- sediment biotransformation screen using a modified OECD 309 test. Biomass collection and handling, biomass solids levels, stirred reactor design, elevated temperature and use of P450 inducers are discussed as approaches to enhance the kinetics typically observed in the OECD 308 test. Two to three case studies will be presented to highlight some of the challenges present in advancing these further.

**MO165 The effect of the autosampler vials glass surface on GC-MS analysis of pyrethroid pesticides at ppb levels.** P. Morgan, Thermo Fisher / Chromatography Consumables. Pyrethroids are a class of synthetically produced insecticides that are mainly used for domestic purposes to control insects such as mosquitoes. They behave very similarly to natural pyrethrins, which are derived from chrysanthemum flowers and are extremely toxic to fish and aquatic organisms. Repeated exposure in humans increases the risk of anaphylaxis and allergic reaction at very low concentrations and can be monitored. In this study, a simple method for isolating pyrethroids from water at low ng/mL level was performed using Solid Phase Extraction (SPE) followed by GC-MS utilizing a Programmable Temperature Vaporizer (PTV) simulated on-column injection. During the development of a method for the high sensitivity analysis of pyrethroids (bifenthrin, permethrin, cyfluthrin and cypermethrin) the calibration accuracy at low ppb levels showed unacceptable linearity and precision for a number of the compounds. Investigation of the sample handling showed that the choice of the autosampler vial was impacting the recovery of the compounds. Previous studies have shown this effect with LC-MS studies when using polar, basic analytes but this had not previously been observed with the GC-MS analysis of pyrethroids. Using a number of different vial types manufactured from different grades of both amber and clear glass the recovery of sample from low concentration standards in the 1-100ppb range were compared. The effectiveness in reducing this surface activity was then explored using a range of chemically treated, physically deactivated and pre-washed vials. Physical characterization of the glass surface was also used to show differences in the glass surface and raw material. The effect was found to be least with vials manufactured from high purity clear neutral borosilicate glass. The use of amber glass without treatment was found to show greater absorbance that could be successfully reduced by silanization.

**MO166 Environmental laboratory practice based on up to date education, knowledge and training. Challenges and trends** M. Vojinovic Miloradov, Faculty of Technical Sciences, University of Novi Sad; I. Spanik, Slovak University of Technology in Bratislava; J. Radonic, M. Turk Sekulic, M. Stosic, D. Milovanovic, N. Senk, Faculty of Technical Sciences, University of Novi Sad. The major water pollution and quality in Western Balkan countries are caused by discharge of untreated or insufficiently treated municipal and industrial waste waters. Other threats to the water quality come from agriculture. Special interest is devoted to the catchment of the Danube and Sava/Vrba River and its tributaries as an important source of drinking water in Europe. TEMPUS Project-Network for education and training for public environmental laboratories-NETREL, aims to train researchers and experts in Serbia and Bosnia & Herzegovina in environmental analytical techniques required to meet the major challenges and trends in the monitoring, assessment and management of pollution and emission of toxic compounds in WBC. NETREL action will allow the development and implementation of state-of-the-art methodologies to improve WB capabilities for monitoring of environmental pollutants. It will provide a unique opportunity to share developed protocols, validated analytical methods and harmonised chemical and biological monitoring methods with Slovakia, Czech and UK regulated by EU legislation. The related list of Priority Substances (2455/2001/EC) was established through the combined monitoring and modeling-based priority setting procedure, combining with modeling approaches. It is essential that attention should be paid to emerging pollutants and that they should be adequately researched and monitored across Europe according to NORMAN list. The

public laboratories in Serbia and Bosnia & Herzegovina monitor only the basic physical chemical parameters of raw and drinking water. The major reasons for the lack of accredited methods for environmental analysis and for absence of systematically monitoring programmes indicated by public laboratories are insufficient experience and up-to-date knowledge in sampling, sample treatment and analytical methodology. Project activities are based on sharing up-to-date knowledge gathered during development of EU environmental policies with academia experts from partner Western Balkan countries, which will further be transferred to staff in public environmental laboratories. The gained knowledge in the fields of QA/QC, analytical methods, environmental monitoring and management would be for the best trends and development of environmental laboratories in WBC.

Acknowledgements – Financial support for these activities are given by 530554-TEMPUS-1-2012-1-SK-TEMPUS-JPHES

#### **MO167 Comparison of speed discs and solid-phase extraction cartridges for drugs determination by GC-MS in water samples**

M. Caban, Faculty of Chemistry, Institute for Environmental and Human Health Protection, Department of Environmental Analysis; A. Fabianska, University of Gdańsk; J. Kumirska, University of Gdańsk; P. Stepnowski, M. Kwiatkowski, University of Gdańsk / Faculty of Chemistry. Nowadays solid-phase extraction (SPE) is the most popular extraction method of environmental pollution (e.g. pharmaceuticals, personal care products) from liquid samples. Polymer (in fact copolymers) sorbent works great for this purpose. The common amount of sorbent is 150-200 mg in a 2 mL cartridge with the maximal sample volume of 500 mL. Environmental samples contain trace amounts of pharmaceutical residue (ng/L), therefore a high concentration factor is needed to evaluate their presence. For this reason speed extraction discs were developed. Their main advantage is quick passing of large volumes of samples (up to 2 L) though the sorbent (packing in large particles). In the presented work we compare the efficiency of two drugs classes ( $\beta$ -blockers and  $\beta$ -agonists) extraction, using speed-extraction discs and normal cartridges, from tap water. The nature of sorbent and applied procedure in both cases were the same. For speed discs the volumes of used organic solvents (for conditioning, rinsing and eluting) and samples were four times greater than those used for cartridges. Derivatization by BSTFA and gas chromatography with mass spectrometry was used for quantitative analysis. It was observed that extraction efficiencies (EE) were similar (73-124%). Matrix effects (ME) were higher (in absolute values) for analytes extracted by speed discs. For this reason analytes absolute recoveries (AR) were smaller when speed discs were used. ME tell us about the influence of matrix components on derivatization and final analysis. Higher sample volumes cause increased amounts of interferences co-absorbed and then co-eluted. The main advantage of speed discs is a high concentration factor which amounts to 20 000 in the presented work. *Acknowledgement* - Financial support was provided by the National Science Center (Poland) under decision number DEC-2011/01/N/ST4/02478.

**MO168 Study of environmental matrix impacts on derivatization and gas chromatography analysis** M. Caban, Faculty of Chemistry, Institute for Environmental and Human Health Protection, Department of Environmental Analysis; A. Puckowski, J. Kumirska, University of Gdańsk; P. Stepnowski, M. Kwiatkowski, University of Gdańsk / Faculty of Chemistry. Much work was attempted to establish the influence of sample matrix complexity on results through the determination of matrix effects (ME), extraction efficiency (EE) and absolute recovery of analytes (AR) in HPLC-MS analysis. There is few literature data describing the influence of aqueous matrices (tap water and waste water) on results obtained by GC-MS methods. For these reasons, the main aims of the present study were to investigate the influence of different aqueous matrices on the solid-phase extraction, derivatization and drugs determination by GC.  $\beta$ -Blockers and  $\beta$ -agonists were chosen as model compounds for this investigation. Wastewater samples were extracted by solid phase extraction. Next, the analytes in extract were derivatized by a popular silylating agent (BSTFA) and by DIMETRIS (manufactured in our lab). GC-MS(SIM) was applied to

quantitative analysis. Afterwards three parameters: EE (Extraction Efficiency), AR (Absolute Recovery) and ME (Matrix Effect) were calculated for all drugs by the following equations:  $EE = (C/D) \times 100\%$ ;  $AR = (C/A) \times 100\%$ ;  $ME = (B/A - 1) \times 100\%$ , where:  $A$  – response for the standard solution,  $B$  – response for the sample spiked with the target compound(s) after extraction, and  $C$  – response for the sample spiked with the target compound(s) before extraction. It was found that the derivatizing agent has influence on analysis results, because each of them possesses unique sensitivity to the matrix components. Each sample extract created individual matrix effects (values may differ from -20 to -80%). Tracking this parameter is important due to big seasonal changes in samples composition and the possibility of false negative or positive results presence. Despite the fact that analyzed drugs are structurally and chemically similar, MEs for them are individual. Presented method of ME, EE and AR calculation is simple and leads to general conclusions about the quality of the results. Nature of MEs are different for GC and for HPLC-MS. Lack of ion suppression / enhancement in EI ion source, results in the statement that GC-MS is a great tool for complex environmental samples analysis. New silylating agent DIMETRIS is a very interesting alternative to popular derivatizing agents. *Acknowledgement* - Financial support was provided by the National Science Center (Poland) under decision number DEC-2011/01/N/ST4/02478.

#### **MO170 Platinum group metals from autocatalysts – emerging contaminants in soils of Czech Republic** L. Sikorova, VSB

Technical university of Ostrava / Faculty of safety engineering Energy research center; M. Sucmanova, TraceLab, spol. s r.o.; T. Vaculovic, L. Simonikova, Masaryk University; J. Štátník, University of Ostrava. Autocatalysts presented a major breakthrough in reducing emissions of gaseous pollutants from transport. However, introduction of new technologies brings new and emerging potential risks. The technology for emissions control is not the exception. Nowadays, autocatalysts are considered the main source of platinum group metals (Pt, Pd, and Rh – PGM) emissions into the environment. Since their introduction, the significant increase of naturally very low PGM concentrations has been observed in different environmental compartments. Concerns, which present the accumulation of these contaminants in the environment, are raised by toxicity of certain PGM compounds well known from the working environment. Soluble Pt compounds are among the most efficient sensitizers. Whereas in many countries there are many studies with respect to environmental burden by PGM, the issue has been solved just several last years in the Czech Republic (CR), and comprehensive information is still missing. To obtain preliminary data for environmental monitoring of PGM with respect to their accumulation, screening of Pt, Pd, and Rh was carried out along the highway D1 between cities Prague and Brno, and in the city of Prague, Czech Republic (CR) in 2009. Sampling was performed at locations with high traffic load, within 5 and 10 m distance from the communication, at depth 5 cm. Before analysis by ICP-MS, soil samples were leached with aqua regia. Direct determination of PGM in extracts was strongly influenced by interferences. With respect to Pd, mathematical corrections were unable to solve them. The concentrations of Pt and Rh in soils along D1 were in the range of 0,24–24,14 ng/g (mean 5,90 ng/g), and 0,04–4,02 ng/g (mean 0,87 ng/g), respectively. In Prague, there were more than 3-fold higher concentrations of Pt, and 4 times higher concentrations of Rh. This is in compliance with traffic intensities, which were almost 4-fold in Prague in comparison with D1. The results indicate a significant increase of PGM concentrations against the background. The question of potential health risks from environmental contamination by PGM becomes relevant in the CR as well. With respect to obtained results, it is necessary to continue in long-term monitoring of PGM in the environment. The attention should be focused on urban areas, especially on sites with regular occurrence of children, since child population is at high risk of exposure to harmful substances from contaminated soils.

**MO171 Monitoring of Heavy metals in Zmit Bay Coast Using Algae (*Gracilaria gracilis*) as a Bioindicator** F. Kocbas, Celal Bayar



University; N. Arslan, Celal Bayar University / Biology; H. Ozciftci, Celal Bayar University; R. Oral, Ege University. *Gracilaria* is a genus of red algae (Rhodophyta) notable for its economic importance as an agarophyte, as well as its use as a food for humans and various species of shellfish. *Gracilaria gracilis* collected from six stations in Izmit Bay, Marmara Sea were analyzed for trace metals (Zn, Cu, Cd, Ni, Pb, Cr, Al) by using Agilent Technologies ICP-MS. Samples were taken between August 2011 and May 2012. The present study showed a concentration of 0,78-4,08 mg/kg Zn, 0,12-1,02 mg/kg Cu, < 0,01 Cd, < 0,001 Ni, 0,6-7,2 mg/kg Pb, 0,2-2,2 mg/kg Cr, 11,56-80,04 Al in *G. gracilis*. The results were compared with literature values reported for similar studies conducted in Izmit Bay, Iskenderun Bay, Çandarlı Bay.

#### **MO172 Heavy Metal Pollution Assessment In *Gracilaria gracilis* of The Izmit Bay, Turkey**

**F. Kocbas**, Celal Bayar University; N. Arslan, Celal Bayar University / Biology; H. Ozciftci, Celal Bayar University; R. Oral, Ege University. Trace metals are an important sources of pollution in the marine environment. Concentrations of Al, Cr, Ni, Cu, Zn, Cd and Pb were determined in *Patella caerulea* samples collected from six stations in Mersin Bay, Turkish coast of Mediterranean Sea. Mersin Bay is under the effects of industrial processes, agriculture and urbanization. The present study showed a concentration of 2,86-50,14 mg/kg Al, 0.08-1,3 mg/kg Cr, 0.28-3,54 mg/kg Ni, 1,14-8,14 mg/kg Cu, 5,46-19,96 mg/kg Zn 0,04-0.88 mg/kg Cd, 0,10-2,52-190 mg/kg Pb in *P. caerulea* collected in Mersin Bay. The results were compared with literature values reported for similar studies conducted in Mersin Bay and adjacent seas.

#### **MO173 Heavy Metal Levels In *Patella* sp In The Coast of Tekirdag, Turkey**

**H. Ozciftci**, F. Kocbas, E. Evsen, Celal Bayar University; R. Oral, Ege University. The coast of Tekirdag is influenced by factors such as rapid urbanization, coastal traffic, port activities, agricultural activities, tourism and fishing. New harbor construction works would place the threat of potential pollution while increasing the region's maritime activities. In this study, collected from the shores of Tekirdag aimed to determine the level of heavy metals in samples of *Patella* sp. Seasonal sampling was performed between August 2011- May 2012. Sample of 25 individuals of *Patella* sp at a station are different points. Agilent Technologies ICP-MS was used in the analysis. The heavy metal concentrations in *Patella* sp samples was: Al > Zn > Pb > Cu > Cr > Ni > Cd.

#### **MO174 Concentration of Heavy Metals from Iskenderun Bay (Turkey) Using *Patella* sp (Gastropoda:Mollusca)**

**B.N. Anil**, Celal Bayar University / Biology; F. Kocbas, H. Ozciftci, Celal Bayar University; R. Oral, Ege University. Bioaccumulation of cadmium, lead, zinc, copper, nickel, chromium, and aluminum in the edible parts of gastropoda species, *Patella* sp, from Iskenderun Bay, the northeastern Mediterranean sea, Turkey was examined. Concentrations of the heavy metals in the examined species was as follows; in *Patella* sp Cd < 0,001-0,8; Pb 0,6-4,6; Zn 14,6-21,96; Cu 13,0-24,54; Ni 2,2-13,4; Cr 1,8-13,6; Al 7,24-50,14 mg kg<sup>-1</sup> dry weight. The results were compared with other studies and discussed. According to these results, The results of the measures for the protection of the marine environment has been required to be increased.

#### **MO175 Heavy Metal Levels In *Patella caerulea* In The Mersin Coast Of The Mediterranean Sea, Turkey**

**F. Kocbas**, Celal Bayar University; E. Yildirim, Celal Bayar University / Biology; H. Ozciftci, Celal Bayar University; R. Oral, Ege University. Trace metals are an important sources of pollution in the marine environment. Concentrations of Al, Cr, Ni, Cu, Zn, Cd and Pb were determined in *Patella caerulea* samples collected from six stations in Mersin Bay, Turkish coast of Mediterranean Sea. Mersin Bay is under the effects of industrial processes, agriculture and urbanization. The present study showed a concentration of 2,86-50,14 mg/kg Al, 0.08-1,3 mg/kg Cr, 0.28-3,54 mg/kg Ni, 1,14-8,14 mg/kg Cu, 5,46-19,96 mg/kg Zn 0,04-0.88 mg/kg Cd, 0,10-2,52-190 mg/kg Pb in *P. caerulea* collected in Mersin Bay. The results were compared with literature values reported

for similar studies conducted in Mersin Bay and adjacent seas.

#### **MO176 Micro-optical oxygen sensors: a new approach for contact-free measurement of ecotoxicological effects**

**X. Zhang**; J. Filser, University of Bremen. Oxygen micro-optical sensors are used widely in marine science to monitor the rate of oxygen production and consumption for profiling biofilms and sediments. It is a sensitive method to analyse the activity and metabolism of aerobic organisms, which can be also applied in ecotoxicological tests in both aquatic and terrestrial systems. In an array of calibration tests for assessment of this new approach, we compared it with Warburg respiratory manometer. First results clearly show that oxygen micro-optical sensors can be well applied to both aquatic and terrestrial tests. Their results can be temperature and atmosphere pressure compensated to get precise measurements. Respiratory activity also correlated well with cell counts of algae, one of the most important advantages of this new method is that respiration and photosynthesis of contaminated soil and water can be measured without contact between sensor and test medium, thus avoiding potential risks of any sensor contamination. The second array of toxicity tests has been planned, in which oxygen micro-optical sensors will be used to detect effects of silver nanoparticles on the growth of soil green algae and the respiration of collembolans. The experiments aim to achieve an improved, efficient and easily understandable way of ecotoxicology assessment. Keywords: micro-optical oxygen sensor, ecotoxicology, respiration, photosynthesis

#### **MO178 Processing Highly Particulate Laden Samples Using Automated SPE Extractors**

**S. Lambert**, ARC Sciences Limited; **N. Hamda**. Oftentimes, aqueous samples collected contain varying amounts of suspended solids or sediment strictly due to either the source of the water being sampled or improper sampling techniques. In any circumstance, samples with high amounts of particulates or sediment have proven challenging to extract using EPA Method 3535: Solid Phase Extraction (SPE). The two options available for SPE are to either use a disk or a cartridge. Processing a high sediment sample with a SPE cartridge will result in instant clogging of the cartridge and failure to extract the "whole sample". A standard 47 mm SPE disk provides many advantages over SPE cartridges. SPE disks can process a wide range of particulate laden samples, but the amount or size of the particulates may sometimes result in longer sample processing times. An alternative is to use a 100 mm SPE disk which has nearly four times the surface area of a 47 mm disk and will process a particulate laden sample at a faster flow rate. However, larger disk sizes require more sorbent and can be very expensive. It would be more cost-effective to use an inexpensive 90 mm prefilter with a 47 mm SPE disk. This poster will demonstrate the development of various extraction methods for a specialized disk holder suited for automated SPE extractors. Multiple sets of data will be presented including data from samples extracted for EPA Method 8270D for Semi-Volatiles and EPA Method 608 for Poly-Chlorinated Biphenyls and Organochlorine Pesticides.

#### **MO179 A Dissolved / Particulate Baseline Study of PAH's (Poly-Aromatic Hydrocarbons) in Particulate Laden Waters**

**S. Lambert**, ARC Sciences Limited. **Introduction:** The purpose of this investigation/method development was to establish a new extraction method using hexane as the extraction solvent for the determination of 209 PCB congeners in 116 chromatographic peaks in various ratios in aqueous samples which would allow for a detection range into the ng/L (PPT) range for 1L and 8L samples while processing the samples through the disk at over 100 mL/min. This was accomplished by optimizing a Solid Phase Extraction (SPE) method based on the Horizon Technology SPE-DEX® 4790 Automated Extractor System using a divinylbenzene (DVB) extraction disk and detection through a GC/ECD. The sample preparation step is an essential element of this method development, and as such, the advancement of an automated disk extraction has resulted in less solvent use, elimination of the solvent exchange step, reduced glassware use, faster extraction time with highly particulate samples, and more consistent and reproducible results. **Results and Discussion:** The results are excellent for all of the selected

persistent organic polluting PCB congeners concentrated down to 1.0 mL on the DryVap®. If there was to be any loss of PCBs it would be during the extraction. The average recovery levels range from 95% for hexachlorobiphenyl (#169) to 112% for dichlorobiphenyl (#15.) from the 209 congeners. The average standard deviation for the entire data set is 4.3. The results of this testing illustrates the method detection limits (MDL) for two initial sample volumes. The results show that this methodology is able to achieve low MDLs of 0.97 ppt for 8-liter samples and 9.34 ppt for 1-liter samples for total PCB. A total of 116 chromatographic peaks were detected, containing 209 PCB congeners in various ratios. This allows an almost complete profile of environmentally occurring PCBs and shows that the SPE-DEX automated extractor can not only process samples at over 100 mL/min but can retain PCB's congeners at a low ppt level with a fast sample flow rate. This method shows that with tighter regulation and lower detection limits being required by regulating authority's analytical laboratories have to find improved methods of extraction and analysis. The challenge for any laboratory is meeting regulatory needs and wants of increased sample throughput, shorten turn around times and achieve reproducible results while providing lower detection limits. With this method based from Automated SPE by GC/ECD detection those requirements can be met. **Acknowledgements** Ann C. Casey<sup>1</sup>, Robert E. Wagner<sup>1</sup>, and Inga C. Hotaling<sup>1</sup>. Northeast Analytical, Inc., 2190 Technology Dr., Schenectady, NY 12308

**MO180 New Developments in Automated Solid Phase Extraction Methodology for Semi-Volatiles** S. Lambert, ARC Sciences Limited. In the laboratory industry, the most challenging methods for extracting water are for Semi-Volatiles. Semi-volatiles that are extracted by traditional techniques are laborious, time consuming and due to the complexity of the matrix can provide poor results if not handled properly. For solid phase extraction with 47mm disks it was questioned due to particulates, solids and the need to change pHs if it could even be extracted effectively. All those complications encountered can lead to frustrating suspensions which in turn lead to long sample process times during SPE. This paper will explain new developments in automated SPE technology, methodology and chemistry. These new developments not only provide a solution for processing troublesome water samples with an automated SPE extractor, but in fact provide an increase in recoveries for troublesome compounds that are experienced with traditional extraction techniques including past SPE methods. The following Horizon Technology instruments were used for this research, the SPE-DEX 4790 Automated Extractor and the DryVap Concentrator System. The analysis will be conducted with an Agilent 6890 GC with an Agilent 5973 mass selective detector. The results will include a list of well over 100 semi-volatile compounds, of which will include many compounds that are known to have recovery issues during the extraction and concentration process. From the troublesome compounds the results illustrate how they were increased to levels well above the quality control acceptance limits. These new developments will provide laboratories with an efficient option for extracting different water matrix types without sacrificing results.

**MO182 Past, present and future role of direct and indirect sources of C4–C14 perfluoroalkyl carboxylic acid (PFCA) homologues in the environment** Z. Wang, Swiss Federal Institute of Technology / Institut für ökologische Chemie; I.T. Cousins, Stockholm University / Applied Environmental Science (ITM); M. Scheringer, ETH Zuerich / Institute for Chemical and Bioengineering; R.C. Buck, E I duPont de Nemours Co Inc / DuPont Chemicals & Fluoroproducts; K. Hungerbuehler, ETH Zurich / Institute for Chemical and Bioengineering. Perfluoroalkyl carboxylic acids (PFCAs, C<sub>2</sub>F<sub>2n</sub> COOH), including perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), are a group of valuable industrial chemicals. PFCAs are highly persistence, ubiquitous in global environmental media, biota and humans and exhibit a range of toxic effects in laboratory animals, inducing concern regarding their environmental and human risks. To characterize the exposure to PFCAs, a holistic understanding of their sources, fate and transport is needed. In

2006, Prevedouros et al. estimated the global emissions of total PFCAs (1951–2004) and highlighted the significance of direct industrial sources to the presence and overall distribution of PFCAs in the environment. However, their work focused mainly on PFOA and PFNA, and a breakdown of PFCA sources on a homologue basis was not provided. Besides, their estimation of indirect sources (degradation of precursors) was oversimplified. In addition, in recent years, major producers and industrial users have taken action to replace the long-chain PFCAs and their potential precursors with chemicals containing shorter perfluorinated chains, while some new producers have begun to make long-chain PFCAs and their potential precursors, suggesting that potential emission sources for PFCAs have changed since the review was published. In this study, we re-evaluated and expanded the work of Prevedouros et al. in the following ways: (a) New information on direct uses of PFCA homologues (C–C) is provided, especially for the short-chain PFCAs (C–C) and their derivatives. These are shown to have been manufactured in large quantities for many years before industry started the transition to alternative substances after 2000. (b) Information on new commercial alternatives to the long-chain PFCAs and their potential precursors (e.g. per- and polyfluoroethers as polymerization aids, fluorotelomer-based surfactants in AFFF, etc.) is collected and their potential contribution to emissions of PFCAs is analyzed. (c) Emissions from indirect sources (degradation of both polymeric and non-polymeric precursors) have been re-assessed in a more realistic way based on recent empirical studies. Thus, we assessed the contributions of direct and indirect sources for each homologue for three periods (1951–2002 (pre-phase-out), 2003–2015 (transition after phase-out) and 2016–2050 (future prognosis)) and identified a homologue-specific source pattern for PFCAs.

**MO183 In-situ calibration of POCIS for Perfluorooctanesulfonate and a China-specific mist suppressant in wastewater** S. Wang, Tsinghua University / School of Environment; J. Huang, Tsinghua University / School of Environment; C. Harman, Norwegian Institute for Water Research (NIVA); Y. Yang, Tsinghua University / School of Environment; Y. Ge, Tsinghua University / School of Environment; T. Larssen, Norwegian Institute for Water Research. Perfluorooctanesulfonate (PFOS) is a well studied compound, having various industrial applications including use as a chemical mist suppressant in chrome electroplating to control Cr (VI) work place emissions for almost twenty years. Conversely another similar, China-specific mist suppressant, F-53B, which was synthesized in 1970s and has been widely used, is almost unknown to science. F-53B (C<sub>10</sub>F<sub>19</sub> KO<sub>2</sub>S, CAS No. 73606-19-6) is the potassium salt of a one-chlorinated polyfluoroethanesulfonic acid. The amount of F-53B used is quite comparable with PFOS in China, and may be increasing since international controls placed on PFOS are a problem that China should face in the coming future. However, there has been no studies concerning its analysis and presence in the environment. The polar organic chemical integrative sampler (POCIS) has been applied for monitoring many organic compounds including pharmaceuticals and pesticides. As a new class of compounds sampled by POCIS, a few perfluorinated compounds (PFCs), including PFOS, were calibrated and measured in water previously. Considering the similar physical and chemical properties of F-53B to PFOS, POCIS may be a suitable sampling tool for this compound as well. In this study, the standard "pharmaceutical" version of POCIS were deployed in a wastewater treatment plant (WWTP) specifically for the electroplating industry for a 7-day in-situ calibration experiment. A HPLC coupled to a triple quadrupole mass spectrometer was employed for analysis. The concentrations of F-53B and PFOS in the effluent of the WWTP were determined and the applicability of POCIS for sampling these two target compounds was examined. The concentrations of F-53B and PFOS in the effluent water were relatively stable at high average values of  $6.4 \times 10^4$  ng L<sup>-1</sup> and  $5.8 \times 10^4$  ng L<sup>-1</sup>, respectively. Both F-53B and PFOS showed linear uptake during the 7-day exposure, with sampling rates of  $0.138 \pm 0.012$  L d<sup>-1</sup> and  $0.170 \pm 0.012$  L d<sup>-1</sup>, respectively. This suggests that the standard POCIS with HLB sorbents are effective for the passive sampling of the two investigated fluorinated mist suppressants. Results are also

compared to laboratory derived sampling rates for a wider suite of perfluorinated compounds, and differences between their uptake kinetics discussed.

**MO184 Transport of Perfluorooctane Sulfonate (PFOS) in Fractured Bedrock at a Well-Characterized Site**D. Lipson, ArcadisUS Inc; B. Raine, M. Webb, ARCADIS-UK. Perfluorinated compounds (PFCs) are becoming increasingly important in environmental investigations due to their previous widespread use, persistence in the environment, and potential toxicity. For example, perfluorooctane sulfonate (PFOS) was manufactured as an aqueous film-forming foam (AFFF) fire-fighting agent prior to 2002, but since then existing stocks of PFOS have been used for emergency fires. As a result, PFOS discharges have occurred due to historical fire-fighting activities. Many questions remain regarding the fate, transport, attenuation, and remediability of PFOS, which is classed as a persistent organic pollutant (POP). Recent advancements in the science of environmental toxicology of PFCs have drawn attention to these chemicals and the need for a better understanding of their behavior in the environment. We evaluated transport of PFOS in fractured bedrock that occurred due to use of AFFF chemicals during emergency fire-fighting activities in relation to a major incident involving petroleum and aviation fuels in December 2005. PFOS was discharged concurrently with the fuels and provided an opportunity to characterize the transport of both PFOS and hydrocarbons including benzene, toluene, ethylbenzene, xylenes (BTEX), and methyl tertiary butyl ether (MTBE). The transport properties of BTEX and MTBE are well known. Therefore, comparing the transport of these hydrocarbons and PFOS at this well-characterized site after a known discharge event has afforded a unique opportunity to gain insight into PFOS transport in groundwater. Site investigation activities included the completion of soil borings and bedrock coreholes, downhole geophysical investigations, and the installation and sampling of groundwater monitoring wells for concentrations of PFOS, BTEX, and MTBE. Routine sampling of monitoring wells has ensued on a near-monthly basis for a period of up to five to seven years, thereby providing a detailed record of environmental monitoring. Contaminant modeling has also been undertaken during the quantitative risk assessment process along with groundwater pumping remediation designed to remedy the potential impacts of the original incident. As a result, significant insights have been gained regarding transport of PFOS in fractured rock at the site, including characterization of background PFOS concentrations and attenuation mechanisms such as retardation and dual-porosity mass transfer characteristics.

**MO185 Fate and Transport of Short-Chain PFASs: Batch, Column, and Field Perspectives from AFFF-impacted Sites**J. Sepulvado, Colorado School of Mines / Hydrologic Science and Engineering; M.E. McGuire, C.P. Higgins, Colorado School of Mines / Civil & Environmental Engineering. Poly and perfluoroalkyl substances (PFASs) have a variety of uses including paper packaging products, nonstick coatings, and aqueous film-forming foams (AFFF). Because of distribution, toxicity, and potential for bioaccumulation, efforts are being made to reduce use of long-chain PFASs in favor of alternative compounds including short-chain perfluorocarboxylic acids (PFCAs) such as perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), and perfluorohexanoate (PFHxA). Additionally, these compounds have been detected in groundwater at AFFF-impacted fire protection training areas (FPTAs) at  $\mu\text{g/L}$ - $\text{mg/L}$  levels, indicating a need to understand transport in the environment. PFASs present in groundwater at AFFF-impacted FPTAs are likely to occur with co-contaminants released during training exercises such as additional AFFF components. Understanding groundwater transport of short-chain PFCAs will include potential impacts of co-contaminants on sorption. Groundwater transport of PFBA, PFPeA, and PFHxA was completed at the batch, column, and field scales. A batch sorption study of PFASs was completed with the objective of investigating equilibrium transport potential in mixed PFAS and co-contaminant scenarios. One-dimensional saturated flow studies of PFAS and co-contaminant systems were then completed to determine how equilibrium observations translated to advective transport. Findings

were compared to an in-depth site assessment of groundwater and soil PFAS contamination at an AFFF-impacted FPTA. During site characterization, PFBA, PFPeA, and PFHxA were detected in surface soil (0.19-2761  $\mu\text{g/kg}$ ), subsurface soil (0.15-384  $\mu\text{g/kg}$ ), and groundwater (0.05-313  $\mu\text{g/L}$ ). At all three scales sorption of short-chain PFCAs did not follow the chain-length dependent trends observed for longer chain length PFASs. Equilibrium vs. column  $K_d$  values indicated that rate-limited sorption may impact transport of PFBA and PFHxA. Co-contaminants generally increased the sorption of PFBA, PFPeA, and PFHxA. Log  $K_d$  values of PFBA increased 32-372% in the presence of NAPL, and non-fluorinated AFFF surfactants. Increased field  $K_d$  vs. equilibrium in column studies may indicate co-contaminant influence. Given the complex nature of short-chain PFCA behavior and co-contaminant interactions and the recent push towards use of short-chain alternatives, these results emphasize the need for additional research on subsurface transport of PFASs.

**MO186 Characterization and biodegradation of two technical mixtures of fluorinated acryl copolymers**C. Eschauzier, KWRWater; P. de Voogt, Institute for Biodiversity and Ecosystem Dynamics, Universiteit van Amsterdam. In this study we characterized two technical fluorinated acrylic mixtures, approved by the Germany and US authorities as oil- and grease resistant coatings for food contact materials. Both technical blends are a cationic FTOH based fluorinated acryl co-polymer mixture, with one being C8-C10 based and one C4-C6 chemistry based. The work shows the transition of C8-C10 FTOH based to C4-C6 FTOH based fluorinated side-chain acryl co-polymers and the resulting environmental sources of perfluoroalkyl acids (PFAA). Accurate mass analysis (UHPLC-ESI<sup>+</sup>-QTOF-MS) showed the presence of unidentified PFAS homologue series with  $m/z = 100$  Da. Residual impurities such as FTOHs and PFAAs were quantified with LC-MS/MS and the analysis showed the presence of 6:2 FTOH, PFHxA and PFBA in the new polymer and 8:2 FTOH, 10:2 FTOH, PFOA and PFDA in the old polymer ( $\mu\text{g/mL}$  range). <sup>19</sup>F-NMR demonstrated the presence of the fluorinated side-chains in both polymers. Biodegradation studies were performed in a laboratory scale bioreactor setup with inoculum being unfiltered wastewater effluent from a sewage treatment plant. Technical mixtures/polymers were spiked at about 110 mg/L. Results of the water analysis show an increase of PFAA and decrease to 18SPE-cartridge, trapped FTOH and was able to account for about 1% of the FTOH that had been added via the initial spiking, and for 10% of the FTOH that had "disappeared" from the aqueous phase in the bioreactor. The produced PFAA (from conversion of residual FTOH present in the polymer mixture) corresponded to 1% of the residual FTOH added initially to the tank on a molar basis. The results show that despite a poor mass balance, PFHxA are released to the water phase and 6:2 FTOH to the atmosphere. An increase in PFBA concentration in the bioreactor was also observed compared to the sterile control experiments. The preliminary results of this study show the presence of yet unidentified residuals in polymer mixtures. After 40 days in a bioreactor, degradation of residuals in "new" 6:2 FTOH-based polymers are a source of PFHxA and PFBA to the aqueous environment and 6:2 FTOH to the gas phase. Although no differentiation between residual degradation and polymer degradation could be made yet at the time of abstract submission, the amount of residual 6:2 FTOH originating from the spiking still explained the formed PFHxA.

**MO187 Identification of novel per- and poly-fluorinated compounds in environmental and biological matrices using LC-MS-TOF**E. Dagnino; A.B. Lindstrom, U.S. EPA; R. McMahan, U.S. Environmental Protection Agency; E.M. Andersen, U.S. Environmental Protection Agency / US EPA; L. McMillan, U.S. Environmental Protection Agency; C. Ball, Agilent Technologies, Inc; M.J. Strynar, US EPA / National Exposure Research Laboratory. Per and Poly-fluorinated compounds [PFCs] are used in industrial products and processes and in a vast array of consumer products, and their presence has been described in environmental and biological matrices worldwide. PFCs are very stable and resistant to biological degradation; therefore, they tend to persist in the environment and human body. Recent studies, describing

significant toxicity in laboratory animals and links between longer chain PFC exposures (such as PFOA and PFOS) and a range of potential human health effects, has led to international regulatory efforts to eliminate the production of certain PFCs. In the United States, these efforts have led to the reduction or elimination of some PFCs in select manufactured products and waste streams and the development of replacement compounds. In general, current manufacturing trends have moved toward a new generation of fluorinated materials that include shorter chain polyfluorinated compounds in order to help reduce the potential for persistence, bioaccumulation and toxicity. At present, very little is known about the transport and fate of these new materials in the environment, their possible toxicity, and their potential risks to humans who may be exposed. Using an Agilent 6200 series time of flight (TOF) mass spectrometer (MS), we have been able to identify a number of these novel per- and poly-fluorinated compounds and some of their corresponding metabolites in environmental matrices (wastewater, surface water, food packaging), in human serum and urine, and dosed rodent. Compound identification has been achieved using TOF MS specific techniques, including determination of accurate mass (ppm accuracy), relative isotopic abundance, generation of diagnostic mass fragments and adducts, and confirmation using authentic standards. This presentation will focus on the use of these TOF MS techniques for the identification of novel fluorinated compounds, their metabolites, and to unambiguously identify historical PFCs like perfluorooctane sulfonate (PFOS) which can be confused with common biological molecules. \n

**MO188 Sources of perfluoroalkyl substances in the Italian drinking and surface waters** M. Rusconi, Water Research Institute Italian National Research Council / Water Research Institute; L. Marziali, M. Mazzoni, Water Research Institute - IRSA-CNR / Water Research Institute; S. Polesello, Water Research Institute CNR / Water Research Institute; F. Rosignoli, Water Research Institute - IRSA-CNR; F. Stefani, Water Research Institute - IRSA-CNR / Water Research Institute; S. Valsecchi, Water Research Institute Italian National Research Council IRSA-CNR / Water Research Institute. A survey of the distribution of perfluorinated carboxylates (from C<sub>4</sub> to C<sub>10</sub>) and perfluorinated sulphonates (from C<sub>4</sub> to C<sub>10</sub>) in the main Italian river basins have been carried out. Concentrations of selected PFAS higher than 1 µg/L were measured in three Italian rivers (Tanaro, Bormida and Brenta rivers). Maximum PFOA concentrations of 1.9 and 6.5 µg/L were measured respectively in Tanaro and in its tributary Bormida which receives the discharge of a fluoropolymer plant. Brenta river showed the highest measured concentration for PFBS (1.4 µg/L), together with significant concentrations of PFOA (292 ng/L), PFPeA and PFHxA (both about 200 ng/L). Sources of PFAS in Brenta river are both textile and tannery districts as well as a factory which produces fluorochemical intermediates. Substances which were present in concentrations > 100 ng/L in the other Italian surface waters were PFBA (in Adda and its tributary Serio rivers), PFPeA (in Adda, Arno, Serio rivers), PFHxA (in Adda, and Serio rivers) and PFOS (in Serio river). From these data two further hot spots for shorter chain PFCAs were identified which were the Arno river, where important textile and tannery districts are present, and Adda river with its tributary Serio. In the latter case it was more difficult to individuate the source of these compounds because the basin is not characterised by specific manufacturing activities. Two other important Italian rivers, Tevere and Adige, were not impacted by PFAS, also downstream to important urban WWTPs. Finally, the concentrations at the basin closure of the river Po, the major Italian river, were 10 times lower (25 ng/L) than those measured in previous campaigns on Po, suggesting a possible decrease in PFOA use. Concentrations of drinking waters in central Italy (from Tevere and Arno river basins) were all under the detection limits, while the detection frequency of PFAS in drinking waters from Northern Italy, the most urbanised and industrialised area of the country, was higher. The highest concentrations were measured for PFOA (up to 1.9 µg/L) in drinking water produced from groundwater which are impacted by discharges from two big textile and tannery districts to which is added a factory which produces fluorochemical intermediates. High concentrations of PFOS (up to 150 ng/L) were also measured in drinking water produced

from groundwater in an area with a diffuse and historical pollution coming from agricultural pressure.

**MO189 Determination of perfluorinated compounds PFC in surface water by HPLC-MS/MS** C. Deblois, M. Duchesneau, Centre d'expertise en analyse environnementale; D. Berryman, Ministère du Développement durable environnement Faune et Parcs. \n \n Perfluorinated compounds have been widely used as surfactants, anti-stain or anti-adhesive. Substances analysed by Centre d'expertise en analyse environnementale du Québec, CEAEQ, are perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctane sulfonamide (PFOSA). These PFC's are well known for their endocrine disruptor potential and are potentially concentrated in biological tissues. PFOA have been detected in human blood almost everywhere in the world. These substances have been detected in North America and Europe in surface water used to produced drinking water in effluents and effluents of municipal wastewater treatment plants. We have adapted a method for the monitoring of these substances in raw, surface and wastewater. Compounds are extracted with HLB SPE cartridge and are analysed and quantified by liquid chromatography mass tandem spectrometry in negative electrospray mode. A monitoring program has permitted to evaluate the occurrence and the concentration of these substances in Canada. Most of the chosen rivers are heavily impacted by wastewater treatment plant effluents. The detection limits for the LC-MS/MS method range from 3 to 6 ng/L.

**MO190 Enrichment of perfluoroalkyl substances (PFASs) in Arctic sea-ice** O.R. Bertrand, Lancaster Environment Centre; C.J. Halsall, Lancaster University; D. Herzke, Norwegian Institute for Air Research; S. Huber, Norwegian Institute for Air Research / Department of Chemistry; P. Carlsson, The University Centre in Svalbard / Arctic Technology Department; R. Kallenborn, Norwegian University of Life Sciences / Arctic Technology; T. Nordstad, Norwegian Polar Institute / Fram Centre; S. Del Vento, The Reach Centre Ltd. / Lancaster Environment Centre; M.H. Hermanson, The University Centre in Svalbard / Arctic Technology Department. Poly- and perfluoroalkyl substances (PFASs) are subject to long range transport and have been reported in the Arctic and to a lesser extent in coastal areas of Antarctica. Here we report concentrations of C<sub>4</sub>-C<sub>10</sub> perfluoroalkyl acids (PFAAs) and other PFASs such as PFOSA<sup>4</sup> (perfluorooctane sulfonamide) measured as part of ship-based campaigns in the Barents Sea and the coastal areas of Svalbard in the Norwegian Arctic. Samples of snow, sea-ice and beneath-ice seawater were collected in April 2011 and again in May 2012. Care was taken to avoid contamination during fieldwork operations and as such method detection limits generated from field blanks were low (~ND-40 pg L<sup>-1</sup> of meltwater or seawater). PFOA and PFNA dominated the PFAAs profile in the late-season snowpack averaging 237 and 333 pg L<sup>-1</sup> (snowmelt), respectively. However, in the sea-ice (multi-year ice) a wider number of PFASs were detected and the concentrations were higher than in snow. In the case of PFOA, concentrations in bulk ice exceeded 3500 pg L<sup>-1</sup>, >>10-fold higher than levels observed in snow or seawater and differences were also apparent between the upper and lower sea-ice. Enrichment factors of ~30-150 were apparent between 'old' snow and lower sea-ice for density-corrected concentrations, with enrichment most pronounced for the even carbon chain-length PFAAs [e.g. C<sub>8</sub>-PFHpA (0.77 here between upper and lower sea-ice) and C<sub>10</sub>-PFNA (1.50) compared to C<sub>4</sub>-PFOA (16.2) and C<sub>12</sub>-PFDDa (50.3)]. Similarly particle-bound PFAAs were close to or below method detection limits in the snowpack, but were present in the sea-ice with possible differences between upper and lower (0.15 m to 0.50 m depth from the base of the snowpack) sea-ice samples suggesting inter-annual chemical processing during ice formation and ageing. Levels of PFAAs in seawater were low and comparable to those measured in the snowpack with perfluorooctane sulfonate (PFOS) only detected in upper sea-ice at a relatively high concentration (397 pg L<sup>-1</sup>). Significant accumulation of PFASs occurs in sea-ice. The timing of ice melt and associated release mechanisms will play an important role in controlling the exposure of these chemicals to the lower marine food web.

**MO191 Transfer of perfluorinated compounds from bottom sediment to benthic invertebrates** D. Bertin, Irstea / Groupement de Lyon; P. Labadie, University of Bordeaux 1 / UMR 5805 EPOC Equipe LPTC; B. Ferrari, J. Garic, Irstea / Groupement de Lyon; H. Budzinski, University of Bordeaux 1 / UMR 5805 EPOC Equipe LPTC; M.P. Babut, Irstea / Water. Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are chemicals of emerging concern, which have been found in almost all aquatic media. Among them the perfluorooctane sulfonate (PFOS) stands as candidate to be a priority substance under the Water Framework Directive (WFD). Although PFASs with 8 or more carbon atoms backbones are known to be bioaccumulative, exposure pathways for aquatic biota remain poorly understood. The first objective of this study was to assess the kinetic of accumulation of different compounds in the larvae of the midge *Chironomus riparius* chosen as model species inhabiting sediment and considered as an important food source for many fish species. The compounds analysed corresponded to 11 carboxylic acids (from the perfluorobutanoic acid, PFBA to the tetradecanoic, PFTeDA), 7 sulfonates (from the perfluoro butane, PFBS to the decane PFDS) and 3 precursors. The second objective was to evaluate influence of environmental relevant temperatures on these kinetics. PFASs come from a natural sediment site along the Rhône river (France) downstream of an industrial platform releasing amounts of PFASs. Sediments were collected with a Van-Veen grab, sieved at 2 mm, pooled in polypropylene jars and stored at 4°C. Chironomids were exposed at their optimum temperature (21°C) for the kinetic experiment in two part, on one side to second instar at fourth instar and on the other hand on fourth instar only. Two other temperature conditions were used for the second experiment (12 and 17°C). PFASs were analysed in all compartments (water, pore water, sediment and organisms) by LC-MS/MS. In the kinetic experiment, PFASs are accumulated by chironomids already at the first intermediate time (two days) for several tested compounds, including the long-chain ones (C>8). Based on these results and the results of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements, we will discuss the pathways and dynamics of PFASs accumulation by the midge (second to fourth instar and fourth instar only) at 3 different temperatures.

**MO192 Simultaneous Quantitative Determination and Time trends of Perfluoroalkane Sulfonates and their Sulfonamide-based Precursors in Fish** S. Ullah, Stockholm University; A. Bignert, Swedish Museum of Natural History; U. Berger, Stockholm University. Perfluorooctane sulfonate (PFOS) and its sulfonamide-based derivatives (PreFOS) have been produced and used extensively during the last decades of the 20<sup>th</sup> century. PreFOS can be transformed to PFOS in the environment. Therefore, the ubiquitous environmental presence of PFOS is the result of a combination of direct emissions of PFOS as well as emissions and subsequent transformation of PreFOS. In 2002 the largest manufacturer has phased out the production of these chemicals. Levels of the highly persistent PFOS were not expected to decline rapidly from the environment after the phase-out, whereas PreFOS were expected to disappear due to transformation, partly to PFOS. In this study, we examined the potential contribution of PreFOS over time to PFOS levels in herring. Herring was chosen both as an indicator species for the marine food web as well as an item for human consumption. An analytical method based on UPLC/MS/MS was developed for the quantitative determination of PFOS and PreFOS including sulfonamido acetates, sulfonamides and sulfonamidoethanols in fish muscle. An aliquot of 2 g homogenated fish sample was spiked with isotopically mass labeled internal standards and extracted with acetonitrile. The extract was cleaned up on graphitized carbon and on a solid phase extraction (SPE) cartridge. In the SPE step ionic and neutral analytes were separated into two fractions. UPLC separation was achieved on an Acquity UPLC HSS T3 column and the MS/MS instrument was operated in electrospray negative ionization. Matrix effects on sorption and/or ionization of the target analytes were observed. Therefore, extraction recoveries were calculated versus a matrix-matched external calibration standard. At a spike concentration of 250 pg/g wet weight absolute recoveries were calculated to 60 – 110% for all analytes except

sulfonamidoethanols, which could not be quantified reliably at this low spiking level, due to their relatively low ionization efficiency. However, these compounds can be analyzed with better sensitivity by gas chromatography coupled to chemical ionization mass spectrometry. Method limits of detection were in the lower pg/g wet weight range for PFOS, sulfonamides and sulfonamidoacetates, and in the high pg/g range for sulfonamidoethanols. Pooled herring muscle samples covering the years 1990 to 2011 were obtained from the Swedish Environment Specimen Bank. Results of the time trend analyses for PFOS and PreFOS will be presented.

**MO193 Screening of Target Food Samples and Packaging for Polyfluoroalkyl Phosphate Esters and Perfluoroalkyl Carboxylic Acids** S. Ullah, Stockholm University; W.A. Gebbink, Stockholm University / ITM; O. Sandblom, U. Berger, Stockholm University. Polyfluoroalkyl phosphate mono-, di-, and tri-esters (mono, di, and triPAPs) are used to water- and grease-proof food packaging materials, and these chemicals are known precursors to perfluoroalkyl carboxylic acids (PFCAs). Dietary intake of PAPs and their subsequent biotransformation could be an indirect pathway of human exposure to PFCAs. Industrial PAP mixtures consist primarily of diPAPs, although mono and triPAPs are present as by-products. Di and triPAPs with varying chain lengths were detected in food packaging materials, and migration of PAPs from packaging material into food simulants has been demonstrated. However, targeted analyses of food samples packed in materials containing PAPs have, to our knowledge, not been performed. Targeted food samples in their original packaging materials were obtained from the Swedish market. Food and packaging materials were solvent extracted followed by a SPE WAX clean-up (only food) and UPLC/MS/MS analysis. In the packaging materials several mono, di, and triPAPs were detected, however, in the corresponding food samples only nine diPAPs were detected with the 6:2/6:2 and 6:2/8:2 diPAPs as the dominant compounds. diPAP concentrations (sum 6:2/6:2, 8:2/8:2, and 10:2/10:2 diPAPs) in the food samples were in the pg/g concentration range, which was comparable to the PFCA concentrations in the same food samples. C5 – C11 and C13 PFCAs were detected in the food samples, however, C6 – C14 PFCAs were also detected in the packaging materials. Comparisons of popcorn before and after heating indicated that migration of PAPs from the packaging material occurred during heating, however, this was not observed for other samples. Among all food samples no relationships were observed between specific diPAPs and PFCAs (e.g., 6:2/6:2 diPAP and PFHxA or PFHpA). The combination of potential migration of both compound classes together with transformation of PAPs to PFCAs potentially occurring in the packaging, during migration, and/or in the food, as well as the presence of PFCAs in food items in general creates a highly complex picture of a variety of sources of PFCAs in the analyzed food samples. Based on the present data, consumption of the food samples would lead to human exposure to nine diPAPs. Subsequent biotransformation of the diPAPs could lead to increased human exposure to PFCAs.

**MO194 Preliminary studies of distribution and correlation of perfluoroalkyl substances in human matrices and food.** F. Perez; F. Fabrega, M. Nadal, Laboratory of Toxicology and Environmental Health; M. Llorca, M. Farre, IDAEA, CSIC; M. Schuhmacher, Schuhmacher; J. Domingo, Laboratory of Toxicology and Environmental Health, School of Medicine; D. Barcelo, IDAEA, CSIC. 1. Introduction Perfluoroalkyl substances (PFAS) are a wide group of emergent pollutants that are present in a different kind of matrices as environmental, humans and animals worldwide [1, 2]. Their properties of stability made them be used in a lot of industrial and domestic application. These characteristics allowed them to bioaccumulate and biomagnify through the food chain. Different studies have shown that PFAS affect the lipid metabolism, disturb the immunity system, can cause liver cancer and can be a cause of human infertility [3]. The attention about the toxicity of these compounds has increased. This work presents a study based on the determination of eighteen PFAS in nine different human matrices and in different food items. The human

matrices analyzed were: liver, kidney, rib, lung and brain from 20 autopsy samples and urine, hair, semen, nails and spittle more than 20 different donors. The aim of this work is present the different profiles of distribution and accumulation obtained in the human matrices analyzed and find a preliminary approximation between the obtained results and the content of PFASs in different food items. 2. Materials and methods The applied method was based in a previous one developed in our laboratory[4] and consists of a simple pretreatment with an alkaline digestion. The clean-up, separation and detection steps were performed using the TurboFlow™ technology coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method is sensitive, with LOD between 0.0003-18 and 0.1-1.25 µg/L for tissue and food respectively and involves minimal sample preparation. The analytical method was validated using animal and human matrices. The method showed recoveries rates in the range from 30 to 150 %. 3. Results and discussion The Figure 1 includes the results obtained for autopsies samples. PFOS and PFOA was the compound found with higher abundance in the majority of the human matrices analysed. PFHxA and PFBA were the more relevant compound found in the food items analysed. 4. Conclusions With this work has proved the accumulation of PFASs in the different human matrices. A preliminary tendency between the subject's age and PFASs accumulation was shown. The obtained results contribute to the information about metabolism and distribution in the body of perfluorinated compounds.

**MO195 Understanding the exposure, body concentration and elimination of PFASs in the North American population.** J. Cousins, Stockholm University / Applied Environmental Science (ITM); F. Wong, Applied Environmental Science (ITM); M. MacLeod, ITM Stockholm University / Department of applied environmental science. Although per- and polyfluoroalkyl substances (PFASs) are ubiquitously found in the human body and are part of many biomonitoring studies in different countries, their pharmacokinetics and exposure pathways remain uncertain. The objectives of this study are i) to model the intrinsic elimination half-lives ( $t_{1/2}$ ) of perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) and ii) to assess the consistency of intake and biomonitoring data in the North American general population. Our objectives are achieved by application of the Ritter model, which is a population-level pharmacokinetic model that relates body concentration, intake and elimination in representative members of a population as a function of time. It is used to solve for the intrinsic elimination rate constant by combining information from biomonitoring and empirical intake data. We derive the  $t_{1/2}$  by fitting the model either to the biomonitoring or empirical intake data, or in different combinations and compare the resulting values of  $t_{1/2}$ . It is hypothesized that if the biomonitoring and intake data are consistent, the modelled intrinsic half-lives should agree with each other. This hypothesis was supported by results for the UK case study for polychlorinated biphenyls (PCBs) (Ritter et al. 2011). However, in a North American case study for polybrominated diphenyl ethers (PBDEs), where the biomonitoring and exposure data do not agree, it was not possible to derive a plausible  $t_{1/2}$  from biomonitoring or exposure data alone. This discrepancy for the PBDE case may be a result of uncertainty in the elimination half-lives, intake estimates, or the biomonitoring (Wong et al. 2012). We are currently in the process of evaluating our modeling results for PFASs. If the biomonitoring and intake data are found to be inconsistent, we will rerun the model for PFASs assuming the chemical has a maximum half-life of 15 years, and calculate the corresponding intake. This intake value represents the minimum intake that is required to explain the biomonitoring data and allows us to compare to our existing intake estimates from the literature. This information provides a bounding estimate of actual intake that can guide further research.

**MO196 Hazardous mixtures without hazard labels – Risk communication about personal care products.** J. Klaschka, University of Applied Sciences. Risks of personal care products are currently communicated as summarized in the following 7 steps: (1) Is there a risk? Yes. According to the Regulation on Classification,

Labelling and Packaging (CLP-Regulation (EC 1272/2008)) cosmetic products need not be classified and labelled in the European Union, even if they contain dangerous substances, which most cosmetic products do. When the author applied the criteria of the CLP-Regulation to a selection of cosmetic product formulas, most of the products analyzed would have to be labelled because of potential negative effects to eyes or skin. (2) What should be communicated? Consumers should be informed that benefits of personal care products can go along with unwanted properties such as the hazards for the human health described above. (3) How should this be communicated? Risk communication for every day products like personal care products should be clear, easily and quickly understandable. (4) How is it communicated? According to the cosmetic regulation (EC 1223/2009), the ingredients must be listed on the containers. In principle, a consumer could read the lists on each product, look up whether an ingredient is classified as dangerous according to the CLP regulation, find out a rough concentration range for each ingredient in the respective frame formulation for cosmetic products and make his or her own risk assessment for each personal care product. On most product containers, there are no indications for the user about potential risks. Instead, they are most often beautifully designed and imply contributions to attractiveness, youth and well-being. Considering No. (1) this can be considered as misleading the consumers and does not correspond to a trustworthy risk communication. (5) Is there a discrepancy between (3) and (4)? Yes, the listing of the ingredients without hazard pictograms of the product can not be considered as a clear, easily and quickly understandable risk communication instrument. Potential reasons why cosmetic products are exempt from the CLP-Regulation will be discussed in the presentation on the SETAC meeting. (6) Does risk communication lead to changed behavior and reduced risk? The data basis to answer this is not satisfying. Surveys showed that even persons suffering from contact allergy are reluctant to rely on the ingredient lists on the containers. (7) Are improvements needed? Yes. It is recommended that the exception for cosmetic products in the CLP Regulation is repealed.

**MO197 Pharmaceuticals for Humans and Environment?** R. Amato, Federal Environment Agency Germany; G. Maack, Federal Environment Agency / Ecotoxicological Assessment. Human pharmaceuticals mainly enter the environment via domestic wastewater. Although concentrations are usually low, first impacts on aquatic organisms have been detected. In the sense of the precautionary principle and consequently in order to reduce the entry of pharmaceutical residues into the environment the Federal Environment Agency, Germany (UBA) and the Institute for Social-Ecological Research (ISOE) prepared recommendations for a communication strategy aiming at sharpening environmental awareness in the handling of pharmaceutical drugs. For this reason the main actors of the health care system, i.e. public, physicians and pharmacists, were identified. Based on results of individual focus groups the current knowledge of the specific target groups and factors, affecting the handling of pharmaceuticals could be ascertained, revealing that the problem of pharmaceutical residues in the environment is widely unknown. Furthermore it could be shown that the examination of the issue *pharmaceuticals in the environment* varies not only between, but also within the individual target groups. Varied response patterns reveal gaps in knowledge and the importance of tailoring communication strategies to specific professionals in the field. The delivery of basic knowledge should help sensitise professionals about the issue and pave the way for further awareness raising campaigns in the professional community to achieve behavioural changes. Since it is known that significant amounts of pharmaceuticals are released into the environment through improper disposal routines, communication strategies as a main message should emphasise that by no means pharmaceuticals should be flushed down the toilet or the sink. In addition recommendations were prepared in order to support proper medication disposal and to promote the return of all unused medication by implementing a feasible take-back scheme. As a second stage it is now envisaged to tackle a further cornerstone of the strategy by addressing the issue of *pharmaceuticals in the environment* in the education of physicians.

#### **MO198 The Students Lab „Fascinating Environment“ – State-of-the-art science and its communication to the public**

**S. Heger**, Institute for Environmental Research RWTH / Department of Ecosystem Analysis; C. Gembé, RWTH Aachen University; C. Schuer, Institute for Environmental Research, RWTH Aachen University; J. Bohrmann, Institute for Biology II, Zoology and Human Biology, RWTH Aachen University; W. Kuebel, Rixdorf-Film Film- und Fernsehproduktion; T. Seiler, RWTH Aachen University / Institute for Environmental Research (Biology V); H. Zielke, Institute for Environmental Research, RWTH Aachen University; H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V). The Students Lab “Fascinating Environment” was established at Aachen University in 2009 to strengthen education and training in environmental sciences by providing state-of-the-art analytical and bioanalytical facilities. Thanks to the generous sponsoring by more than a dozen industry partners, modern lab equipment is available and enables both, excellent education as well as excellent research. This unique cooperation between university and industry provides interested companies a platform to present their products, and simultaneously the university benefits from the utility of sponsored equipment for education and research. This approach has resulted in a large number of bachelor and master theses and also several high-ranked international peer-reviewed scientific publications. Beside the involvement of the Students Lab in education and research, skills in presentation and science communication of the students are actively advanced. As of winter term 2010/11 the new project FILM started in association with the Students Lab. The aims of FILM are (a) to capture scientific results on film, (b) to plan and produce short movies about scientific test systems, (c) to process both for a broad variety of possible audiences and (d) to comprehensively and unambiguously present one’s own research in a filmed interview situation. The project was included into an already existing skill course system for students of biology and ecotoxicology. Here, student skills in presenting scientific results to non-professional public can be trained by the example of their own work in the laboratory. The interaction between first semester students, interested pupils and experienced researchers is further planned to be increased by regularly organized events, such as the “Open Day of the Aachen Biology and Biotechnology (ABBT)” or the “Students Lab’s Industry Day”, which will give the experienced researchers as well as the sponsoring companies the opportunity to present their work and communicate their knowledge and experience to a new generation of students.

**MO199 Actual situation of rodenticide intoxication in Brazil: prior assessment** **S. Papini**, Secretaria municipal de Saúde de São Paulo / Coordenação de Vigilância em Saúde; L.E. Nakagawa, Instituto Biológico / Laboratório de Toxicologia. The chemical control of rodent in Brazil is legally authorized with the use of anticoagulant products specially coumarin derivatives chemical group, whose active ingredients and formulations are registered in National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária – ANVISA). These products are effective in the control, because the animal dies some days after the contact with bait, enabling that all animals of colony have intoxicated, and secure to the people once they have low concentration and they have antidote. Research made in 2005 showed stability of molecule formulated as paraffin bloc and pellet (legal rodenticide) for 10 weeks in fieldwork, research in 2012 showed efficacy of these rodenticides paraffin blocks by 10 weeks too. These works proved that legal products are effective in the control. Currently, in many Brazilian cities the use of illegal “rodenticide” has increased, which kill the animal immediately after the contact with product, what, unfortunately affect the colony control, since only some animals die, but they appeal the people, because the people can see the animal dying. Some these illegal “rodenticides” are products used in agriculture, being the most common the active ingredient aldicarb (carbamate), known as chumbinho, although actually this product is prohibit. It has observed an increase of suicides, homicides and intoxications by rodenticide. The data of National System of Toxic Information (Sistema Nacional de Informações Toxicológicas - SINITOX-FIOCRUZ) registered the number of rodenticide intoxication,

included suicides, but the System does not differentiates legal and illegal rodenticides, highlighting that rodenticide intoxication outperforms illicit drugs and pesticides. A survey, in Brazil, from 2005 to 2011 showed an average of 3,500 intoxications by year, being about 50% suicide. Therefore, it can assume that the intoxications are by illegal rodenticides, some of them with chemical composition unknown, masking data of intoxication by rodenticide. After SINITOX data evaluation, we understand be necessary to improve the notification chips so that the term rodenticide to be used properly. The intoxication by other products that are not rodenticides, although erroneously used for this purpose, they should not be classified in the same field of rodenticide intoxication, improving the quality of information.

#### **MO200 Environmental sustainability of coal ash utilization of for in-situ treatment of Acid Mine Drainage (AMD)**

**S. Stefaniak**, E. Miszczak, Polish Academy of Sciences / Institute of Environmental Engineering; J. Szczepanska-Plewa, AGH University of Science and Technology / Faculty of Geology, Geophysics and Environmental Protection; **I. Twardowska**, Polish Academy of Sciences / Institute of Environmental Engineering. The idea of coal ash/coal fly ash utilization for treatment of acid mine drainage (AMD) separately or by co-disposal with sulfidic mining waste arouses recently growing interest, and the results presented by different authors are mostly positive. Having in mind general irreversibility of non-point introduction of a material into environment, coal ash (CA) properties, directions of its weathering transformations, and sustainable development as a major principle of the environmental policy, a study has been carried out to assess long term efficiency of *in-situ* treatment of AMD with the use of CA. In the column experiments, freshly generated (CA-f) and 15-years old (CA-15) class F coal ash was treated with simulated averaged AMD sulfate solution (Me-SO<sub>4</sub>) containing Potentially Toxic Elements (PTEs) in equivalent proportions 1 (Cu) : 2 (Zn) : 8 (Fe) : 1/30 (Cd), and actual concentrations 153 mg Cu/l : 377 mg Zn/l : 852 mg Fe/l and 11.4 mg Cd/l at pH 2.48. Both materials were found to be very good sorbents of Fe ions that were bound by 96.9 % and 90.3% of input load, respectively, in a full range of leachate pH, from pH 8.16 to 2.41-2.96, in the full cycle of experiments. Sorption capacity of both CA for Zn, Cu and Cd were about one order of magnitude lower than for reference mono-metallic system at input pH 4.0 due to competitive interaction with other metals at a dominance of Fe and much lower input pH. For these metals, the binding efficiency was Cu>Zn>Cd, while CA-f appeared to be better sorbent for Cu (4120 mg/kg, at pH<sup>limit</sup> 4.47) and distinctly worse than CA-15 for Zn (7400 mg/kg at pH<sup>limit</sup> 4.67) and Cd (106 mg/kg at pH<sup>limit</sup> 7.54), at corresponding values for metals binding capacity onto CA-T5: 2940 mg Cu/kg at pH<sup>limit</sup> 6.12, 13990 mg Zn/kg at pH<sup>limit</sup> 3.83 and 161 mg Cd/kg at pH<sup>limit</sup> 7.16, respectively. After exceeding pH limit values, release of both previously bound and originally occurring in CA matrix metal loads in amounts exceeding threshold values for good groundwater chemical status were observed. At different stages of leaching, mobilization of elevated loads of macrocomponents (Na, K, Ca, Mg) and other trace elements (Ni, Al, Cr, Mo, Ti, and B) from CA matrix also occurred. The mechanism of binding/release was explained with use of PHREEQC geochemical program. The results showed that at uncontrollable non-point application of CA, environmental sustainability might be compromised, thus precautionary principle must be considered.

#### **MO201 Heavy Metals Contamination in Different Environmental Sectors in Mining and Smelting Areas of Daye, Central China**

**W. Chen**, Lancaster University / Lancaster Environment Centre; S. Qi, China University of Geosciences / School of Environmental Studies. This study compares the heavy metals (As, Cd, Hg, Pb and Zn) in different sectors (soil, surface water, sediment and plant) collected in various years from four mining and smelting areas (Daye Smelter, Tonglvshan Mine, Tieshan Mine and Longjiaoshan Mine) of Daye, a typical mining city in the Central China. It can be found there was serious pollution in these areas. For example, the heavy metals in some soil samples at all the sites were higher than the guideline (GB15618-1995). And when comparing with the heavy metals concentrations in the



soil from the background area in the region, the contamination were even more serious. The similar situations happened to the water, sediments, plants in these areas. Comparing the data in different years and analyzing by different institutes, there was no significant changes and distinctions except data of the water and sediments from the Daye smelter-Xiguang River area in 2005 and 2009. According to the field survey, the dramatic drop of concentrations of heavy metals in the Xiguang River was due to the elimination of the discharge and clearing and digging the sediments from the riverbed. Because of the high concentrations of heavy metals in soil, water and sediments, these elements can be detected from the plants including the vegetables and rice. Local residents may suffer from relatively high health risk because of the heavy metals contamination. Therefore, the government and all the people in the region has to take the stringent measures to restrict and eliminating the heavy metals contamination and reasonably carry out the mining and smelting activities.

#### **MO202 Influence of mining activities on the accumulation of trace elements in edible plants: the case study of mines located in the Iberian Pyrite Belt**

P. Alvarenga, I. Simoes, Polytechnic Institute of Beja / Technologies and Applied Sciences; P. Palma, Instituto Politécnico de Beja; O. Amaral, Polytechnic Institute of Beja; J.X. Matos, Laboratório Nacional de Energia e Geologia / UI Recursos Minerais e Geofísica. The Iberian Pyrite Belt (IPB) is one of the largest metallogenetic provinces of massive sulphides deposits in the world. Its exploitation dates back to the time of the Roman Empire, and was very active during part of the XIX and XX centuries. In some cases, populations have developed around these activities and tended to explore small farms in the vicinity of the mining areas. The abandonment of some of these mines did not reverse this situation and they still live there and farm soils that are potentially affected by mining activities. The development of agriculture activities in mine wastes (e.g. tailings) is the worst risk scenario, and it is important to evaluate the contamination of soils with potentially toxic trace elements and ascertain their accumulation in edible plants produced in these soils. These plants may accumulate certain elements to levels that exceed current standards and acceptable daily intake for humans. Eighteen different sampling sites were selected in small farms from three Portuguese IPB mines (São Domingos, Aljustrel and Lousal) and were analyzed considering: soil nutritional status, total trace elements concentrations and bioavailable fractions (As, Cu, Pb, and Zn). The same trace elements were analyzed in three different species of edible plants: lettuce (*Lactuca sativa*), coriander (*Coriandrum sativum*), and cabbage (*Brassica oleracea*). The soils could be considered contaminated with trace elements, since the total As, Cu, Pb, and Zn concentrations surpassed the Canadian Soil Quality Guideline Values for agricultural use in 100, 72, 94 and 75% of the sampling sites, respectively. The maximum total concentrations for those trace elements were extremely high in some of the sampling sites (e.g. 1851 mg As kg<sup>-1</sup> in São Domingos, 1126 mg Cu kg<sup>-1</sup> in Aljustrel, 4946 mg Pb kg<sup>-1</sup> in São Domingos, and 1224 mg Zn kg<sup>-1</sup> in Aljustrel). However, the soil pH (H O) were mainly neutral, a factor that contributes to the low bioavailable fractions for the same trace elements, especially the effectively bioavailable fraction (extracted with CaCl<sub>2</sub> 0,01 M). As a consequence, despite the high concentrations found for the total As, Cu, Pb, and Zn concentrations in the soils, the plants were not as effected as could be expected. Nevertheless, the trace elements contents in some plant species were significantly different depending on the mining areas, namely, lettuce and cabbages grown in soils from the Aljustrel mining area had higher Cu and Zn content.

#### **MO204 Development of a strategy for addressing risks for the environment, human health and safety of abandoned mine sites**

B. Griffin, Golder Associates; M. Rankin, AECOM; R. Moraes; M. Parot, Golder Associates. Mining of various metals and coal has been an important economic activity in Chile for over 300 years. Approximately 230 mines have been abandoned in various conditions which may pose public and environmental risks. The national government is responsible for orphaned mine environmental liabilities, and commissioned its National Geological and Mining Service (SERNAGEOMIN) to develop

a practical and cost-effective approach to assess mine site risks to prioritize remediation efforts. This approach is based on risks for human health, safety and for the environment and the process consists of several components: (i) *Pre-Screen Orphaned Site Inventory* which is based on limited site chemistry, site reconnaissance and photographs. (ii) *Identification of Hazard Scenarios* which is based on site reconnaissance and review of existing site information considering possible receptors associated with each identified risk scenario related to security or contamination (e.g. people, aquatic life, terrestrial life, protected areas or economical activities such as agriculture, aquaculture and fishing). (iii) *Simplified Risk Assessment (SRA)* which targets people, the environment and related economic activities as the entities at risk; and employs a risk matrix approach to estimate the severity of the risk scenario consequences (e.g., catastrophic, high, moderate, low or negligible) and their associated likelihoods (e.g. high, moderate, low, negligible), both according to defined criteria. The output of the SRA distinguishes three risk categories: (a) Class I - *clearly significant risk* qualifying the site as an *urgent priority* requiring remediation; (b) Class II - *clearly insignificant risk* qualifying the site as *not requiring remediation* and (c) Unresolved - *uncertain risk* which warrants more detailed risk assessment (DRA) to resolve the classification status. (iv) *Detailed Risk Assessment (DRA)* which involves application of conventional contaminant-based human and/or ecological health risk assessment to provide a more refined understanding of these risks, and to resolve the uncertainty preventing classification of the site as either Class I or II. (v) *Prioritization of Class I sites for Remediation*: After completing both SRA and DRA steps (where required), those sites identified as Class I are then assembled into a prioritized list based on risk rank for urgency of remediation. An overview of the process and example application will be provided.

#### **MO205 EQS approaches for waterbodies impacted by abandoned metal mines under the Water Framework Directive**

P. Simpson, A. Peters, WCA Environment Ltd; B. Brown, R. Smith, WCA Environment; G. Merrington, H. Potter, V. Greest, Environment Agency. Abandoned metal mines are significant and unregulated sources of metal pollution in England and Wales. As part of a programme to manage impacts from abandoned mines under the Water Framework Directive (WFD) this work aimed to clarify the ecological risks when metal concentrations exceed their respective EQS in mining-impacted catchments. Additional project objectives were to provide guidance on 1) the course of action when biological and chemical measurements of status differ, 2) how to implement water quality targets for metals that represent good chemical and ecological status in mining-impacted rivers, and 3) the evidence required to enable refinement of water quality targets in mining-impacted rivers. Field surveys of four mining impacted waterbodies were undertaken across Wales and South West England. These surveys included the collection of chemical, ecological and physicochemical data (for metal bioavailability calculations), including from headwaters. These field data, in combination with existing Environment Agency chemical and biological data, were then analysed to determine the potential of various candidate alternative approaches for applying EQS in mine impacted waterbodies during WFD classification, including the use of Biotic Ligand Models (BLMs), Bioavailability Screening Tools, Ambient Background Concentrations (ABCs) and site-specific quality targets based on the macroinvertebrate community. The conclusions of the project were: Site-specific data can be used to refine the predictions of bioavailability screening tools made using default values, and are likely to result in improved compliance (particularly for the copper EQS). The BLM for copper provides less precautionary estimates of site-specific PNECs than the bioavailability screening tool and could be readily applied to refine EQS compliance where risks remain after application of the bioavailability screening tool. Ambient Background Concentrations (ABC) for zinc can be estimated by sampling in the headwaters of waterbodies, but care must be taken to ensure that these estimates are reliable. Site-specific quality targets for zinc, based on the predicted or observed macroinvertebrate community, can result in improved compliance compared to the use of both conventional and

bioavailability-based EQS. However, the methodology for deriving site-specific quality targets requires additional validation.

#### **MO206 Establishing the Environmental Risk of Metal**

**Contaminated Sediments** S. Lynch, School of Geography, Earth and Environmental Sciences; L. Batty, R. Bartlett, University of Birmingham / School of Geography, Earth and Environmental Sciences. Estimates indicate that 9% of rivers in England and Wales are failing to meet Water Framework Directive targets due to mining pollution. Remediation efforts have in some cases reduced metal contaminants in the water column to 'below detection'. Metal contaminants in the sediment remain very high. There are currently no mandatory standards for sediments in the UK, largely due to the difficulty in predicting metal behaviour in these high spatially and temporally variable environments. Climate change predicts longer, dryer antecedent conditions followed by more frequent, large flood events. That may increase the mobility and bioavailability of toxic trace metals such as lead and zinc within metal contaminated sediment. Flooding and drought can alter redox (reduction and oxidation) conditions in the sediment resulting in biogeochemical changes that alter the behaviour of di and tri valent metals such as iron and manganese and anions such as sulphur. Trace metals zinc and lead are often partitioned with iron, manganese and sulphur minerals in the sediment. The mobility of these trace metals can therefore be influenced by the precipitation and dissolution of iron, manganese and sulphur. Research indicates that freshly precipitated metal oxides and sulphides may be more 'reactive' (prone to dissolution when conditions change) than older crystalline forms. The frequency and duration of the wet and dry cycle, therefore, may influence the reactivity of secondary minerals that form in the sediment and the concentration of dissolved trace metal release. This study involved subjecting metal contaminated sediment to various wet and dry cycles over a 90 day period in a laboratory. Dissolved (< 0.45µm) metals (Pb, Zn, Mn, Fe, Ca) and anions (NO<sup>-</sup>, Cl<sup>-</sup>, SO<sup>2-</sup>) were monitored. The pH remained low (~5) but not acidic. Variable wet/dry cycles were contrasted with constant flood and field capacity to detect how changing the sediment environment altered dissolved metal concentrations. Dissolved trace metal concentration for sediment exposed to certain wet and dry cycles did, over time, increase to a greater extent than for constant flood and field capacity. Further study will involve sequential extraction and electron microscopy to gain knowledge of how sediment 'reactivity' and metal behaviour changes for certain wet and dry cycles. Bioavailability techniques will determine the environmental risk these metal releases pose.

#### **MO207 Reconstructing the historical effects of mining and metallurgical activities on lake ecosystems: The paleoecotoxicology toolbox**

L. Doig, S. Schiffer, K. Liber, University of Saskatchewan / Toxicology Centre. For various reasons, historical (including pre-operational) environmental data are often lacking for lakes affected by mining and metallurgical activities, especially for older operations. Without long-term data, managers are not able to: show how much a system has degraded or recovered; determine thresholds for undesirable consequences in ecosystems; or set realistic mitigation goals. Aquatic sediments contain a tremendous amount of information that, once interpreted, can help fill these knowledge gaps. Stratigraphic analysis of depositional sediments is commonly used in paleobiogeography to study temporal changes in the distribution of taxa, in paleoecology to reconstruct historical ecosystems, and in paleolimnology to infer past environmental conditions in inland lakes. Depositional sediments can also be used in paleoecotoxicological investigations to reconstruct the timeline of contamination and toxicant-induced changes in aquatic ecosystems. Using Ross Lake (Manitoba, Canada) as a test case, we discuss various tools useful in reconstructing the ecological effects of almost 80 years of mining, metallurgical and municipal activities on this northern lake. Physicochemical variables (e.g., trace metals, stable isotopes) and subfossil remains (diatom, chironomid, cladoceran, chaoborid) will be discussed as lines of evidence.

#### **MO208 Assessing the environmental impact of mining activities in the Alqueva reservoir (Guadiana Basin, Southern of Portugal)**

Palma, L. Ledo, Instituto Politécnico de Beja; I. Barbosa, Centro de Estudos Farmacêuticos, Faculdade de Farmácia, Universidade de Coimbra; P. Alvarenga, Instituto Politécnico de Beja. Pollution of aquatic environment by trace metals is a worldwide problem. Trace elements from natural and anthropogenic sources continuously enter the aquatic ecosystem where they carriage a serious threat because of their toxicity, long time persistence and bioaccumulation. Thus, metals tend to accumulate in sediments from where they may be released and induce toxic effects on aquatic organisms, as well as moving up through the food chain and reach the human. The Alqueva reservoir, in the Guadiana River Basin, was chosen as a case study once it constitutes the most important water supply source in southern Portugal, a semi-arid region with high levels of water scarcity. The risk assessment of trace elements in Alqueva sediments was performed by the following analysis: (i) physical and chemical analysis (grain size, pH, organic matter, nitrogen, phosphorus); (ii) total metal quantification (Cu, As, Pb, Cr, Cd, Zn and Ni); (iii) chemical speciation of potentially toxic elements (Cu, As, Pb, Cd and Zn) using the BCR sequential extraction procedure; and (iv) ecotoxicological evaluation with *Vibrio fischeri*, *Thamnocephalus platyurus*, *Daphnia magna* and *Heterocypris incongruens*. Total trace elements concentrations indicated that As, Cd and Pb surpassed the Canadian levels for the protection of aquatic life, in some of the sites of Alqueva. This contamination may be due to the acid mine drainage (AMD) resulting from the mines (most of them already inactive) located around the reservoir. In general, the sequential extraction showed that the oxidizable and residual fractions were the dominant fractions for the majority of the trace elements. These results are in agreement with the observed ecotoxicity effects, which showed that the direct bioassay with the benthic *Heterocypris incongruens*, which evaluated the whole-sediment, was more sensitive, comparing with the indirect tests that assess the bioavailable fraction. This research confirms the need to use different forms of exposure and several organisms from different trophic levels in the ecotoxicity assays. Further, the present study shows the pollutant potential of AMD in this reservoir and highlights the ecologic impact of the metallic substances analyzed that, despite being detected in less bioavailable fractions of the sediment, promote high toxicity effects to the benthic species exposed to them.

#### **MO209 Use of native versus transplanted tissues as a biomonitoring tool at the historic Britannia Mine site**

B.G. Wernick, Golder Associates Ltd. The former Britannia Copper Mine operated from the early 1900s to 1974, during which time it generated more than an estimated 40 million tonnes of tailings. A large proportion of the tailings was deposited in Howe Sound and as fill along the Britannia Beach shoreline. The acid-generating tailings and former mine workings had been leaching dissolved copper and zinc into Britannia Creek and Howe Sound. In recent times the provincial governmental began various ambitious remediation works intended to intercept, collect and treat metals discharging to the environment. As part of the remediation works, a monitoring program was initiated to monitor for resulting changes in water quality and shoreline ecology, with the long-term objective of defining and supporting an eventual ecological risk assessment. The analysis of tissue chemistry is a risk assessment tool commonly used to provide an indication of time-averaged metals exposure and the Britannia Mine monitoring program has involved the use of both native and transplanted mussels (*Mytilus* sp.) and a brown alga (*Fucus gardneri*). However, the use of native versus transplanted tissues can introduce a confounding factor in the assessment of potential for on-going contaminant uptake and thus evaluation of the success of remediation activities. The collection of native tissue growing at a given site provides relevant exposure data; however, there is evidence that fucoid algal species can inherit tolerance to metals (e.g., have lower uptake) over time. Therefore relying on native tissues for biomonitoring could potentially underestimate the significance of contaminant loading to the intertidal community. Conversely, in the absence of native tissues, as initially was the case at Britannia Beach following commencement of remediation, it may not be possible to evaluate potential for uptake without using transplanted organisms. Moreover, the use of transplanted organisms clearly defines the exposure period and allows to a greater

extent the ability to correlate tissue loadings with biological effects such as differences in growth between sampling areas. Data from the Britannia Mine monitoring program illustrate these principles.

**MO210 Effects of red mud on aquatic organisms** M.O. Bianchi, Universidade Federal Rural do Rio de Janeiro / Soil Department; M. Correia, Embrapa; M. Moreira-Santos, R. Ribeiro, Universidade de Coimbra / IMAR-CMA; A. Resende, E. Campello, Embrapa Agrobiologia; J. Sousa, Universidade de Coimbra / IMAR-CMA. Brazil is the third largest producer of alumina ( $Al_2O_3$ ), obtained from the reaction of mineral bauxite and the sodium hydroxide at high temperature and pressure during the Bayer process. Red mud is a waste, generated in this industrial process in a proportion of 0,3 to 2,5 ton/alumina ton produced. It is highly alkaline, has a clay texture and high contents of sodium and aluminum. Sealed tanks are used to dispose up to 16 million tons of red mud in Brazil each year, but potential alternatives include the use as a soil amendment to rise pH of acidic soils. In addition to the possible effects on soil, the rainy climate of these tropical regions could favor the leaching of salts to streams, impacting aquatic environments. The objective of this study was to test the impact of the exposition of aquatic organisms from different trophic levels to red mud. A red mud eluate was prepared to perform the toxicity assays. The acute toxicity was tested for *Vibrio fischeri* (Microtox® system) and *Daphnia magna* (mortality as an endpoint). Chronic toxicity was evaluated for *Pseudokirchneriella subcapitata*, considering growth inhibition as an endpoint, and reproduction for *D. magna*. An extremely sensitivity was observed for *V. fischeri*, only five minutes after exposition to the lowest concentrations of red mud tested ( $EC_{50} = 0,046\%$ ,  $EC_{10} = 0,097\%$ ). The growing of *P. subcapitata* was also affected by the red mud ( $EC_{50} = 18,89\%$ ,  $EC_{10} = 38,99\%$  of red mud), but the nutrient addition reduced the toxicity ( $EC_{50} = 27,60\%$ ,  $EC_{10} = 47,53\%$  of red mud). The mortality of *D. magna* in acute test was related to exposition period. After 24 h, the mortality was only observed in the highest concentration, but after 48 h, the mortality occurred at lower doses too. It was not observed a dose-response effect of the red mud concentration and the numbers of juveniles produced. However, at the dose of 40% of red mud, no juvenile was produced. Despite variability in the responses of organisms to a possible contamination with red mud, this residue has a strong deleterious effect above aquatic environments.

**MO211 Abstract** P. Cirpus, BASF SE. In the new EU directive 1107/2009 for registration of plant protection chemicals a new data requirement for investigation of the *aquatic mineralization* of a compound according to OECD guideline 309 was implemented. Since for the aquatic risk assessment of plant protection chemicals already several mandatory tests existed under EU directive 91/414/EEC (hydrolysis, aqueous photolysis (sterile), aqueous photolysis in natural water, water/sediment study), the additional value and contribution of the *aquatic mineralization* study to the overall understanding on the behaviour of a compound in the aquatic environment is discussed.

**MO212 Terrestrial Field Dissipation Studies: Experiences with Regulation No. 1107/2009** H. Bayer, P. Cirpus, BASF SE. In 2011, Council Directive 91/414/EEC was replaced by Regulation No. 1107/2009. As a consequence, new data requirements and new guidelines came into force. Among other studies, Terrestrial Field Dissipation (TFD) studies were also affected by this change. This presentation covers first experiences of a chemical company with TFD studies conducted under the new regulation. Critical issues, conflicting guidance and consequences thereof will be discussed.

**MO213 Aerobic Mineralisation in Surface Water (OECD 309): First experiences with a new data requirement for plant protection products** G. Tarara, N. Eckermann, K. Stroech, Bayer CropScience / Environmental Safety; D. Schaefer, Bayer CropScience / Environmental Safety - Environmental Fate. Following the introduction of EU Regulation No 1107/2009, the data requirements for active substances have been updated. In the section on Environmental Fate, a test for

aerobic mineralization in surface water according to OECD Guideline 309 has been defined as a new data requirement. Experience with this kind of test (as applied to plant protection products) is very limited to date. This applies to technical details of the experimental set-up and conduct as well as to the interpretation of the test results in the regulatory framework for plant protection products. In this poster, first practical experiences with this new study type are presented for several agrochemicals that cover a wide range of chemical and biological properties. Based on the experimental results, the scope of the test and its technical limitations are discussed. The results are also compared to results of similar studies such as hydrolysis and aerobic aquatic metabolism, to put them into context. This allows for first conclusions on the use of OECD 309 data in hazard and risk assessments, including PBT assessments.

**MO214 Development and Validation of a Conceptual Model describing the Environmental Fate of a Maize Herbicide** A. Sapiets, Syngenta / Syngenta; N. Peranginangin, Syngenta Crop Protection, LLC.. Commission Regulation 1107/2009 and the linked Communication (SANCO 11844/2010 Test Methods and Guidance Documents) require that the US EPA Guideline for terrestrial field dissipation is used to conduct field studies to support EU registration of agrochemicals. A key part of the EPA guideline is the development of a conceptual model describing the fate and behaviour in the environment of the chemical under test. This presentation describes a conceptual model for the maize herbicide bicyclopyrone, that specifically addresses the question "Why dissipation and mobility in the field differed from those predicted from data generated under laboratory conditions." The assumptions underlying the model hypotheses were used to design new studies and experimental apparatus to understand the mechanism by which photolysis acts as a major route of degradation in the field. The conceptual model was tested against predicted and measured data sets. A simple methodology for incorporating the photolytic half-life into the PRZM model has been described.

**MO215 Rapid and Preferential Interconversion of the Enantiomers of the Chiral Herbicide Haloxyfop in Soil** T. Poiger, Agroscope ACW / Plant Protection Chemistry; M.D. Mueller, I.J. Buerge, Agroscope Changins-Wädenswil Research Station ACW. According to Commission Regulation 1107/2009, active substances with substantial content of inactive isomers are "candidates for substitution". Also, in recent conclusions on active substances consisting of 2 or more stereoisomers, the European Food Safety Authority (EFSA) has requested applicants to provide additional information on behavior and fate of individual isomers. However, guidance on generation of such data is not yet available. The example of haloxyfop-methyl demonstrates that depending on the chiral stability of a compound and the stereochemistry of its metabolites, potentially quite numerous experiments are required to characterize the fate of all stereoisomers of an active substance in soil. Haloxyfop-methyl is a selective herbicide controlling grasses in various broad-leaved crops. The compound was introduced into the market as racemate and later replaced by the enantioenriched "haloxyfop-P-methyl", mainly consisting of the R-enantiomer. This enantiomer carries the desired biological activity. Concurrently, several authors investigated the fate of racemic and enantiopure haloxyfop-methyl in soil and found a fast ester cleavage followed by conversion of the S-enantiomer of the herbicidally active acid metabolite into its antipode. However, the mechanism, rates, and contribution of soil microbiology was not yet fully understood. In this work, we investigated the sequence of ester cleavage of R- and S-haloxypop-methyl and further degradation and chiral inversion of the acid enantiomers in three different soils. Our results confirm the rapid ester hydrolysis of haloxyfop-methyl with half-lives of a few hours and indicate that this hydrolysis is only weakly enantioselective. Further degradation of the acid was slower with half-lives of a few days. In all three soils, the S-acid was rapidly converted to the herbicidally active R-acid. These processes are biologically mediated, as the sterile control showed no degradation and no inversion during the first 4 days of incubation. In experiments where approx. 50 % of soil water had been

replaced by deuterium oxide, a significant H-D exchange in haloxyfop acid was observed, pointing to a reaction mechanism involving abstraction of the proton at the chiral center of the molecule.

**MO216 Enantioselective transformation of the chiral herbicide beflubutamid in soil** I. Buerge, Research Station Agroscope Changins-Wädenswil ACW / Plant Protection Chemistry; I. Hanke, Plant Protection Chemistry; T. Poiger, Agroscope ACW / Plant Protection Chemistry; M. Mueller, Research Station Agroscope Changins-Wädenswil ACW / Plant Protection Chemistry. According to the European regulation No 1107/2009, active substances with a significant proportion of non-active isomers are “candidates for substitution”. Furthermore, in recent conclusions on active substances consisting of two or more stereoisomers, the European Food Safety Authority (EFSA) has asked applicants to provide additional information on behaviour and fate of individual isomers. However, no guidance on generation of such data is available so far. The example of the chiral herbicide beflubutamid shows that depending on the chiral stability of a compound and the stereochemistry of its metabolites potentially quite numerous experiments are required to fully characterize the fate of all stereoisomers of an active substance in soil. Beflubutamid is a recently introduced contact herbicide against dicotyledonous weeds in winter cereals. It acts by inhibition of the enzyme phytoene-desaturase, which is involved in the biosynthesis of carotenoids, and causes bleaching of sensitive plants. The racemic compound is applied although only the *S*-enantiomer is herbicidally active. In soils, beflubutamid is degraded to a transient primary amide and further to the corresponding acid. Both metabolites are still chiral. The degradation of beflubutamid and sequential formation and degradation of its two metabolites was determined with laboratory incubation experiments using the racemic compounds and also the pure enantiomers. The analytes were extracted from soil with methanol, acetone, and water, and were analyzed with enantioselective GC-MS. Incubation studies with the pure enantiomers showed that beflubutamid and the amide metabolite were both configurationally stable, whereas for the acid, enantiomerization was observed. Degradation was enantioselective, particularly for the amide. To fully characterize the fate of beflubutamid and its chiral metabolites in two soils, a total of 18 parallel incubation experiments in combination with an elaborate fitting procedure were required.

**MO217 Analysis of the ecotoxicity data submitted within the framework of REACH Regulation** M. Sobanska, R. Cesnaitis, T. Sobanski, B. Versonnen, V. Bonnomet, J. Tarazona, European Chemical Agency. The REACH Regulation has been operational for the 5 years now. With the first registration deadline in November 2010, the European Chemicals Agency (ECHA) has received a large amount of scientific and administrative information related to the registered substances. In order to understand what type of data has been submitted under the REACH framework a detailed analysis of the availability and content of information on ecotoxicity endpoints has been performed. To avoid unnecessary testing, the REACH Regulation provides registrants with the possibility to build testing strategies and to adapt the standard information requirements based on the specific conditions listed in the Regulation. When well documented and justified, (Quantitative) Structure-Activity Relationships, grouping, read-across, weight-of-evidence approaches or other possibilities for fulfilling standard information requirements can be used instead of new experimental studies. The types of information submitted by registrants to fulfil data requirements for aquatic, sediment and terrestrial toxicity endpoints were analysed. The availability of experimental results per registered substance for different environmental compartments and trophic levels has been reported. Finally, information was gathered on the test guidelines used and the species tested.

**MO218 PBT assessment methodology – development needs** J. Peltola-Thies, European Chemicals Agency ECHA; C. Tissier, L. Ribeiro, P. Lepper, European Chemicals Agency (ECHA). The needs to develop further the methodology for PBT assessment are two-fold. Firstly, the PBT-concern behind the requirement to conduct PBT

assessments in several regulatory schemes calls for a science based methodology independent of the regulatory schemes under which the PBT assessment is carried out. The second challenge is to integrate and implement the science based methodology in a specific regulatory scope without neglecting any relevant information. At scientific methodology level, some examples of the development needs are provided in the following. These are: development of an understanding on how to interpret field –data derived from monitoring, e.g., TMFs and BMFs in bioaccumulation assessment and monitoring data in persistence assessment; refinement of the methodology to assess UVCB substances, but especially elaboration of more detailed testing approaches for these substances; role of the non-extractable residues fraction in persistence assessment; role of (pre-)adaptation in biodegradation tests; interpretation of experimental data derived with biodegradation test guidelines other than the conventional OECD screening tests and simulation tests; how to consider data from the terrestrial compartment; identification of substances which clearly do not fulfil the persistence criteria but nevertheless due to different environmental fate mechanisms persist and accumulate in the environment similarly to substances clearly fulfilling PBT/vPvB –criteria. Furthermore, more experience on how results of fish feeding studies and aquatic biomagnification/bioaccumulation data may best be used and interpreted is still needed although much work in this area has already been carried out to establish clear guidelines. There also may be a need to develop a quantitative weighting system for weight-of-evidence approaches, although such a method could not replace expert judgement based weight-of-evidence assessment but rather provide additional support for data rich substances. In the poster the scientific issues with regard to methodology development are addressed more in detail. Furthermore, an analysis of the relevance and priority of the individual issues of method development from a REACH perspective will be reflected.

**MO219 Stakeholder involvement at the different steps of the Authorisation process under REACH** J. Ruoss, European Chemicals Agency / Risk Management Identification; L. Ribeiro, ECHA; B. Muller, P. Lepper, E. Karhu, European Chemicals Agency. Subjecting a substance to authorisation is one of the options for regulatory authorities to manage the potential risks exerted by Substances of Very High Concern (SVHC) under REACH. The authorisation process comprises three steps: - Identification of Substances of Very High Concern (SVHC) - Inclusion of substances in Annex XIV, the ‘List of substances subject to authorisation’ - Granting of authorisations Furthermore, before initiating the authorisation or other regulatory processes, authorities (Member States Competent Authorities or the European Commission) normally evaluate the available information on substance properties and on uses to identify the substances for which further regulatory action is deemed necessary and to identify the appropriate risk management route. Principal data source both for this preceding step and for identification of SVHCs and inclusion of substances in Annex XV are the registration dossiers although other available information will also be taken into account. In order to ensure that authorities’ decisions on the need for supplementary regulatory risk management measures (beyond those already in place) and on the instrument to use are appropriate, it is important to keep registration information up to date and as accurate and detailed as possible, in particular with regard to data on hazard properties and environmental fate of the registered substance and the description of its uses, related tonnages, and the resulting routes for exposure to the substance. In case a proposal to identify a substance as SVHC is submitted, there is an opportunity for interested parties to comment on the SVHC proposal and, if the substance is identified as SVHC, further opportunities to comment are available during the follow-up steps of the authorisation process such as inclusion of the SVHC in the ‘List of substances subject to authorisation’ (Annex XIV) and in the ‘application for authorisation’ phase. In the poster it will be discussed which kind of comments and information would be most relevant to be submitted from the perspective of different stakeholders during the public consultations at the different steps of the authorisation process and further explained how the submitted information is being taken into account by ECHA, its

Committees and eventually the European Commission.

**MO220 Development of exposure scenarios for mixtures under REACH. A case study.** E. Uotila, Ekoiz-Berrilur Res. Consortium, CBET Res. Grp., Dept. Zoology and Animal Cell Biology.; A. Rodriguez Ruiz, M. Soto, University of the Basque Country. / Ekoiz-Berrilur Res. Consortium, CBET Res. Grp., Dept. Zoology and Animal Cell Biology.; M. Ramos Peralonso, Green Planet Environmental Consulting SL. The new chemicals regulation of the European Union, REACH, has introduced exposure scenarios (ESs) as a new mechanism of communication of safety information on chemicals along the supply chain. ESs describe the conditions and risk management measures that ensure the safe use of hazardous substances regarding human health and the environment. Formulators have to pass on ES information received from the suppliers of the substances used in their mixtures to downstream users to ensure the safe use of the mixtures. The most straightforward way to communicate the safety information of mixtures is to merge the information of different substance ESs into mixture ESs. However, it can be complicated to find the most relevant conditions and risk management measures from different substance ESs to ensure the safe use of a mixture. Thus, there is a need to develop a method that enables formulators to define the most appropriate set of conditions of use for their mixtures in an efficient and reliable manner. The aim of this study was to explore different approaches proposed for the development of ESs for mixtures. Two case studies, a flame retardant paint and a laboratory reagent, were used to examine the usefulness of the approaches and whether they enable the development of meaningful ESs for mixtures on the basis of the information available in the substance ESs. Three approaches were found; 1) Critical component approach 2) DPD+ methodology and 3) GES-approach. Only DPD+ methodology was used in the case studies. It was not possible to apply the other two approaches because there was lack of practical information on their use and there was not sufficient (eco)toxicological information on some substances. The findings of this study showed that at the moment there is no methodology that enables formulators to merge effectively the information of substance ESs into mixture ESs, and that there are deficiencies in the amount and quality of ES information on substances and raw materials which difficult the development of ESs for mixtures. Thus, there is a need to improve the existing methodologies or to develop new ones, and to increase the communication between substance suppliers and mixture formulators in order to generate information on substances that enable formulators to develop meaningful ESs for their mixtures.

**MO221 Norwegian monitoring of contaminants** N. Eckbo; B. Nordboe, Norwegian Climate and Pollution Agency. The Norwegian Climate and Pollution Agency (Klif) is responsible for monitoring contaminants in the environment. The primary goal is to get an overview of the state of the environment and its development, in relation to Norwegian environmental goals or international obligations. During 2010 and 2011 a broad assessment of the national Norwegian monitoring programs operated by Klif was conducted. There was a need to cover new environmental challenges such as climate change, emerging hazardous substances and a wish to link monitoring closer to the generation target "An environment without contaminants". The assessment concluded to increase the activity on hazardous substances, especially on emerging contaminants, biomagnification in humans and terrestrial wildlife, and pollution of hazardous substances from diffuse urban sources. New substances like phosphorus flame retardants, perfluorinated organic substances, brominated flame retardants, siloxanes and chlorinated paraffin's was incorporated in the national monitoring programs. In addition, three new programs (Great Lakes, Supersite Oslo and Contaminants in terrestrial and urban environment) are being developed. These also include monitoring of biomagnification and linking concentrations in the environment to effect-scenarios. Some of the existing monitoring activities was reduced or cut, some of the time trends on hazardous substances are no longer part of Klif's monitoring programs, but will be continued through other monitoring systems, especially time trends important to the scientific community. In

addition, monitoring of hazardous substances far from sources was reduced by terminating the program to assess fluxes of contaminants I the open sea areas. We would like to present the new national monitoring programs with one poster.

**MO222 Assessing whether a substance is of equivalent level of concern under REACH Article 57(f)** S. Doyle, European Chemicals Agency / Risk Management Implementation Unit; L. Ribeiro, ECHA; N. O Farrell, P. Lepper, European Chemicals Agency. There are certain chemical substances, which are considered to be of particular concern due to the very serious effects they have on human health and the environment. These "Substances of Very High Concern" or "SVHCs" are those which meet the following criteria for human health and the environment, in line with Article 57 of the REACH Regulation. - Article 57(a-c): classification as category 1A or 1B carcinogenic, mutagenic or reprotoxic (CMRs); - Article 57(d-e): persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB); - Article 57(f): ...for which there is scientific evidence of probable serious effects to human health or the environment, which give rise to an equivalent level of concern (ELoC) to points (a) to (e) and which are identified on a case by case basis. In collaboration with experts from Member State Competent Authorities and the Commission, ECHA has been considering possible factors, which might be pertinent for assessing whether substances with certain intrinsic properties could be identified as SVHCs, under the 'equivalent level of concern' route set out in Article 57(f). For example for human health, factors relating to health effects (type, irreversibility and delay) and other factors (quality of life impaired, societal concern or possibility to derive a safe concentration) could be important considerations. It is the intention that such factors could be used to assess whether the impacts caused by substances on the health of the affected individuals or the environment and on the society as a whole are comparable to those elicited by substances meeting the 57(a) to (e) criteria. In such cases it might be justified to conclude, on a case by case basis, that such a substance is of ELoC in accordance with REACH Article 57(f). This poster will present these factors and explore what other information would be pertinent to support a conclusion on ELoC.

**MO223 Eco-epidemiology works!** L. Posthuma, RIVM / Lab. for Ecological Risk Assessment; K.E. Kapo, C.M. Holmes, Waterborne Environmental Inc; S.D. Dyer, The Procter Gamble Company / Central Product Safety; D. De Zwart, RIVM / Centre for Sustainability, Environment and Health. Fifty years ago Silent Spring (sept, 1962) was - in fact - a comprehensive eco-epidemiological analysis of the severe impacts of toxic compounds in the living environment. Due to measures taken, large scale mass kills and problems as those reported then have been reduced. It took nearly 25 years to define eco-epidemiology, by Bro-Rasmussen and Lokke (1984), and another 25 years to conclude now that eco-epidemiology works! This presentation presents the state of art in eco-epidemiological diagnosis of impacts of chemicals and their mixtures in our landscapes, both terrestrial and aquatic. Since recent policy targets have been set not only for chemicals but also holistically ("Good ecological status", "Sustainable soil use"), we have considered how to merge the following issues: existing data, holistic policy targets, impact reduction measures, sustainable management, ecological (trait) data, and of course ecotoxicological theories and approaches. Given the fact the major monitoring data bases are being collected due to the current policy frameworks, we were able to develop the field of eco-epidemiology. Taking the holistic policy target and looking at the efficient use of existing investments in monitoring, we developed a suite of analyses and case study results that show *that* and *how* eco-epidemiological analyses may help to forward holistic policy aims in protecting and restoring water, sediment and soil quality. Keywords in the examples are, amongst others: diagnosis, multiple stress analyses, relative impacts of chemicals, natural variability, traits, sensitivity, foodweb, stability, diagnosis and prognosis. The examples will illustrate the key issues of which one can conclude that eco-epidemiology works are going on, and that they can work in the support of environmental protection and restoration.

**MO224 A biotest based classification of waste according to criteria HP14 in the waste frame work directive harmonizing with the chemical legislation** S. StiernströmITM / Department of applied environmental science; M. Breitholtz, Department of applied environmental science; O. Wik, Swedish Geotechnical Institute.

According to the Waste Framework Directive (WFD; 2008/98/EC), waste and hazardous waste should be classified in accordance with the List of waste, in which so-called mirror entries concern waste types with the potential to be either hazardous or non-hazardous. The WFD further states that "classification of waste as hazardous waste should be based, inter alia, on the Community legislation on chemicals, in particular concerning the classification of preparations as hazardous..". In this context, Regulation No 1272/2008 (CLP Regulation) implements globally harmonized criteria for the classification of substances and mixtures according to their physical, health and environmental hazards. Annex III in the WFD lists 15 properties of waste that may render it hazardous. Of these, ecotoxicological properties should be classified based on its inherent hazardous effects under criterion HP14 ("Ecotoxic"). A consultation on the review of the HP-criteria from the EU Commission, proposes that the HP14 classification should be done with computing models. The proposed models are based on the sum of substances that the waste contains, classified as aquatic ecotoxic, according to the CLP. However, this should in our opinion only be an option if the composition and component toxicity of the waste is fully known. To classify waste with unknown composition and component toxicity, the characterization should be done in a biotest. Currently, there are neither harmonized quantitative criteria for the HP14 classification nor threshold values. Considering harmonization of waste and chemical legislations, this classification will involve drastic changes as opposed to more recently approached proposals for ecotoxicological evaluations of waste both related to choice of leaching conditions and ecotoxicity tests. Here, we present a study that out-lines a proposal for a biotest-based classification of solid waste for HP14, harmonizing with the CLP in the following steps; (1) rules for classification of hazardous waste according to waste legislation and rules for ecotoxicological aspects according to CLP, (2) adaptations of the classification rules in the CLP needed when designing a biotest based classification for solid waste with respect to HP14, (3) recommendations on test procedures for preparation of eluates from solid wastes for subsequent ecotoxicity testing as well as choice of biotest battery and (4) recommendations on criteria and limit values.

**MO225 NO TIME TO WASTE: development of a refined methodology to assess the waste life stage for metals and metal compounds under REACH** M. Eliat, Arche consulting; M. van Gheluwe, I. Vercaigne, ARCHE. Manufacturers or importers of a substance as such, in mixtures or in articles subject to registration under REACH are obliged to take the waste life cycle stage of the substance into account when undertaking the appropriate (exposure and risk) assessments. The waste, in which a substance is contained, includes waste from manufacture, waste occurring as a consequence of the use (on its own or in mixtures) and waste formed at the end of service life of articles. The conditions ensuring control of risk in the waste life stage of a substance need to be documented in the CSR and also communicated down the supply chain by means of the exposure scenarios and the extended Safety Data Sheet. A generic methodology for the environmental exposure assessment for the waste life stage has been proposed by ECHA (guidance Chapter R18, ECHA, 2012). Applying the suggested methodology together with the proposed defaults leads to overestimation of the risks, especially for high volume chemicals such as metals. The parameters for which default values are proposed in the guidance are: fractions of a substance becoming waste at the different life cycle stages, number of waste treatment facilities and emission factors depending on the type of treatment facility (e.g. landfilling or incineration of municipal/hazardous waste). The guidance explains possibilities and limits for refining such default values. ARCHE has developed a methodology to refine the default values for metals based on actual waste statistics and the use of measured data. These data were extracted from the scientific literature, online databases and compiled

from questionnaires sent to the manufacturers. This methodology has been applied for different data rich metals like Zn, Cd, Ni, Pb and also applied to some less data rich metals like Ag, Sb and Co. By means of a questionnaire sent to the manufacturers and sometimes downstream users the default fraction of substance going to waste could be reduced by one or two orders of magnitude. The default emission factor to water for the landfill could also be reduced by one order of magnitude with information from the scientific literature. On the other hand the default emission factors to water and air for incinerators were rather in line with the measured emission factors. Finally exposure estimates were calculated for a generic landfill and incinerator using the refined parameters and also taking into account realistic waste water volumes and dilutions.

**MO226 Illustration of the methodology for deriving EQS for specific pollutants in surface water in Slovenia according to the guidelines of the WFD** M. Kos Durjava, Public Health Institute Maribor; F. Balk, Royal Haskoning; B. Kolar, Public Health Institute Maribor; W.J. Peijnenburg, RIVM / Laboratory for Ecological Risk Assessment. According to the European Water Framework Directive (2000/60/EC), the EU member states are required to prepare a list of specific pollutants and their environmental quality standards in the aquatic environment for the purpose of evaluating the ecological status of water. In addition, for the purpose of evaluating the chemical status, defined by the Directive on environmental quality standards 2008/105/EC, a country must select biotic species that are relevant to surface water and determine the list of substances for monitoring in biota and/or sediment. In the period 2005-2010 we have successfully developed all the above mentioned standards and lists for Slovenia. In this article we present a list of river basin specific pollutants for surface water in Slovenia and their environmental quality standards. We present the species that will be monitored and a list of substances to be monitored in sediment or biota. The approaches presented serve as exemplary for similar evaluations to be carried out by various countries inside and outside Europe.

**MO227 The Derivation of Water Quality Limit Values for Chemicals Derivation: An International Perspective** D. Leverett, WCAEnvironment Ltd. A water quality limit value is a numerical concentration of a substance against which water quality objectives are measured. Such limits are termed differently in different countries and regions but the derivation of all involves the assessment of ecotoxicological data to derive a concentration of a substance below which environmental effects are not expected to occur, or to occur in an acceptably small percentage of species or life stages. There are considerable differences between countries and regions with respect to the specific procedures used in the derivation of limit values, specifically in relation to the use of deterministic or probabilistic determination methods, *de minimis* data requirements, study reliability assessments, the potential for taking water chemistry into account, the degree of prescription in guidance and requirement for expert judgment, and the allowance for the use of field data in the assessment. While the guidance and procedures for the derivation of limit values is relatively mature in the so-called 'developed' countries and regions, the procedures applied in the developing regions of the world are relatively immature, and in some cases, non-existent. Arguably, it is in these regions that reliable limit values for those specific substances known to be an environmental issue are most needed. Faced with limited resources for the development of bespoke country-specific guidance, regulatory agencies in developing areas often turn to the guidance developed by other regions, and in most cases this amounts to the direct application of the procedures for deriving guidelines published by the USEPA (1985) or indeed the direct application of the US guideline values themselves. This poster will present a review of the guidance applied in different countries and regions (EU, Canada, USA, Australia, New Zealand) to derive limit values, including comparisons of the procedures and methods used to assess ecotoxicological data and derive different types of limit values, and an assessment of the potential for limit values derived in one jurisdiction to be adopted or adapted for use elsewhere.

**MO228 Platform and databases of the different ecotoxicological impacts aquatic indicators for the evaluation of the ecotoxicological impacts in LCA and ERA** J. Payet, O. Hugonnot, E. Maillard, Cycleco. Subject: AiiDA (<em>Aquatic Impact Indicators Database</em>) Currently, a scientist that wants to get ecotoxicological data on a substance of interest is facing different difficulties, such as the inexistence of ecotoxicological data on the substance, the difficulty to obtain an efficient and global view on data sources and databases, and the complexity for analysing and classifying the existing data for a substance. Therefore, there is a need for the ecotoxicity data user to manage these different problematics in order to obtain values for water quality criteria assessment, risk assessment, life cycle impact assessment and so on. The AiiDA data are mainly based on 8 databases of ecotoxicological data on the Web (OPP, Fathead, Aquire, ECHA, CERC-USGS, EAT, OECD, IUCLID) using nearly 500 000 results which provide the user with 200 000 different test results after test selection. Thus more than 7500 substances are documented with 3600 species. This tool allows the automatic calculation of various ecotoxicological water quality criteria and their uncertainties according to official recommendations and advice. 5400 different substances are covered with data produced on at least 3 phyla, giving a reliable ecological representativity. The following water quality criteria are proposed in AiiDA: - HC<sup>50</sup> calculated with the AMI (*Assessment of Mean Impact*) method provided with its confidence interval for more than 10 000 HC<sup>50</sup> Acute and Chronic. - HC<sup>50</sup> and confidence interval 95% based on the *Aldenberg review (2000)*<sup>50</sup> or based on *US-EPA method*. - PNEC calculated with the recommendations of the *Technical Guidance Document on Risk Assessment (2003)* The obtained values are available from an interactive platform on the internet and allows to provide the calculation details. The comparison of the substances, and the calculation of Species Sensitivity Distributions and Phyla Sensitivity Distributions is provided to the user.

**MO230 Catch 22 - what shall I do with my fish? Protection levels are inconsistent for European fish consumers** C. Heiss, J. Koschorreck, Federal Environmental Agency. The European PBT-policy requires phasing out of substances that are persistent, bioaccumulating and toxic (PBT and PBT-like). Several of these chemicals are also regulated under the water framework directive (WFD) and food safety regulation (FSR). Inconsistent generic approaches for deriving quality standards within these two regulatory frameworks may result in a Catch 22 for authorities and consumers of wild fish. Human health assessment under the FSR has been setting maximum residue concentrations for chemical substances in wild fish since the 1960s. A generic assessment derives safe concentrations for human consumption. These levels are based on toxicity data and exposure modeling. Regional authorities use these concentrations to safeguard the marketing of wild fish to consumers. Fish with chemical concentrations above the respective maximum levels are not allowed on the market. The most relevant pollutants affecting European water bodies are regulated in the Directive EC/2008/105. In this concept, environmental quality standards (EQS) are used to assess the chemicals status of European waters and to indicate need for mitigation measures. Environmental concentrations above the EQS indicate risks to or via the aquatic environment. Due to secondary poisoning EQS for PBT- or PBT-like substances refer to concentrations in fish or other suitable taxa. Recently, 11 EQS for substances with PBT and PBT-like properties have been proposed for wildlife fish. The respective EQS are derived from generic models taking into account human consumer safety and secondary poisoning in wildlife under the Water Framework Directive. In effect, in Europe there are two regulatory frameworks in place which protect human consumers from exposure to contaminated wild fish. These two frameworks use different generic models which result in different quality standards for wild fish. This may lead to a catch 22 situation for authorities and fish consumers, e.g. anglers. The German environmental specimen bank (ESB) has been collecting and archiving wild fish samples in a highly standardized manner for over 20 years. We used data for some of the 11 priority hazardous substances with PBT

and PBT-like properties (Mercury, PAH, PBDE, Dioxin, PFOS) from these samples to characterize inconsistencies between the level of protection in EU food safety regulations and the Water Framework Directive. Results are presented and options of improving the integrated risk assessment are discussed. Key words: integrated risk assessment; PBT-substances; Environmental quality standards;

**MO231 Scientific evidence justifies the inclusion of endocrine disrupters, mixture effects and nanomaterials in EU legislation – we need action now** L. Mikkelsen, E. Aggerholm Kristensen, Danish Ecological Council. Six years after its adoption, the European Commission is reviewing the REACH regulation. This replaces a real revision in 2012 and 2013. If appropriate, the Commission will propose amendments to the regulation based on the review outcomes. The Danish Ecological Council urges, that EU chemicals legislations are strengthened upon three areas; endocrine disrupting chemicals (EDCs), mixture effects, and nanomaterials. EDCs are currently not covered by any procedure of authorization and mixture effects are not sufficiently accounted for since relevant legislations mainly consider toxicity of single chemicals. Before June 2013 the EU must decide whether there is a threshold below which EDCs are not harmful. If not, EDCs must be included in the full authorization procedure of REACH – like for instance CMRs. Many studies indicate that such a threshold does not exist for many EDCs. Additionally, it has been found that current legislations have a number of limitations when it comes to nanomaterials, including unclear terminology and inadequate registration and risk assessment, due to the small size. It is crucial that these issues are included in all relevant EU chemicals legislation (e.g. REACH, RoHS and the Water Framework Directive) as the body of evidence showing that these groups of chemicals may contribute to health and environmental problems is growing. EDCs and mixture effects are interfering with the hormone system, which control many biological functions, including reproduction and metabolism. In addition, EDCs have been increasingly linked to a range of health problems including altered brain development giving rise to behavioral or attention deficit disorders, cancers (particularly breast, prostate and testicular cancer), diabetes, reproductive disorders, and impaired fertility. Furthermore, scientific studies indicate that certain nanomaterials increase the risk of e.g. cardiovascular disease where the cardiovascular effect of the nanomaterial is dependent of size, surface area, and chemical composition. Unless action is taken, exposure to EDCs and nanomaterials will continue to increase, with human exposure arising through a wide range of everyday consumer products. In three “Call for Action” Policy papers The Danish Ecological Council calls on the EU to act on 22 specific points concerning endocrine disrupting chemicals, mixture effects and nanomaterials.

**MO232 Knowledge uncertainty and variability uncertainty in Life Cycle Inventories: A case study on bath powder** M. Budzinski, DBFZ Deutsches Biomasseforschungszentrum gemeinnützige GmbH DBFZ / Bioenergy Systems; R. Rieckhof, E. Guenther, TU Dresden. Life Cycle Assessment (LCA) as a decision support tool is affected by uncertainty. A variety of methodologies exist to assess uncertainty in order to improve reliability in LCA. All are related with additional efforts for the analyst. From a practitioner's point of view, the aims are to minimize the effort and to draw significant conclusions about the quality of data and results. While not all sources of uncertainty are in the direct control of analysts, they can improve the quality of the Life Cycle Inventory (LCI). Inventory data covers process data and emission data. For both data types knowledge uncertainty (epistemic uncertainty) and variability uncertainty (aleatoric uncertainty) exist. In this study, we apply uncertainty importance analysis to investigate the significance of uncertain LCI data. Variability uncertainty of the amounts of unit processes is considered by estimating the appropriate frequency distributions. Knowledge uncertainty of emission data is analyzed separately with data quality indicators (DQIs). We then develop a matrix by merging both uncertainty types to determine key unit processes, which are based on low data quality and contribute significantly to the total variability of impact assessment results. With the help of this



uncertainty importance analysis we can systematically determine the unit processes for which additional data collection is necessary to improve the reliability of studies. In order to assess the proposed approach for determining the uncertainties in LCI we perform a carbon footprint case study on a bath powder. In the case study different scenarios are analyzed. For each scenario the overall variability uncertainty of global warming potential (GWP) is estimated by Monte Carlo simulation. Furthermore, the uncertainty importance analysis reveals that the key unit processes are the heating of the bathwater (resulting from variability uncertainty) and the disposal of the paper and the board (resulting from knowledge uncertainty). No unit process can be identified that includes high levels of both uncertainty types. Hence, the unit processes which significantly contribute to the overall GWP are based on better data quality compared to marginal processes. The additional efforts to conduct uncertainty importance analysis depend on the number of unit processes within the analyzed product system and the used method for uncertainty propagation in LCA software.

**MO233 Variability of LCA results of biodiesel as a consequence of inventory modelling choices** E. Kiss, University of Novi Sad / Faculty of Technology.

Biofuels are being promoted as possible solutions to address global warming and the depletion of fossil resources. Significant disagreement and controversies however exist regarding the actual environmental benefits of biofuels, as shown by a large number of life-cycle studies that have varying and often contradictory conclusions. A previous research has revealed three distinct sources of variation in the LCA results of biofuels: (1) 'real' variability in parameters due to different production technologies of biofuels and local conditions; (2) 'methodological' variability due to different ways to calculate the LCI results; and (3) 'uncertainty' due to parameters rarely included and/or poorly quantified. This article points out the significant differences between LCA studies stemming from modelling choices about system boundaries, function and functional unit, reference systems and allocation methods (system expansion or partitioning methods). The extent to which these choices can influence the results of LCA is demonstrated and measured on the example of biodiesel produced from rapeseed oil. The environmental performance of biodiesel is evaluated by performing number of LCI analysis each representing one of the possible inventory modelling choices. The results of scenarios are then compared and analysed. The results show a large difference in LCI and LCA results of biodiesel with a high sensitivity to the following factors: the method used to allocate the impacts between the co-products, the assumptions made regarding land use modelling, including high uncertainty associated with N<sub>2</sub>O and other gaseous emissions from cultivated soil, and the choice of the functional unit. The author came out with the conclusion that an important source of the variability of results can be found in the often arbitrary interpretations of the ISO 14040 and ISO 14044 standards which allow the use of several methods to address the same methodological issue. This research was supported by the Ministry of Science and Technological Development of the Republic of Serbia (Project No. OI 172059).

**MO234 Decision uncertainties in LCA: database, software and LCIA methodology choice** A. Bonou, Corporate Technology; S.I. Olsen, Technical University of Denmark.

The types of LCA uncertainty include: random data variation (variability), lack of knowledge about quantities (parameter uncertainty), model structuring (model uncertainty), model choices (decision uncertainty). The last one of these types is of particular relevance to LCA practitioners when it comes to choosing inventory data, impact assessment methodology and software. In this study the most used of these alternatives are consistently compared *ceteris paribus*: a) *LCI database choice*: Generic processes representing the same function from PE and ecoinvent database have been compared. More than 200 unit processes have been sorted under different process clusters and within each cluster the processes describing the same functional unit have been compared. b) *Software choice*: GaBi and SimaPro have been compared with respect to modelling and assessing the same ecoinvent LCI data. c) *LCIA*

*methods*: The characterisation factors for the common impact categories of different LCIA methods have been compared. The combined effect of the three choices will be presented on an industrial case study of Siemens A/S. Further work is on progress to systematically quantify such decision uncertainties as an integrated part of the modelling process.

**MO235 Prioritizing data collection to minimize uncertainty due to characterisation factors** G. Bourgault, CIRAIG; P. Lesage, CIRAIG / CIRAIG; R. Samson, Ecole Polytechnique de Montreal / Department of Chemical Engineering; M. Margni, Ecole Polytechnique de Montreal / Department of Mathematical and Industrial Engineering.

Except for a minority of foreground production sites, the geographic location of elementary flows is usually at best known at country resolution. This resolution forces the use of aggregated characterization factors (CF), whose uncertainty is larger than the native CF calculated by method developers. A primary data collection could help practitioners to reduce the uncertainty by identifying the location of certain plants in the supply chain. We propose a method to prioritize this primary data collection to maximize this reduction. The uncertainty increase indicator (UII) is calculated for each country, substance and impact category. When very different native CF are aggregated together, the UII is higher, indicating a greater benefits in finding spatial information. The UII is reported for each contributor to impact, allowing calculate a potential global uncertainty change with better spatial information.

**MO236 Time matters: better refurbish or demolish and construct a new residential building?** M. Hiete, University of Kassel / Center for Environmental Systems Research (CESR); U. Neumann, University of Kassel / CESR / Center for Environmental Systems Research (CESR).

Residential buildings differ in many respects from other industrial products. The extraordinarily long lifetime of often more than a century and the fact that buildings can be subject to renovations, refurbishments and reconstructions belong to the most important differences. Refurbishment and reconstructions are strategies to extend the service lifetime of a building. This is important from an environmental point of view as the construction stage and upstream processes such as the production of building materials like cement or steel are responsible for a large share of the environmental impacts of a building over its entire life cycle. However, refurbished residential buildings are in general not as energy efficient as newly constructed ones. Therefore, if there are the two options, to refurbish a building or to demolish it and to construct a new one afterwards, a detailed analysis is necessary to find out which option is in the end the more environmentally sound one. This question has become highly important and controversially discussed given the fact that the residential building stock is one of the key sectors of final energy consumption and greenhouse gas and air emissions. Both options reduce these environmental aspects but differ in terms of when and to which extent these burdens are reduced. Demolition with subsequent new construction can be thought of as an investment in terms of environmental burdens which is paid back over years during the use stage by further reduced energy demand and emissions. Taking a given residential building as an example, the environmental aspects and impacts of the two options are compared. Special attention is paid to dynamic effects and how to take them into account, in particular comparing today's and future emissions and the effects the changing energy supply system has on the assessment. A set of scenarios is developed and assessed to account for these issues as well as different options of for example the energetic performance of the building envelope and the heating system.

**MO237 The importance of considering product loss rates in LCA to reduce uncertainty of results: the example of closure systems for bottled wine** S. Humbert, Home; A. Kounina, Quantis EPFL; E. Tatti, R. Pfister, Ing. oenologist; O. Jolliet, University of Michigan / School of Public Health.

The environmental impacts of wine and closure systems have been assessed by several studies. However, different types of closures, such as natural cork stoppers, synthetic stoppers or screw caps, have different properties, offering different levels of product

protection and consequently presenting more or less risk for wine losses. To date, the influence of the closure type on the overall environmental impacts of bottled wine, taking losses into account, has not been studied in a life cycle assessment context. This study analyses the influence of closures on the environmental impacts of bottled wine, accounting for differences in loss rates for two selected closure systems: cork stoppers and screw caps. The system studied relates to the functional unit “a 750-ml bottle of **drinkable** wine” and includes the wine bottle production, 750 ml of wine, the wine closure production, the wine loss associated with the closure systems, the wine poured down the drain. The indicators assessed in this study are: global warming over 100 years (in kg CO<sub>2</sub> eq), non-renewable primary energy use (in MJ), atmospheric acidification (in g H<sup>+</sup> eq), photo-oxidant formation (in kg ethylene eq) and eutrophication of surface water (in kg PO<sub>3</sub>-eq). The main conclusions show that: The different closures<sup>4</sup> and associated wine losses represent less than 5% of the total life cycle impact of bottled wine. The wine loss rate resulting from the type of closure and its specific properties is a key parameter to consider when assessing the impact of different wine closures and can result (especially for cork stoppers) in a higher impact than the closure itself (provided the effective loss rate of cork stoppers is higher than 1.2%). This study exemplifies the importance of considering the full implication of different components on overall product functionality to draw conclusions regarding environmental preferences. This study evaluates the importance to consider the full life cycle in LCA studies, since, even if it increases complexity of initial analysis, it may reduce uncertainty of final results by increasing their robustness.

**MO238 Understanding the local implications of societal scale technology deployment** H. Breunig; Y. El-Hasnaoui, Institut national des sciences appliquées de Lyon; T.E. McKone, University of California / Sustainable Energy Systems Group. Environmental technologies are designed under green engineering principles to provide solutions that significantly mitigate the negative environmental and human health impacts associated with societal growth. However, the capability of a technology to achieve an environmental goal can vary regionally and temporally. Life cycle assessment (LCA) is a proven method for understanding the broader impacts of a technology's life cycle or incremental management decisions thereof. But, due to a lack of spatial and temporal sensitivity, current LCA methodologies do not have the capacity to capture dynamics in socioeconomic, environmental and technological landscapes. We predict that a LCA method that can capture heterogeneity in data will have the spatial sensitivity to account for local or global scale scenarios of technology scale-up. Our second hypothesis is that geographic information science (GIS) may be valuable for reducing uncertainty if it can improve the specificity of LCA data and reduce the arbitrary nature of spatial scale decisions in LCA given limited available data. The case study we have chosen to facilitate this research focuses on by-product management for geologic carbon dioxide capture and sequestration (GCS) scale-up in the United States (US) at coal fired power plants (CFPP). We selected twelve saline aquifers previously targeted for GCS in different parts of the US to introduce geographic and regional economic variability. GCS is likely to be adopted at a national scale in the US and in other countries. We determined in previous research that there are numerous alternative GCS deployment scenarios that have widely different implications at local and global scales. Conclusions drawn from the LCA of one CFPP would not support sustainable national decisions. Our initial LCA provided regional averages, but not site-specific values since we were working in Microsoft Excel and were data limited. The regionalized LCA we are developing for scenarios of GCS scale-up will require GIS to interpolate and discretize data at region and aquifer scales and allocate them to the point representing a power plant. Our research will be a step towards developing a spatially sensitive LCA method for assessing large-scale technology deployment. We believe this innovative approach for conducting regionalized LCA in a scenario analysis is a salient answer to the call for uncertainty management in emerging technology analysis.

**MO239 Automatic calculation of land use indicator values in LCA**

**implemented in the R Programming Environment** K. Flammang, U. Leopold, Public Research Centre Henri Tudor / Resource Centre for Environmental Technologies (CRTE); I. Vázquez-Rowe, CRP Henri Tudor / Resource Centre for Environmental Technologies CRTE; A. Marvuglia, CRP Henri Tudor / Cork Constraint Computation Centre; E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies (CRTE). The need to assess environmental impacts linked to land use and land use changes is recognised in the literature and in a number of international conventions and agreements. Land use was first proposed as an important impact category in Life Cycle Assessment (LCA) in 1996 and since then it has continuously grown in significance within the LCA community. Many approaches have been developed, providing suggestions for indicators that are suitable for modelling these impacts in LCA, but few deliver detailed guidelines on how to calculate quantified indicators. This prolonged effort to report land use impacts in LCA has led to the use of two major perspectives: quantification of land occupation and transformation based on land surface, on the one hand, and evaluation of soil quality through the measurement of different parameters, such as soil organic content or erosion resistance, on the other hand. In this study the Land Use Indicator Value Calculation Tool (LANCA®), which can be defined as a method developed to allow the quantification of a cluster of land use parameters within LCA, was implemented in the open source Programming Environment R to automatically calculate and regionalise the potential land use impacts associated with the land use patterns in Luxembourg from 2006 to 2012. Geo-referenced information for a wide range of parameters relevant in assessing soil functionality, such as precipitation and evapotranspiration, soil properties, land use class or declination, are stored in a geographic database and mapped using R's geographic data management and mapping capabilities. These data are used as input for the R LANCA model, which calculates the environmental impacts associated with the five indicators considered in the model (erosion resistance, physicochemical filtration, mechanical filtration, biotic production and groundwater replenishment) for all the cultivated areas in Luxembourg on an annual basis. The values of the different indicators are presented as geographic maps using R's capabilities for geospatial visualisation. R implementation allows an automatic calculation of the land use indicator values offering a spatial differentiated assessment of the land use impacts linked to any specific land coverage with the advantage of permitting an easy and fast update of the calculation every time new updated values (e.g. coming from new local measuring campaigns) are available for the input parameters.

**MO240 Valuing the Greenhouse Gas Emissions from Wind Power. A META-Review** S. Chapman, HeriotWatt University / Institute of Infrastructure and Environment; E. Owens, Heriot-Watt University / Institute of Infrastructure and Environment, School of the Built Environment. The growth in both onshore and offshore wind power has been rapid over the past few decades and has led to a need for comparable, consistent and reliable life cycle carbon assessment of wind power in order to provide decision-makers with robust information. The current published estimates for wind power range from 2 to 81gCO<sub>2</sub>e/kWh. This study reduces this range through a meta-analysis of 82 estimates gathered from 17 independent studies. Through harmonisation of lifetime, capacity factor and recycling, the published range of life cycle carbon emissions estimates is reduced by 56% to between 2.9 & 37.3gCO<sub>2</sub>e/kWh. Average values for onshore and offshore wind power are estimated as 16 & 18.2gCO<sub>2</sub>e/kWh respectively after harmonisation and onshore and offshore wind power technologies exhibit similar characteristics in relation to their life cycle carbon emissions. Key differences with previous studies are that this study benefits from inclusion of data from a recently published comprehensive offshore wind farm assessment, and harmonisation is conducted for recycling procedures which results in an increase in the lower band of the range of life cycle carbon emissions estimates. This study concludes that much can be gained from harmonisation of historic life cycle estimates and moving towards a standard approach for carbon assessment of given technologies should be seen as an industry imperative. Wind power estimates lying within the interquartile range of

meta-analyses such as presented in this paper and elsewhere should be considered to be reliable. However, the effects of wind generation intermittency on the carbon efficiency of thermal generation plant elsewhere in a supply network is not fully quantified and must be investigated to improve our knowledge of the overall carbon emissions produced by the deployment of wind generation plant. Keywords- Life Cycle Assessment; Wind Power; Harmonisation; Meta-Analysis

**MO241 The World Food LCA Database project: towards more accurate food datasets** L. Peano, Quantis; A. Deschryver, ETHS, Humbert, Home; Y. Loerincik, Quantis; G. Gaillard, J. Lansche, T. Nemecek, Agroscope Reckenholz-Tänikon Research Station ART. The food and beverage sector is moving rapidly regarding sustainability issues (such as labelling purposes or “food eco-design”). This concerns both institutional and private organisations; consumers and environmental organizations also claim for more transparency on the environmental performance of food and beverage products. Life cycle assessment (LCA) has proven to be an effective method to assess the environmental impact of a product or service throughout its life cycle. However, currently, major limitations in doing such analyses are the lack of inventory data on food products and processes and a lack of consistency between existing food datasets. Therefore, there is a need to develop detailed, transparent, well documented and reliable data in order to increase accuracy of food LCA. In this context, Quantis, the Agroscope Reckenholz-Tänikon Research Station ART and some leading companies in the food sector have decided to launch in 2012 the World Food LCA Database project. The database will include datasets concerning agricultural raw materials (including, when possible and relevant, differences between production systems such as organic or non organic, intensive or extensive), inputs (such as pesticides and fertilizers), processes, processed food products, food storage and food transportation. The data will come from existing LCAs on food products (partners’ LCA, Agroscope and Quantis existing databases), literature review on LCA of food products, statistical databases, environmental reports from companies, technical reports on food and agriculture, partners’ information on food processes as well as collected primary data. Background datasets from the ecoinvent database will be used and new datasets will be compatible with ecoinvent. To guarantee its transparency, the database will be fully documented, unit processes will be visible (except for confidential data provided by the companies) and information sources identified. The user will be able to differentiate among different stages of the process and to identify the main contributors of a specific dataset. The project has started in March 2012 and will be completed in March 2015. The presentation will present the project (results of the literature review of the existing food datasets, involved companies, time schedule) as well as current state of the results.

**MO242 Comparative Life Cycle Assessment of rheumatoid arthritis treatments** C. Catalan, Quantis; L. Hamon; B. Affeltranger, INERIS / Chronic Risks Division; A. Kounina, Quantis EPFL; O. Jolliet, University of Michigan / School of Public Health. Rheumatoid arthritis is a chronic inflammatory degenerative disease that affects millions of people worldwide leading to painful side effects and shorter life span. The treatment remains a challenge since the active ingredient is not specific, potentially causing important side effects. A comparative LCA has been performed within the European project NANOFOL, between two existing treatments (based on pills or subcutaneous injections) and two prospective nanovesicle treatments (based on liposome or bovine albumin serum nanovesicles), permitting to reduce by a factor of 5 the amount of active substance (methothrexate, MTX) administered. Specific characterization factors have been calculated to evaluate the potential toxic and ecotoxic impacts of MTX and its metabolite, using the model USEtox. In contrast to most LCA of medications, the End-Of-Life ecotoxicity and human toxicity impacts of MTX excreta released to surface water after WasteWater Treatment (WWT) were also included. The results show the foremost importance of nurse transportation for scenarios needing it, representing more than 95% of the overall impacts, followed by packaging steps. When excluding transportation, new treatment strategies have the same order of magnitude than the

subcutaneous injection existing treatment and are more than one order of magnitude more impactful than the oral treatment. The toxic impacts associated with the excreta emissions after WWT have rather low impacts on general population, one to three orders of magnitude lower than the impacts linked to particulate matter for drug production without transportation. The patient side-effects are of the same order of magnitude as the nurse transportation impacts. Thus the MTX dose reduction in the nanodelivery scenarios, leads to an important absolute reduction in impact for the patient himself. Sensitivity study shows that WWT abatement rate and the MTX metabolic rate little influence final results. On the contrary, the MTX dose administered and the distances driven by caregiver play an important role in all impact categories. This study has enabled to identify the main drivers of the impacts and the trade-off between conventional delivery of MTX and nanodelivery. It also permits to highlight the methodological developments regarding the toxic and ecotoxic impacts and to put in perspective the substantial benefits for the patient to receive the treatment versus the limited impacts on the overall population of the MTX excreta.

**MO243 PlasticsEurope Programme of update of polymer data to support reliable LCA** G. Castelan, ATOFINA / LCA.

PlasticsEurope has developed and provided to the LCA community the environmental data of the major monomers and plastic materials since the early 1990’s. In order to report about the improved performances of chemical processes a general update of eco-profile (Life Cycle Inventory) and Environmental Product Declaration has been carried out over the last 3 years. The methodology, crucial to guaranty the consistency of the programme, was updated to include the latest developments, as for example a more detailed inventory of water flows. This will be kept moving thanks to the involvement of qualified LCA consultant to develop and verify the eco-profiles, workshops with experts, and support to treat the questions and comments of users. One of the next challenges will also consist in working with the chemical process experts upstream to polymerisation to follow key values and update accordingly the Ecoprofiles. Beyond the main mission to provide reliable environmental data for polymers, PlasticsEurope is engaged to support the dissemination of good practices of LCA by joining initiative like UNEP-SETAC LCA or contributing to the European projects like the European Life Cycle Database and the Environmental Footprint.

**MO244 Life cycle inventory of Asian Aquaculture Feeds - including estimates for dispersion** P.J. Henriksson, Leiden University /

Institute of Environmental Sciences. Over the last decade, LCA has been implemented to evaluate several aquaculture production chains. A common conclusion amongst all these studies is that feed production is a major contributor to most environmental consequences. In the meantime, there is a shortage in current LCA databases of processes identifying most of the underlying raw materials used in Asian aquaculture feeds. Therefore, as part of the on-going EU FP7 SEAT project (www.seatglobal.eu), we here have evaluated aquaculture feed production in Asia, including estimates of dispersion, defined as the sum of inherent uncertainty, spread and representativeness. Primary data was collection was collected from feed mills in Bangladesh, China, Thailand and Vietnam, during 2011 and 2012. From these a number of raw materials were identified as common ingredients. However, the data quantity and quality available in literature differed greatly, and was simply missing in some cases (e.g. Apple snails from Bangladesh). In other cases, where processes were well described in literature (e.g. soybeans, Brazil), large differences amongst datasets were experienced. In order to commonly meet these challenges, and produce dispersion estimates around point values, the methodology proposed by Henriksson et al. (in review) was adopted. This approach promotes subjective sourcing of data using a decision tree, a weighting procedure amongst values based upon representativeness, and the inclusion of inherent uncertainty, spread and unrepresentativeness in the overall dispersion parameter. The findings identified a highly globalised trade of resources, which posed clear challenges for consistent data sourcing. Measurement error and modelled uncertainty most strongly influenced inherent uncertainty, while spread was the result of horizontal averaging. Finally,

representativeness captured the additional uncertainty introduced by decision stakes and data characteristics. Overall, the here presented research showed that overall dispersion estimates can be produced and implemented for all sources of data.

**MO245 Applying text mining to characterize the use of Computational Sustainability techniques in LCAA.** Marvuglia, CRP Henri Tudor / Cork Constraint Computation Centre. Since its beginning in the late 60's LCA has evolved significantly both on the methodology side and the data availability side. While at that time the problem was mainly on data availability, nowadays a considerable amount of data is available at the inventory level. Nonetheless, with the current evolution of LCA towards spatial differentiation, a new trend started in the direction of data hunting at an ever increasing level of spatio-temporal detail. Consequently, an additional problem lies now in the existence and mastering of efficient software to manage big amounts of data and computational tools able to fill the existing practical and conceptual gaps in LCA. Several computational techniques, spanning from spatial statistics to data mining, from economic modelling to mathematical optimization (to mention only a few) have been developed over the last decades and successfully applied to several case studies in the Computational Sustainability research field. Their application in the LCA domain is certainly promising and some instances already exist. Several issues remain however still open and a number of techniques could be proficiently applied to address them. For example, the utilization of data mining and ML algorithms could be highly beneficial to address the problem of data gaps with toxicity-related impact categories; the use of visual analytics and advanced processing of remotely sensed images could support the evaluation of the status of ecosystems goods and services. Text mining is an advanced analysis technique more and more applied in several disciplines. This study consists in the application of text mining to trace the evolution of several computationally advanced techniques in LCA. In particular, heterogeneous co-occurrence maps have been built using a corpus extracted from the ISI Web of Knowledge (WoK) website, thus unveiling clusters of closely related textual items for the period 1995 – 2012. Even though we recognize the set of selection criteria used to extract the corpus and the use of only one database are limitative factors for the analysis, it is still remarkable the fact that the number of applications detected is not very high (except for the increasing application of linear programming especially from 2010). This probably means that in the LCA field there is still significant a lack of cross-domain interaction especially with the areas of applied mathematics and artificial intelligence.

**MO246 LCA-GIS INTEGRATION FOR THE ENVIRONMENTAL ASSESSMENT OF WASTE MANAGEMENT SYSTEMS** V. Pérez., Universitat Jaume I Department of Mechanical Engineering Construction / Mechanical engineering and constructions. Life Cycle Assessment (LCA) is the most appropriate methodology for the environmental assessment of waste management systems. There are a number of computational tools in the market with the goal of performing this task. Nevertheless, these tools seldom allow the user to incorporate own inventory data, making it difficult to model close to reality scenarios. Besides, spatial or temporal aspects are rarely considered in the analysis. The capabilities offered by Geographic Information Systems (GIS) allow tackling efficiently these issues. In the context of an LCA-GIS integration, a computational tool has been developed using Model Builder application by ESRI. The theoretical framework of LCA has been taken into account to consider every stage of a waste management system: pre-collection, collection, transport, treatment and disposal. GIS provide the user-friendly workspace where the appropriate input data overlaying and final results presentation will be performed. This computational tool is easily shareable between GIS software users. The developed tool allows the user to assess a variety of waste management scenarios from an environmental perspective, taking into account primary inventory data to define the characteristics of waste fractions and facilities which form part of the system. Network analysis applications are used to increase

the accuracy in the assessment of waste transport stage, considering optimal routes among facilities. Site-specific and temporal issues are incorporated to the analysis within the land use impact category. A proposal for the assessment of this controversial impact category is presented here, using aboveground biomass as an environmental indicator. The elaborated tool provides results for a variety of indicators, which consistently define the environmental performance of a waste management system. The value of these indicators can be numerically presented or graphically represented in map format. In order to validate the functioning of the presented tool, an environmental assessment will be performed, being its aim to determine the environmental consequences of incorporating biowaste separate collection to a system where paper, glass and light packaging are already collected separately.

**MO247 The worth of assessing Ecosystem Services in Luxembourg from a biophysical perspective** B. Rugani, University of Siena / Resource Centre for Environmental Technologies (CRTE). The concept of ecosystem services (ES) is used to represent and measure the benefits humans derive from nature. Despite being one of the most relevant topics in environmental science nowadays, this concept is still lacking commonly accepted definition and framework. For example, The Economics of Ecosystems and Biodiversity (TEEB) project is spreading worldwide and becoming a standard. It intends to give economic valuation of the consequences of biodiversity loss and support to ES assessments. However, prior to assign an economic price to ES, it would be worthwhile assessing functions and properties of ecosystems. This gives ultimately the extensive- and ecological-oriented basis for a consistent analysis that does not neglect limits, complexity and constraints of the ecosystems. Further, ES are not yet comprehensively assessed in LCA, which usually inventories biophysical inputs and assesses impacts on the biophysical dimension of the cause-effect chain. This work aimed at performing a preliminary biophysical ES inventory in Luxembourg. Since this was the first national-scale attempt on this matter in the country, we also identified the availability, limitations and challenges for data collection. Moreover, a comparison and combination with an Emergy application was carried out. Indeed, Emergy is a valuable tool to synthesize the dynamics of complex territorial systems, as well as a common denominator for physical evaluations of ES in LCA. After a literature review, we selected 20 ES, which were regrouped into five categories (provisioning, regulating, supporting, cultural and other services), as well as related indicators. We found out that, while provisioning, regulating and other services are relatively well documented, supporting and cultural services are not. Data were then mapped using QGIS®. In addition, we built a matrix for qualitative assessment with scores of 'service relevance'-by-'land cover type', including also those services showing data lacks and high uncertainties. These results were compared to the Emergy values calculated with an Emergy conventional perspective, which can also monetize physical flows, and the scores combined to observe the relationship between Emergy and the most relevant ES. We finally identified pertinent opportunities to improve the proposed methodology, which focused on the combination of very different but apparently complementary tools to assess the complexity and worth of ecosystems.

**MO248 External costs per unit of emission of Non-Toxic Air Pollutants based on TM5-FASST** P. Preiss, econcept AG / Institute for Energy Economics and the Rational Use of Energy (IER); R. Van Dingenen, F. Dentener, Joint Research Centre - Ispra / Institute for Environment and Sustainability, Climate Change Unit. Within the life cycle assessment characterisation factors represent a global average. However, in many studies it is suggested that human health damages due to air pollutants need to be assessed in a spatially resolved context in order to increase the accuracy of impact assessment results. The assessment of impacts to human health due to air pollution has several spatial and temporal aspects. The spatial aspects include the dispersion and chemical transformation modelling, which is dedicated to derive spatially resolved ambient concentrations of pollutants for individual source regions. This enables the use of spatially resolved information on receptor areas in order to calculate impacts more precise, and it enables

to weight these impacts appropriately to area-specific details like population density, age distribution or income. The actual impact and external costs per unit of emission depends on the location and time of the corresponding emission. Within the TM5-FASST dispersion modelling framework, the world is divided into 56 source regions and receptor grid cells with a resolution of  $1^\circ \times 1^\circ$ . Within this assessment population data and area specific concentration response functions are applied in order to calculate the relevant diseases. This paper presents new and spatially explicit characterization factors (CF) in terms of human health impacts and corresponding damage cost factors per unit of emission for various air pollutants for the whole world. The considered air pollutants are ammonia (NH<sub>3</sub>), oxides of nitrogen (NO<sub>x</sub>), sulphur dioxide (SO<sub>2</sub>), non-methan volatile organic compounds (NMVOC) and primary particulate matter (PPM). The impact assessment is accounting for secondary inorganic aerosols (SIA) consisting mainly of ammonium nitrate, ammonium sulphate and other nitrates and sulphates and ozone. Finally, the application of monetary valuation according to the “willingness to pay” concept is used to derive specific external cost estimates per unit of emission. Monetary valuation of the corresponding impacts is done in two ways, namely a) including equity weighting by using European average monetary values and b) without equity weighting by purchasing power parity adjusted monetary values. The result emphasise the importance of spatially resolved dispersion modelling, and demonstrate the effect of different value systems with regard to the welfare loss.

#### **MO249 A novel life cycle impact assessment methodology for assessing the direct and indirect impacts of fossil resources depletion**

**C. Bulle**, CIRAIQ Polytechnique Montreal / Chemical Engineering. Various life cycle impact assessment (LCIA) methods have characterized the impacts associated with the depletion of fossil resources. Methods vary widely in their approach, however, the ILCD handbook, which recently performed an analysis of existing LCIA methods, recommends ReCiPe as the most developed LCIA method for endpoint impact characterization of fossil resources depletion, but it is still classified as an interim method due to its limitations. In this work, a new LCIA methodology for the depletion of fossil resources is defined based on ReCiPe's definition as our starting point with the goal to make certain enhancements to it in line with the ILCD Handbook recommendations. The World Energy Projection System Plus (WEPS+), an energy modelling system used to produce the International Energy Outlooks by the US Energy Information Administration, was used to model the shifts in the energy market as a consequence of the marginal depletion of a fossil resource. A change in the price of the resource as a consequence of depletion is applied to the price of the fossil resource in the model. The changes in the world energy market up to 2035 are calculated and compared to the reference scenario. The changes in the world energy market present themselves as 1) changes in demand and production of the fossil resource itself and 2) changes in the production and prices of other energy resources. The direct impacts are defined as the total difference in costs that the world competing users will have to pay in order to meet its energy needs as a consequence of the change in the price of the resource due to marginal depletion. Characterization factors are calculated for petroleum, natural gas and coal in \$/MJ and are integrated in IMPACT World+ LCIA methodology. \n

#### **MO250 Monetisation of fossil resource depletion by assessing the surplus cost**

**T. Ponsioen**, PRe Consultants / Consultancy. For the life cycle impact category fossil resource depletion, monetisation can be applied by assessing the future consequences of resource extraction. This is then based on the concept that resources will be extracted under more and more challenging conditions and with alternative technologies in the future. The additional efforts can be described by surplus costs, which have an increased impact on society. Surplus cost is defined here as the global future cost increase due to marginal fossil resource use. This approach has been applied in the past for the ReCiPe method, but the data gaps for fossil resources in this method compromised its robustness. Here, we present an improved indicator for surplus cost of fossil resource depletion and its underlying assumptions. First, the

marginal cost increase (MCI) is calculated as an intermediate parameter for crude oil, natural gas and coal separately. Its calculations are based on production cost and cumulative future production data per production technique or country. To calculate the surplus cost from the MCI, several value choices were made regarding fossil resource production scenarios and discount rates. We chose for three different societal perspectives with different production scenarios as published by the IPCC and fixed discount rates. The results show that the ratios between the indicators of the different types of fossil resources (crude oil: natural gas: coal) are rather constant in most cases, only in the egalitarian perspective the ratio deviates because only there zero discounting is applied (egalitarian: 100:47:21; hierarchist: 100:54:1.1; individualist: 100:39:0.7). The MCI gives a similar ratio (100:48:1.0); so, in general, the MCI gives a good indication of the impact indicator. However, there are large differences between the surplus cost indicators for each perspective in cost per gigajoule (egalitarian > hierarchist > individualist). From Monte Carlo simulations, we found that data uncertainty is low. However, the uncertainty of total available resources per production technique or country was not included, because there is no information available to quantify it. We also found that the results are extremely sensitive to the discount rate assumed. When applying the present approach to other resources, such as minerals, water, and land, consistent modelling of the discount rate for different perspectives is therefore recommended.

#### **MO251 Integrating Payment for Environmental Services into Life Cycle Analysis: Metal Removal from Run-off from Abandoned Mine Sites in North Yorkshire**

**H.A. Baxter**, University of Hull / Centre for Environment and Marine Sciences joint with Department of Engineering. Payment for Environmental Services (PES) schemes are becoming more widely used globally to encourage practices which actively improve local ecosystems, in order to improve/maintain environmental services (ES) which are of value to humanity. Through linking potential purchasers for the ES with those who maintain the land, negotiations about the value of benefits derived imposes a value upon the range of services which result from actions taken by the land custodian. Life cycle analysis techniques can determine the impacts of implementing land management practices, identify potential purchasers and provide information for negotiations between the parties about appropriate levels of monetary remuneration and realistic expectations of what can be achieved by the service provider. PES schemes in turn will provide LCA practitioners with real-world data relating to the value that society places on different impact categories within an LCA framework. Conditionality is a requirement of the most PES schemes, this presents an opportunity for collection of monitoring data to improve future LCA models and determine the levels of uncertainty and error present by comparison with a predictive LCA used for a specific PES scheme. By using LCA techniques to determine the impacts of different remediation methods for specific sites in North Yorkshire integrating PES methodology, for the use phase of the LCA, to identify the potential monetary benefits that can be derived from remediation the specific size is being undertaken so that a determination can be made about; 1. whether the benefits derived from site remediation outweighed the impacts which result from the production, deployment, and use of different remediation techniques 2. the potential for recovery of the upfront investment and continuing costs related to the remediation method deployed 3. the additional economic value derived from metal removal provided by improvement in the four categories of environmental services (provisioning, regulating, auditing/habitat and cultural services)

#### **MO252 Socioeconomic analysis of the use of nickel sulphate in the manufacture of bathroom fixtures and fittings**

**P. Simpson**, WCA Environment Ltd. Most bathrooms in the EU have fittings (e.g. taps) made using a nickel sulphate (NiSO<sub>4</sub>) based manufacturing process. This poster describes a socio-economic analysis (SEA) for this use of NiSO<sub>4</sub> undertaken within the hypothetical context of REACH Authorisation. Nickel substances are not subject to Authorisation under the EU REACH regulation. Where possible, SEA impacts are

monetised, enabling direct comparison of “continued use” versus “refused REACH Authorisation”. An analysis of alternatives found that the most likely outcome is that fittings made using NiSO<sub>4</sub> will be imported into the EU. Results were, as follows: **Economic costs:** Net loss of production value in the EU of €350-388 billion Net Present Value (NPV) over 20 years. This would be offset by production outside the EU.

**Health benefits:** Based on conventional risk assessment, OELs and DNELs for NiSO<sub>4</sub> are not exceeded. Therefore, impacts on workers or the public should be negligible. An economic assessment based on a unit risk factor approach for carcinogenicity, estimates of the costs of allergic contact dermatitis and unit damage costs (€/kg) for airborne emissions of nickel suggests total avoided damage to health of €16-98 million NPV over 20 years. If nickel salts are assumed to have a practical threshold for carcinogenicity the lower bound for this estimate would be close to zero. **Environment benefits and costs:** Approximately 100-150 waterbodies in the EU-27 may improve (to some extent) as a result of a refused Authorisation. However, this conclusion requires site-specific data to confirm. This benefit should be balanced against the impacts of an increase in CO<sub>2</sub>, NO<sub>x</sub> and SO<sub>x</sub> emissions from transportation (partial estimate of €12 million<sup>x</sup> over 20<sup>x</sup> years in Europe only) due to imports if Authorisation was not granted. **Social costs:** Up to 150,000 EU jobs could be at risk. **Wider economic costs:** Up to 0.22-0.24% of EU GDP may be lost. The costs of a refused authorisation are dominated by the loss in EU production of bathroom fittings. Potential health benefits are small relative to this (well below 0.1%) and improvements in water bodies are uncertain. These benefits are also partially offset by the health and environmental impacts of a refused authorisation (e.g. from transport emissions linked to additional imports). Given the scale of economic and social benefits relative to the risks of continued use, the benefits of Authorisation outweigh the risks to human health and the environment.

#### **MO253 The plant uptake factor (PUF): A small value with a high impact. Is a PUF default value still reliable?**

**L. Bonath**, Federal Environment Agency / Section IV Pesticides Fate and Behaviour Groundwater Risk Assessment; **A. Nehls**, R. Herr, K. Kuppe, C. Pickl, Federal Environment Agency / Section IV 1.3: Pesticides Fate and Behaviour & Groundwater Risk Assessment. The EU registration processes require the assessment of the leaching potential of an active ingredient (AI) and its metabolites of plant protection products (PPP) to groundwater (GW) as described in FOCUS (2000, 2009). AI as well as metabolites in soil can be taken up by plants via the root system. The transferred amount is no longer available for leaching processes. Therefore for GW simulations using FOCUS-PELMO or PEARL in the EU registration procedure a PUF has been considered for several substances. Here either a PUF default of 0.5 or a calculated value based on a single study (Briggs, 1982) is applied or experimental data are used without having an agreed experimental study design. In some cases even rotational crop studies serve as a basis for decisions whether to apply a PUF default value although no quantitative conclusion can be made from such a study. In fact, for systemic, non ionised substances FOCUS (2000) recommends the use of a PUF default on the basis of the Briggs equation that provides a relationship between the transpiration stream concentration factor and octanol/water partitioning coefficient (logK<sub>ow</sub>) under specific test conditions. This correlation may not be applicable for all substances, crop combinations or experimental conditions as already published and due to the high variability of the PUF found for substances having a similar logK<sub>ow</sub> in different crops. Therefore EU member states should question the still ongoing general practice of applying a PUF default while knowing about its variability influenced by certain factors at the same time. Recent analyses showed that using a PUF default of 0.5 has an high impact on reducing the GW entries from 20 up to 60% for the majority (89.3%) of simulated substances (n=75). Only 10.7% of tested substances showed minor deviations between 0 and 20%. Therefore the importance of applying a reliable PUF on the admission of a PPP might be higher than commonly assumed. According to FOCUS (2009) the PEC<sub>gw</sub> can be refined by experimental determination of a substance specific PUF. Currently, in Germany experimental conditions for a PUF determination are under

investigation. But still, we need more information on parameters influencing the PUF and to develop an agreed guidance regarding the PUF on EU level. The information presented here may contribute to a discussion in handling and assessing the PUF and to a harmonization regarding the exposure assessment for PPP between national, zonal and EU level.

#### **MO254 Proposal for the Use of the Plant Uptake Factor in Regulatory Leaching Modelling**

**R. Sur**, Bayer CropScience LP / Environmental Safety; **W. Schmitt**, Bayer CropScience AG / Environmental Modelling; **G. Goerlitz**, Bayer CropScience AG / Environmental Safety; **R. Bongartz**, Bayer CropScience AG; **V. Gourlay**, Rheinland-Pfalz AgroScience GmbH. Plant uptake of agrochemicals from soil porewater is a process contributing to the dissipation in the soil compartment. This loss process is quantitatively accounted for by the plant uptake factor (PUF) relating the concentration of a compound in the water taken up by transpiration to its concentration in soil porewater:  $PUF = \frac{c_{\text{uptake}}}{c_{\text{porewater}}}$  with  $c_{\text{uptake}} = \frac{m_{\text{uptake}}}{V}$ . The PUF in turn is used by leaching models to calculate the amount of a compound taken up according to:  $m_{\text{porewater}} = c_{\text{porewater}} \times V \times PUF$ . Presently, the recommendation for regulatory groundwater modelling in Europe is to use a default PUF of 0.5 for systemic and of 0 for non-systemic compounds and ionic species. Recently, some regulators have questioned the default of 0.5 as being not appropriate especially for very lipophilic compounds as the underlying dataset of Briggs et al. (1982) shows smaller numbers for those compounds. However, Briggs et al. determined the TSCF (transpiration stream concentration factor) as a surrogate measure for plant uptake rather than the PUF itself. But the TSCF is not the appropriate measure for leaching modelling. It substantially underestimates the amount taken up from soil porewater, because it only considers substance translocated to shoots and does not take into consideration additional amounts residing in roots and lower parts of the stem. In the present study PUF values have been determined with a study design appropriately considering the entire amount of compound taken up by plants. The intact plant was exposed to the test compound in nutrient solution via the roots for the duration of one week and mass and volume losses were recorded over time. In total, some 15 compounds covering a wide range of polarity (ionic, polar, medium polar, lipophilic) in a variety of agricultural crops were investigated. The vast majority of active substances and soil metabolites showed a PUF significantly above 0.5, for about half of the compounds values above 1 were measured. A PUF of 0 indicating complete exclusion from uptake was not observed in any case. The results of the study support the proposal to use a default PUF of 0.5 in leaching modelling on tier-1 for all compounds. On tier-2 a measurement of the PUF according to the used protocol should allow the determination of a refined value. The PUF in the simulations may be restricted to an upper level of 1, unless there are scientific reasons to do otherwise.

#### **MO255 Comparison of plant uptake models used in exposure assessment tools**

**K. Takaki**; C.D. Collins, Reading University / Department of Geography and Environmental Science. Plant uptake of organic chemicals is an important process in considering the chemical exposure to humans. There have been many studies to estimate the amount of organic chemicals entering plants via the environment and a number of models for plant uptake have been constructed (Collins et al. *Environ. Sci. Technol.* 2006, 40). The regulatory authorities have added these plant uptake models into their chemical exposure assessment tools used for the determination of risks to human health. These models have been used to set the environmental quality standards in soils, and to evaluate the risks of new and existing chemicals. Therefore, the accuracy of plant uptake models is important to society. In this research, we focused on five different models of vegetation set in governmental tools, that is, RAIDAR, EUSES, CSOIL, CLEA and CalTOX. Following an in depth literature search a validation was undertaken followed by a sensitivity analysis. The results are presented in the poster.

#### **MO256 A dislodgeable foliar residue study to determine DT50 of**

**pesticides on plant surface for calculation of foliar wash off** H. Shbaita; H. Penning, BASF SE / Environmental Fate. The wash off of pesticides from plant leaves (surfaces) is getting more in focus. It is considered in FOCUS surface water calculation for both runoff and drainage entry pathways. It is also mentioned in the new EFSA recommendation for FOCUS groundwater and in the EFSA guidance on PEC soil. Furthermore, the refinement of the wash off factor based on the empirical equation described in the FOCUS surface water manual is increasingly being questioned by different authorities within the registration of plant protection products. Such refinements are sometimes necessary to show a safe use of the plant protection products. One possible option to refine the wash off would be the refinement of the standard foliar DT50 of 10 days. In this study the authors presents a novel and simple experimental setup to measure the dislodgeable foliar residue on the plant after the application of an herbicide at an early post-emergence growth stage. The experimental set is based on an experimental set used for the worker re-entry assessment. The dislodgeable foliar residues on the whole plant (without roots) are measured at different time points by two subsequent dislodging procedures with acetone. This was done for a short time (~ 20 seconds) to ensure that the wax layer (including the spray deposit) had been removed from the leaf surface and not from the leaf itself. The absolute mass of the plant protection product was then used to derive a refined foliar DT50.

**MO257 The impact of developed scenarios on the risk evaluation of PPP used in soilless cultivation of greenhouse crops** L. Wipfler, Alterra Wageningen UR; T. Van der Linden, RIVM; A. Cornelese, Ctgb; D. Ludeking, E. Van Os, T. Vermeulen, Wageningen UR, Greenhouse Horticulture. Greenhouse horticulture is an important agricultural sector in the Netherlands. 10,000 ha of high tech greenhouses are responsible for the production of flowers and vegetables with a total production value of 7.7 billion EURO. Generally, crops are grown within high-tech greenhouses in which climatic conditions as well as water/substance fluxes are highly controlled. Currently, in the Netherlands and in many other EU member states, the emission of plant protection products (PPP) from greenhouses to surface water is assessed as a diffuse emission process, using a fixed percentage of 0.1% (as in drift deposition), independent of the type of greenhouse, cropping system or application method. Vermeulen et al. (2010) showed that the assumption of diffuse emission processes is no longer valid and that it is highly probable that the currently used percentage of 0.1% may underestimate the emission from greenhouses to surface water. The emissions appear to vary widely dependent on application technique, crop characteristics and water management. To improve the risk assessment of PPP use within greenhouses for aquatic organisms, new realistic worst case exposure scenarios have been developed and parameterised, while using a suit of models that enables the calculation of water and PPP fluxes within a greenhouse and exposure concentrations within the receiving water course. These scenarios account for modern horticulture practices in the Netherlands such as the use of rainwater, recirculation of irrigation water, condensation water reuse, discharge management of recirculation water and specific crop characteristics. All regularly used application techniques are considered for the scenario development. The possible impact on the risk evaluation and, consequently, approval of current products is yet unknown. In this study a comparison is made between the currently used risk assessment method and the proposed new method, for a selected number of products, application types and crop types used in substrate horticulture in the Netherlands. The considered application types are spraying application, fogging, low volume misting and application with the nutrient solution. Crops range between crops with a high water demand and a low sodium tolerance and crops with a low water demand and a high sodium tolerance. Aim of the study is to identify the consequences of using the new scenarios for the risk evaluation of PPP used in soilless cultivation.

**MO258 Understanding Pesticide Photodegradation and Persistence in Protected-Crop Environments** E. Hill; C.J. Halsall, N. Paul,

Lancaster University; J. Moore, BPI Visqueen. Improved understanding of the fate of pesticides in food, in agricultural production, and in the wider environment is of growing importance. The effects of pesticides on non-target organisms cause environmental concerns, which extend beyond the agro-ecosystem, potentially to the global scale in the case of persistent organic chemicals. Pesticide residues are also a significant commercial problem for farmers, growers and retailers, since there are consumer concerns even when residues are below the maximum permissible levels, especially with fresh produce such as salads and soft fruit. Fresh produce is a highly valuable sector of agriculture, in 2008 the total value in the UK alone of production of soft fruit and fresh vegetables was £331M and £1,101M respectively, and protected vegetables (those grown under plastic or glass) alone were worth £282M. This very high-value sector is especially prone to concerns over pesticide residues, but there is little understanding of why pesticide degradation may be slower in protected crops compared to open field conditions. Photochemistry is an important degradation route for many pesticides, but what is needed is a way of predicting degradation under different light conditions; whether those conditions result from agronomic practices (different cladding materials in protected cropping) or natural variation (for example due to season or latitude). The degradation of fenitrothion under different protective claddings and of pirimicarb with no cladding was used to find the rates and half-lives of the chemicals. Using degradation rates of fenitrothion at different spectral band widths, a 'Pesticide Action Spectra' or PAS for the degradation can be plotted which gives the effectiveness of the light at each wavelength. The PAS can then be used to weight the light dose against the % degradation, and this gives a much more realistic view of how light will affect the degradation of pesticides in real crop environments. With this information a model can be produced which will equip growers with the information needed to limit the pesticide residues present on their crops at harvest.

**MO259 How protective is FOCUS Groundwater Modelling? – A Comparison of Simulated and Measured Leachate Concentrations** W. Koenig, Federal Environment Agency UBA; G. Holdt, P. Klaas, Federal Environment Agency (UBA); M. Klein, Fraunhofer IME; A. Osterwald, C. Pickl, Federal Environment Agency (UBA). In the registration procedure of plant protection products in Germany the groundwater risk assessment for active substances and their metabolites at tier 1 and 2 is based on modelling results using simulated leaching concentrations of the FOCUS Hamburg scenario from the FOCUS PELMO model. Dependencies of pesticide sorption and degradation data from different soil properties as well as the parameter variability are considered in the endpoint selection for modelling. At tier 3, the measured leachate concentrations for active substances and metabolites from outdoor lysimeter studies are accepted as higher tier endpoints and could overwrite predicted concentrations from simulation runs. This is generally justified by comparable soil and climate conditions in the Hamburg model scenario and during lysimeter experiments. The protection level of the tiered approach for the groundwater risk assessment in Germany is currently under investigation. The objective is to discover discrepancies and their possible causes between simulated and experimentally derived endpoints and to determine, whether tier 1 and 2 calculations are still more conservative than higher tier results from lysimeter studies. Therefore, the 80<sup>th</sup> percentile of predicted groundwater concentrations for the Hamburg scenario simulated over 20 years with FOCUS PELMO 4 and the maximum average annual leachate concentrations from lysimeter experiments are compared for 33 active substances and 71 metabolites. Results show higher predicted leachate concentrations from modelling compared to measured concentrations from lysimeter cores for all active substances (except one substance, where the lysimeter conditions differ from the lysimeter test guideline) and almost all metabolites in the range important for regulatory decisions. These results indicate a regulatory save prediction of the leaching risk to groundwater for all active substances and most metabolites, but with the uncertainties resulting from the limitations of lysimeter experiments. Further investigations using the methodology of inverse modelling are ongoing on the main critical point, how the short



duration of lysimeter studies in combination with a single application finally affects the prediction of the leaching behaviour. The outcome of the analysis shall indicate under which conditions lysimeter studies can be evaluated as endpoint or as process studies for active substances and/or metabolites in the context of groundwater risk assessment.

**MO260 Recommendations, how soil photolysis could be considered in higher tier groundwater modelling of plant protection products with FOCUS PELMO** A. Osterwald; P. Klaas, M. Winkler, W. Koenig, G. Holdt, A. Nehls, C. Pickl, Federal Environment Agency (UBA). Simulating the leaching behaviour of plant protection products, soil photolysis is generally not considered as a degradation pathway. Although it is known that for some substances soil photolysis may play a significant role under environmental conditions, there has been no concept for a regulatory implementation. In the context of the FOCUS groundwater report (2009), leaching models were updated and the possibility to calculate degradation via soil photolysis was technically realised. Within the development of a revised methodology for the assessment of exposure of soil organisms, the EFSA Panel on Plant Protection Products and their Residues (PPR) provided a scientific opinion on how to derive the half-life for degradation from the results of field dissipation studies (EFSA 2010). The Panel recommended, among other things, to use the calculated half-life of the slow phase of biphasic degradation kinetics (DegT50) as input parameter for fate modelling, suggesting that this part of the residue decline represents biodegradation in the soil matrix, whereas the fast phase represents the sum of loss processes from the soil surface (mainly soil photolysis, but also volatilisation and runoff). The German Federal Environment Agency (UBA) welcomes the concept and agrees that it is also applicable for deriving input parameters for leaching models. Based on the scientific opinion (EFSA 2010), recommendations for a higher tier approach considering soil photolysis when simulating the leaching behaviour of plant protection products with FOCUS PELMO 4.4.3/5.5.3 were developed by UBA. Some general thoughts on how to deal with DegT50 values from laboratory and field dissipation studies and criteria for mixing these data to derive endpoints for modelling are presented. Furthermore, the requirements to accept soil photolysis as a significant loss process from the soil surface as well as options for inclusion of photolytic metabolites in the simulation runs are explained and the corresponding input parameters (transformation rate and reference radiation) for modelling are defined.

**MO261 Pragmatic approach to estimate the influence of the Freundlich exponent 1/n on leaching** C. Hoerold, Rifcon GmbH / Environmental Fate and Modelling; G. Wiedemann, Rifcon GmbH; N. Seiterle-Winn, . In leaching calculations for pesticides with the groundwater FOCUS models, the adsorption behavior of a substance is usually described by the Freundlich adsorption isotherm which is defined by the sorption coefficient K<sub>foc</sub> and the Freundlich exponent 1/n. If experimental data is not available, an appropriate Freundlich exponent needs to be selected for modelling in a pragmatic way. In this case, the FOCUS guidance (2011) recommends using a default value for 1/n of 0.9. The strong influence and sensitivity of the Freundlich exponent was already discussed in some studies and model manuals. However, there is no evaluation of a huge data set giving a possibility to estimate the influence of this parameter on the results in advance. Therefore test calculations with 26 different substances (with K<sub>foc</sub> values ranging from 10 to 18000 L/kg) combined with three different 1/n values at six application rates for each substance. Calculations were done for winter cereals in FOCUS-PEARL 4.4.4 for all nine FOCUS groundwater scenarios. Influences of other substance specific parameters like e.g. degradation in soil were reduced to a minimum by using the same values for all substances. Evaluating the resulting predicted groundwater concentrations (PEC<sub>gw</sub>), we were looking for a systematic pattern of the behavior of PEC<sub>gw</sub> for different K<sub>foc</sub> and 1/n values. Concentrations in groundwater generally increased with increasing 1/n exponent. But above a certain application rate, this trend is reversed. Moreover it could be shown, that the influence of the 1/n on the PEC<sub>gw</sub> increases with decreasing 1/n value as well as with

decreasing application rates. This pattern was found for low and medium K<sub>foc</sub> values, but not for substances with very high K<sub>foc</sub> values (> ca. 1900 L/kg) for which the influence of the application rate disappeared. This allows a rough estimate whether a modification of 1/n may have a relevant effect on the PEC<sub>gw</sub> values for the substance of concern.

**MO262 Refinement of pesticides PEC Groundwater by Anses :Implementation and Feedback on Risk Assessment** a. conrad, Boivin, V. Poulsen, ANSES. At Anses pesticide risk assessment in the framework of Regulation (EC) n° 1107/2009 is based on EU FOCUS groundwater scenarios (Tier 1). PEC<sub>gw</sub> calculations for PPP uses are based on 80th percentile of annual average PEC<sub>gw</sub> at 1-meter depth for all relevant EU FOCUS groundwater scenarios. Using EU FOCUS groundwater scenarios further mitigation measures may then be recommended such as limitation of the maximum number of applications per year, modified timing of application or dose reduction. For refined risk assessment at national scale (Tier 2), if appropriate, simulations using specific French groundwater scenarios (FROGS, French refinement of groundwater scenarios) may be run. FROGS model is based on national soil/climatic/agronomic distributed data. FROGS was aimed to produce PEC<sub>gw</sub>, and risk mitigation measures if needed, representative of specific agro-pedo-climatic conditions. The use of the specific representative scenarios is only considered for a higher tier risk assessment. A feedback on PEC<sub>gw</sub> simulations performed with FROGS and submitted in registration reports in order to refine the risk assessment at national level is presented. For this purpose, a review of the groundwater risk assessment conclusions performed using both FOCUS and FROGS models has been performed. A comparison of simulation modelling outputs is presented through different cases submitted in the registration dossiers. For most of the dossiers assessed, the FOCUS modellings were adequate to finalise the risk assessment. For only few cases, FROGS modellings were used to refine the risk. Specific scenarios were aimed to improve the confidence in PEC<sub>gw</sub> simulations. The results from this first review indicate that the PEC<sub>gw</sub> relevance using EU FOCUS models is adequate. Consequently, results from specific scenarios do not lead to PEC<sub>gw</sub> drastically different than those resulting from FOCUS tool. In only few cases, specific national scenarios allowed to provide more accurate mitigation measures.

**MO263 Modelling pesticide leaching at the regional scale in Austria with GeoPEARL** M. Stemmer, Austrian Agency for Health and Food Safety / Institute for Plant Protection Products; E. Murer, Federal Agency for Water Management; M. Schwarz, Austrian Agency for Health and Food Security / Department for Data management; B. Moebes-Hansen, Austrian Agency for Health and Food Security / Institute for Plant Protection Products; C. Poettinger, Austrian Agency for Health and Food Safety / Institute for Plant Protection Products; A. Tiktak, PBL Netherlands Environmental Assessment Agency. In order to evaluate the impact of pedoclimatic conditions in Austria onto the leaching behaviour of pesticides and their metabolites, a national version of the spatially distributed leaching model GeoPEARL was developed. GeoPEARL was originally developed to support the Dutch decision tree for evaluating the leaching potential of pesticides. The Austrian version of GeoPEARL, which is based on a one square kilometre raster covering the entire arable area, was created by combining national data on soil properties (digital soil map), climate (national climate stations), crop use (invekos) and irrigation. The convection/dispersion model adequately describes total yearly amounts of leachate as measured in several lysimeter in Austria. Based on results from lysimeter tracer experiments water flow dynamics in shallow soils are reasonably described as well, preferential flow in structured soils is not adequately addressed. Modelling results on several pesticides and their metabolites as well as the applicability of regional mitigation measures to reduce leaching will be presented and discussed in detail.

**MO264 Prediction of large-scale temporal-spatial distribution of pesticides for paddy field and use for ecological risk assessment** Y. Imaizumi, F. Shiraishi, S. Serizawa, National Institute for

Environmental Studies; J. Goukon, Miyagi Pref. Inst. Pub. Health & Env.; Y. Imazu, Shizuoka Inst. Env. & Hygiene; R. Rio, Kagoshima Pref. Inst. Env. Res. & Pub. Health; K. Kawata, Niigata University of Pharmacy and Applied Life Sciences; H. Yamamoto, The University of Tokushima; D. Nakajima, T. Sakurai, N. Suzuki, H. Shiraishi, National Institute for Environmental Studies. Ecological risks are usually assessed based on the ratio between the predicted environmental concentration (PEC), which is usually calculated based on a fate model or survey results, and the predicted no effect concentration (PNEC). Recently, spatially explicit wildlife exposure models become a hot issue, which are considered as tools used for ecological risk assessments. As for ecological impacts, temporally explicit exposure models are also important because of seasonal ecological dynamics. We have been developing the emission estimation method (PeCHREM: Pesticide Chemicals High Resolution Estimation Method) for pesticides used typically in paddy fields, and the multimedia environmental fate model (G-CIEMS: Grid-Catchment Integrated Multimedia Modeling System). The combined PeCHREM/G-CIEMS model could predict environmental concentrations of many pesticides with high temporal-spatial resolution (5km × 5km grid in atmosphere, average 9.7 km<sup>2</sup> of catchments, and average 5.6 km of river segments) in areas all over Japan. This study focused on applicability of this model for ecological risk assessment based on model validation. We performed field survey of river water in 7 sites for the validation. Totally 38 pesticides (25 herbicides, 7 fungicides and 6 insecticides) were used. The maximum concentrations and the maximum days, *i.e.*, the days when concentrations come up to the maximum value, were compared between predictions and observations for the quantified 244 combination from total 266 combinations of 7 rivers and 38 pesticides. The 159 combinations had a prediction error of less than one order of magnitude. On the other hand, this model could not accurately predict the maximum days. We also calculated ecological hazard quotients (HQ) which is the ratio of the predicted or observed maximum concentration to the national environmental standard concentration. The predicted HQ values agreed satisfactory with the observed ones. We confirmed that this model was useful for ecological risk assessment.

**MO265 Toxicokinetic relevant processes in the bee hive – An overview.** K. Szonn, RWTH Aachen University / Institute for Environmental Research; C. Maus, A. Nikolakis, Bayer Crop Science AG; H. Ratte, Research Institute for Ecosystem Analysis and Assessment – gaia; M. Ross-Nickoll, RWTH Aachen University; W. Schmitt, Bayer Crop Science AG; S. Siehoff, A. Toschki, Research Institute for Ecosystem Analysis and Assessment – gaia; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research. Pollination is an important factor of the food economy and the honey bee *Apis mellifera* is the most important commercial pollinator. Over the last few years, potential effects of insecticides on bee colonies in Europe and the US have been discussed, and there is largely consensus that the risk assessment for bees will benefit from refinements that can be developed on the basis of a deeper understanding of mechanisms of bee exposure to pesticides. To understand the effects of toxicants in the bee hive it is important to fully understand their fate within bee colonies. As toxicokinetic usually comprises absorption, distribution, metabolism and elimination on the level of the organism, here the so called superorganism honey bee colony is the sample of the examination. Literature data were analyzed to determine the relevant toxicokinetic processes in the bee colony and how these processes influence the fate of chemical substances in the bee hive. To realistically estimate the quantity of chemical substances bees are exposed to within the hive, the whole process of resource collection and processing has to be taken into account. Consequently it is crucial to define all the possible routes of exposure (e.g. collection of contaminated nectar, pollen, water and resin from the environment). Additionally the uptake and transport by forager bees have to be determined in order to estimate the overall amount of a toxicant taken up by the colony. Within the hive, material is stored and processed to honey, bee bread, royal jelly, and wax; active ventilation reduces the amount of water in the hive. The food resources are consumed by worker bees, processed and fed by nurse bees to other

worker bees, the queen, the larvae and the drones. This processing by nurse bees and especially the fate of the compound during this process has to be examined. Worker bees move through the whole hive and have intense contact with other bees. We assume bee-to-bee-contact as the most important factor for the distribution of chemical substances in the hive; physicochemical properties of the materials used in the hive have to be considered, as well. A conceptual model shall be developed that comprises all processes deemed relevant and that will be the basis for a model simulating bee hive toxicokinetics.

**MO266 Laboratory experiments to validate 3D numerical modeling of chlorine decay in industrial cooling water discharge** S. Saeed, N. Deb, ExxonMobil Research Qatar; R. Campbell, URS; V. Kolluru, S. Prakash, Environmental Resources Management (ERM); E.J. Febbo, ExxonMobil Research Qatar. A series of experiments are conducted to characterize the effects of temperature, chlorine dose, and natural organic matter (NOM) concentration on trihalomethane (THM) and haloacetic acid (HAA) formation, and chlorine demand in treated seawater. Results of kinetic experiments show that the disappearance of chlorine concentrations was rapid within the first 2 hours with concomitant increases in total THMs (TTHMs) and in bromoform concentrations. Rapid decrease of UV absorbance at 254 nm is also observed which may be indicative of natural organic matter degradation into smaller organic molecules including THMs and other by-products. Results from these experiments are used to evaluate a 3D hydrodynamic numerical model that was developed previously to predict the formation of chlorination by products (CBPs), and to estimate the dispersion of CBPs and residual chlorine from discharged industrial cooling water into the Arabian Gulf waters off North East, Qatar. The 3D model has also been shown to accurately predict the temperature effects (dispersion) of heated water plumes into the receiving waters under study. The 3D model takes into account the complexity of reactions between organic precursors and chlorine, which usually involve several parallel pathways leading to a great variety of CBP formation products. This complexity makes it difficult to develop more generic models for simulating CBP formation however, and it is therefore very important to perform calibration and verification of the model. Comparing model predictions using inputs from laboratory results obtained in this study versus results using empirically derived relationships enables the calibration and tuning of the numerical model. Future work to verify model predictions against field measurements, and extension of the model for use to predict fate and transport of various other constituents will provide a valuable tool for predicting distribution and fate of chemical compounds for use in risk assessment and environmental management activities.

**MO267 SeSaME (Sino Simplebox Model) a level III (steady state) multimedia model for China: accounting for spatial variation in environmental properties** Y. Zhu; O. Price, Unilever / Colworth Science Park; S. Tao, Peking University / College of Environmental Sciences; K.C. Jones, Lancaster University / Lancaster Environment Centre; A.J. Sweetman, Lancaster University. With the rapid development of the Chinese economy, the production, manufacture and consumption of chemicals has increased sharply. Many chemicals have, and continue to be detected across the region in water, air, soil and food. Increasingly, the environmental fate and behaviour of these chemicals has been attracting scientific and political interest, arising from concern over environmental and human exposure to these chemicals, both close to sources, and in remote environments far from point of release. Multimedia environmental fate models have been used to investigate chemical exposure and potential risk to humans and the environment, both as research tools and for regulatory assessment. In this study a multimedia fate model (SeSaME) has been developed to study the fate and behaviour of persistent chemicals across China. SeSaME explores the importance of spatial variability of environmental data across this diverse country which covers six climatic zones. In total, 16 kinds of environmental parameter datasets were collected, with the majority at a 1 km resolution, including the area fraction of land type, soil organic carbon content, wind speed, temperature, precipitation and the fraction

of it infiltrating into the soil and running to the water, soil erosion rate, surface water depth, river discharge, population density and sewage treatment connectivity. The spatial variation in all input variables was investigated and the impact on model output explored. All the maps were overlaid in the same projection system in Arcgis and processed in the 200 × 200 km boxes as the regional scale of SeSaME to predict the fate of the target chemical.

#### **MO268 Suggestions for improvement of the model SimpleTreat**

**A. Hein**, GKSS Research Centre Geesthacht / Pharmaceuticals, washing and cleansing agents, nanomaterials; **K. Ziegler**, Federal Environment Agency (UBA) / Biocides; **J. Struijs**, . Model calculations are an important component for the exposure estimation of human pharmaceuticals and biocides within the environmental risk assessment following the EMA guideline (EMA/CHMP/SWP/4447/00, Juni 2006) and Technical Guidance Document on Risk Assessment (TGD, 2003). For pharmaceuticals the predicted entry into the surface water (PEC<sub>surfacewater</sub>) is refined in Phase II Tier B with the model SimpleTreat by estimating the fate in a conventional activated sludge process. In regulation of biocides the results of SimpleTreat calculation are used directly dependent on their intended uses for PEC calculations of the different environmental compartments (soil, surface water and air). SimpleTreat was designed to compute the emission of neutral hydrophobic chemicals from communal wastewater treatment plants and was originally developed by the Netherlands National Institute for Public Health and the Environment (RIVM) as a spreadsheet model (Struijs et al., 1991). The program has been revised (Struijs, 1996) to be generically applicable for countries in the EU and has not been changed since 2003. The German Federal Environmental Agency (UBA) commissioned RIVM to check the validity of SimpleTreat regarding default operational parameters and the applicability of SimpleTreat for so-called “difficult chemicals”. Especially pharmaceuticals or biocides are often ionisable and persistent compounds. This poster is focusing on the evaluation of the operational aspects of this survey: The review of the implemented default values regarding operational parameters of sewage treatment plants reflecting the European standard in sewage technology 2000+ (e.g. capacity of local sewage treatment plant, sludge loading rate) since the model SimpleTreat describes sewage treatment technology of 25 years ago. Update and refinement options for the estimation of chemicals distribution in sewage treatment plants for a more realistic prediction. As a result of this project, suggestions will be presented to adjust and improve SimpleTreat. Thus a modified SimpleTreat will serve in a long-term as a realistic exposition model to the specific requirements in the authorisation processes of pharmaceuticals and biocides.

#### **MO269 Evaluation of SimpleTreat for biocides and pharmaceuticals**

**K. Ziegler**, Federal Environment Agency (UBA) / Biocides; **A. Hein**, GKSS Research Centre Geesthacht / Pharmaceuticals, washing and cleansing agents, nanomaterials; **J. Struijs**, . SimpleTreat is a software program to estimate the fate of chemicals in a conventional activated sludge process. It was designed to compute the emission of chemicals from communal wastewater treatment plants and originally developed by the Netherlands National Institute for Public Health and the Environment (RIVM) as a spreadsheet model (Struijs et al., 1991). The program has been revised (Struijs, 1996) and adapted to be generically applicable for countries in the EU. It functions as a central exposure assessment device in the EUSES system (TGD, 2003). The model has not been changed since 2003. This project aims to clarify the validity of SimpleTreat and the domain of applicability, especially for biocides and pharmaceuticals. UBA commissioned RIVM to indicate alternative application modes of the model in case these substances fall beyond the applicability domain due to their specific properties. Suggestions are made to modify and improve SimpleTreat to evaluate so-called “difficult chemicals” like e.g. ionised organics, surface active substances. The poster will present the outcome of the survey and will examine and summarise the following aspects: How realistic is the prediction of the environmental exposure in the model SimpleTreat compared to measured data from communal

wastewater treatment plants? Based on a data set on Kow, measured Koc- values derived from soils and sludges available for pharmaceuticals and biocides suggestions will be made for the use of partitioning coefficients in exposure estimation in SimpleTreat for environmental risk assessment. The gained experiences of the survey should allow the adaption to the specific requirements in EU legislation of biocides and pharmaceuticals. Consequently, the results will serve to guarantee for long term sustained environmental protection in the authorisation processes.

#### **MO270 Biocides: a higher Tier risk assessment approach in soil. A two-dimensional case study.**

**T. Schröder** BASF SE. 1. Introduction Addressing the environmental risk of biocides by means of modeling at a higher tier (beyond standard emission scenarios) requires creative approaches. Currently only basic guidance is given for some biocidal scenarios, lacking more realistic and hence higher tier approaches by complex environmental modeling. This is among others caused by the fact that biocidal uses are extremely diverse in their application method, and as such the environmental risk assessment that has to capture these uses. 2. Modelling Approach In this study modeling techniques from plant protection products (PPPs) are used in combination with a biocidal application scenario to investigate the environmental behavior of a biocide in soil. To address the fate of a substance in soil in a realistic way, a mechanistic model is used. This model accounts for the spatial component of the local application of a biocide in soil in two dimensions. The case study deals with a soil injection of a termiticide around a house. In the model a geometric domain is defined that suits the purpose of covering the application scenario on the one hand and registration needs on the other hand (areas in the domain where a safe use should be shown). Soil properties and boundary conditions are imposed based on a scenario which was originally defined for PPP's. Furthermore, initial conditions are imposed based on soil physical assumptions in conjunction with the biocidal use rate and type of application. Finally two application patterns were investigated for a period of 30 years. Then the concentration over time is analyzed in the liquid phase, as well as the total concentration in soil for various defined areas of the domain. Such procedures are common in PPP and are adapted here to a biocidal scenario. This case study shows that combined knowledge from both areas can lead to an improved environmental risk assessment for biocides with sometimes very specific use characteristics.

#### **MO271 Predicting the fate and elimination of chemicals in sewage treatment plants: evolution of the SimpleTreat model**

**A. Franco**, T. Gouin, Unilever / Safety and Environmental Assurance Centre; **O. Price**, Unilever / Colworth Science Park; **J. Struijs**, . Given the large number of chemicals under regulatory scrutiny, models are typically applied in risk assessment to estimate fate and elimination of chemicals in sewage treatment plants (STPs). In Europe, the STP model recommended for environmental risk assessment chemicals discharged down the drain is SimpleTreat. SimpleTreat is a multimedia box model of a conventional activated sludge STP with primary and secondary sedimentation. As for other similar models, the accuracy and the representativeness of SimpleTreat are limited and measured data in STPs are preferred, when available. SimpleTreat is usually run with a basic input dataset, its parameterization reflects a worst case scenario, predictions tend to be conservative, and therefore only used for lower tier assessments. A probabilistic parameterization, based on actual STPs conditions for the EU, was applied to achieve a more realistic representation of the variability of raw sewage characteristics, STP design and operational parameters. The uncertainty of chemical input properties, including degradation rates derived from higher tier biodegradation studies (OECD 314B, OECD 303A) was incorporated in the simulations. A validation study with ten selected chemicals showed good agreement with measured concentrations collected from the literature. The uncertainty analysis highlighted the importance of refined data on partitioning and biodegradability in activated sludge to achieve realistic estimates. The study indicates that the best strategy to refine the exposure assessment of down-the-drain chemicals is by integrating

higher tier laboratory data with probabilistic STP simulations and, if possible, by comparing them with monitoring data for validation.

**MO272 Volatile anaesthetics – an approach assessing environmental risks** K. Krome; S. Hahn, G. Koennecker, Fraunhofer Institute for Toxicology and Experimental Medicine. According to the European guideline on the environmental risk assessment of medicinal products for human use (EMA/CHMP/SWP/4447/00, 2006) the calculation of a worst case predicted environmental concentration (PEC) in surface water is required for human pharmaceuticals. This calculation is based on the assumption that 100% of the parent active ingredient enters the human body and subsequently is excreted from the human body into the wastewater before reaching surface waters. However, commonly used volatile anaesthetics predominantly leave the human body via pulmonary exhalation and are introduced into the atmosphere as the main target compartment. Only minor amounts (? 5%) of administered anaesthetics are excreted via urine as parent compound or as metabolite(s). The described approach intended to determine a reliable risk for the environmental compartments air, water and soil arising from three commonly used anaesthetics because the standard assumption of predominant urinary/faecal excretion is clearly not appropriate for volatile anaesthetics. Modeling tools were applied to determine the predicted environmental concentrations in air, water and soil after administration to humans and excretion directly into the atmosphere. In addition, for those amounts of the parent compounds as well as for its metabolites, which are expected to reach the water compartments via urinary excretion, the conventional method of exposure estimation according to EMA/CHMP/SWP/4447/00 (2006) was used. PEC values of the parent substances in water and soil as well as PEC values of the metabolites were compared to predicted no effect concentrations (PNEC) retrieved from literature data to calculate a reliable risk towards aquatic and terrestrial organisms. Until now, no guidelines for the estimation of environmental risks arising from atmospheric contamination have been developed in Europe. Thus, ozone depletion potential, potential contribution to global warming and long distance transport were assessed using literature data. We propose that the described procedure enables an appropriate way of assessing risks of volatile anaesthetics towards air, water and soil.

**MO273 Assessment of the Influence of Climate Change on Multimedia Fate of Persistent Organic Pollutants in South Korea** Y. Lee, Seoul National University / Dept. of Environmental Studies; H. Kim; L. Chang, Seoul National University; J. Cai; D. Lee, Seoul National University / Graduate School of Environmental Studies. The influence of climate change on multimedia fate of 10 VOCs (BTEX, and chlorinated hydrocarbons), 16 PAHs, and 17 PCDDs/Fs was assessed with a region-specific multimedia fate model. This model described the heterogeneity of environmental characteristics (hydrological network, land use, rainfall, and soil properties etc.) of South Korea using Geographical Information System (GIS). Two simulations were performed, one with 3-hour meteorological data obtained from climatic simulation for the period 2000-2050 driven by SRES A1B scenario (A1B run), and the other with a current climatic condition (Not Climatic Change, NCC run). In this work, monthly and annually averaged concentrations in environmental media (i.e. air, soil, water, and sediment) and inter-media flux calculated from A1B run and those from NCC run were analyzed. The air annual average concentrations of VOCs and most of PAHs (except benzo(b)fluoranthene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene, and indeno(1,2,3-c,d)perylene) from A1B run were not significantly different from those from NCC run. On the other hand, those 4 PAHs and PCDDs/Fs concentrations of A1B run were slightly lower than those of NCC due to the increase in wet deposition of particle bound chemicals in A1B run. It is suggested that the increase is mainly caused by the increase in the frequencies of rain events rather than the increase in annual precipitations. The increment of wet deposition is proportional to the magnitude of temperature-induced changes in vapor pressure. For three chemical groups, the annual average concentrations in soil from A1B run were slightly decreased compared with the results from NCC run. The decrease in annual average of PAHs and PCDDs/Fs

concentrations in soil was mainly due to the increase in solid runoff caused by the increase in annual precipitations. The water and sediment annual average concentrations in A1B run were higher than those in NCC run for three chemical groups. The increased solid runoff from soil contributed to the significant increase in PAHs and PCDDs/Fs in water and the magnitude of concentration change in water and sediment was determined by the octanol-water partition coefficient. Ratios of the monthly averaged concentrations of PAHs and PCDDs/Fs in water in A1B to those in NCC ranged up to 10 to 25, suggesting a possibility of substantial increase of both human health and aquatic ecosystem risks posed by certain chemicals.

**MO274 Characterizing Uncertainty in non-testing information for the prioritization of chemicals** M. Iqbal, B. Bergback, U. Sahlin, Linnaeus University / School of Natural Sciences. Under REACH several thousand chemicals must undergo risk assessment before 2018. A large number of chemicals will have to be tested in order to fill the information gaps on their hazardous properties and risks to support regulation. The information gaps can be filled by non-testing information, such as QSARs. However, non-testing information are subject to added uncertainty compared to testing information, which may be important to consider in chemical prioritization for testing. The objective of this study was to show if the consideration of uncertainty in the QSAR predictions result in a more efficient prioritization of chemicals. For this purpose, we ranked a set of PBDEs according to "urgency to test" based on QSAR-based assessments of toxicity and persistence. Uncertainties in QSAR predictions were considered in three different ways: no consideration using best estimates; classical uncertainty analysis using probability distributions; and extended uncertainty analysis by enlarging the individual QSAR predictions lying on the border (or outside) the QSAR's domain of applicability. Without considering uncertainty, those believed to be of highest risk were regarded as most concern. Under uncertainty, ranking was done according to the value of information, which evaluates the increase in expected welfare from reducing uncertainty by testing. The results showed that highly brominated PBDEs were of high concern and should be given priority for testing. Under different treatment of uncertainty, the ranking for chemical testing changed for BDE-153 to BDE-209, however, no difference in ranking was observed from BDE-03 to BDE-99. The consideration of uncertainty in QSPR predictions resulted in improving the efficiency of chemical ranking strategies for testing.

**TU001 Can flood events affect rainbow trout? The biomarker-cascade after exposure to PAHs in sediment suspensions** M. Brinkmann, RWTH Aachen University Institute for Environmenta / Department of Ecosystem Analysis; S. Hudjetz, M. Hennig, J. Kuckelkorn, RWTH Aachen University, Institute for Environmental Research; C. Cofalla, S. Lorke, RWTH Aachen University, Institute for Hydraulic Engineering and Water Resources Management; U. Kammann, Thünen Institute of Fisheries Ecology; M. Hecker, Toxicology centre, University of Saskatchewan; J.P. Giesy, Toxicology Centre & Dept. of Veterinary Biomedical Sciences, University of Saskatchewan; H. Schuttrumpf, RWTH Aachen University, Institute for Hydraulic Engineering and Water Resources Management; A. Schaffer, H. Hollert, RWTH Aachen University, Institute for Environmental Research. In context of the scientific discussion about the potential ecotoxicological impacts of flood events, it is important to understand the detailed mechanisms of contaminant uptake from suspended particles and related effects on aquatic biota. Rainbow trout (*Oncorhynchus mykiss*) were exposed to suspensions of natural sediment from the River Rhine (Ehrenbreitstein Harbour). Prior to suspension, the sediment was spiked with the polycyclic aromatic hydrocarbons pyrene, phenanthrene, chrysene, and benzo[a]pyrene at environmentally relevant concentrations (4.1, 5.0, 3.3 and 8.3 mg kg<sup>-1</sup> dw, respectively). A control treatment without addition of PAHs was included in the experimental design. The experiment was conducted first at an average temperature of 24 °C and then repeated at 12 °C. The nominal concentration of suspended solids was 10 g L<sup>-1</sup> dw in both experiments. After 0, 1, 2, 4, 6, 8 and 12 days of exposure, physicochemical

parameters, concentrations of PAHs in suspended matter, as well as biomarkers of exposure in rainbow trout (biliary PAH metabolites, hepatic 7-ethoxyresorufin *O*-deethylase (EROD) activity and lipid peroxidation) were measured. Chemical analyses (GC/MS) revealed that concentrations of pyrene and phenanthrene in suspended solids decreased over time, while no significant degradation was observed for chrysene and benzo[a]pyrene. Concentrations of biotransformation products of PAHs in bile of fish increased slightly in the control group at 24 °C, while average levels increased to 166 µg mL

#### **TU002 Do flood events matter? Biomarker response of rainbow trout following exposure to re-suspended sediments in an annular flume**

**M. Brinkmann**, RWTH Aachen University Institute for Environment / Department of Ecosystem Analysis; S. Hudjetz, H. Herrmann, RWTH Aachen University, Institute for Environmental Research; U. Kammann, Thünen Institute of Fisheries Ecology; C. Cofalla, S. Lorke, RWTH Aachen University, Institute for Hydraulic Engineering and Water Resources Management; A. Schaffer, RWTH Aachen University, Institute for Environmental Research; M. Hecker, Toxicology centre, University of Saskatchewan; H. Schuttrumpf, RWTH Aachen University, Institute for Hydraulic Engineering and Water Resources Management; H. Hollert, RWTH Aachen University, Institute for Environmental Research. Re-suspension of contaminated sediments during flood events is a significant secondary source of persistent pollutants in rivers. Especially in the context of global climate change it has been predicted that both frequency and intensity of flood events will increase locally and periodically in the upcoming decades. Thus, the assessment of risks associated with sediment re-suspension will be a key issue for the protection of the aquatic environment. The interdisciplinary project FLOODSEARCH II provides an important contribution to this field by experimentally combining methods of hydraulic engineering and ecotoxicology. In this study, rainbow trout (*Oncorhynchus mykiss*) were exposed to native sediments with different contamination levels for seven days in an annular flume, a device that can be used for erosion and deposition studies. Different biomarkers of exposure and effect were measured in exposed fish: Biliary metabolites of polycyclic aromatic hydrocarbons (PAHs), concentration of copper in gills, hepatic enzyme activities, lipid peroxidation, and metallothionein concentrations, as well as micronuclei in peripheral erythrocytes. Concentrations of the 16 EPA-PAHs and copper in suspended particulate matter were measured as selected representatives for contamination with persistent organic pollutants and heavy metals, respectively. In summary, the project intended to broaden our knowledge of the interactions of sediment dynamics and ecotoxicological potential of sediment-bound pollutants in support of sediment risk assessment and management plans. The research project FLOODSEARCH II was funded by a boost fund project at RWTH Aachen University that was financed by the German Excellence Initiative to promote innovative and interdisciplinary research fields.

#### **TU003 Skewed sexual differentiation induced by combined effects of temperature and an endocrine disrupter is greater for inbred compared with outbred fish**

**A.R. Brown**, AstraZeneca / Dept. of Aquatic Toxicology; J.R. Peters, AstraZeneca Brixham / Environmental Effects; G.C. Paull, University of Exeter; P.B. Hamilton, University of Exeter / Biosciences; S. Owen, AstraZeneca; D.J. Hosken, University of Exeter; C.R. Tyler, The University of Exeter. Endocrine disrupting chemicals (EDCs) with the potential to cause reproductive impairment in individuals and/or lead to biased population sex ratios, could combine with other environmental stressors to reduce effective genetic population size in wildlife. Geographically isolated, inbred wildlife populations may be particularly at risk due to their already limited genetic variation and adaptive capacity. Here we investigated effects on sexual differentiation and development of combined exposure to an EDC (the aromatase inhibitor, clotrimazole, at 2 or 10 mg l<sup>-1</sup> compared to a predicted environmental concentration PEC<sub>local</sub> of 0.2 mg l<sup>-1</sup>) and elevated environmental temperature (33°C versus 28°C) in inbred and outbred zebrafish (*Danio rerio*). F<sub>0</sub> generation inbred and outbred zebrafish family lines (n=20) were both derived (at F<sub>0</sub>) from the

outbreeding of two wild-sourced populations under standard laboratory conditions (28°C) and were subsequently exposed in a partial life-cycle study from 35-95 days post hatch (dph). Two of the 20 inbred families displayed considerable inbreeding depression with high embryo/larval mortality rates and these family lines thus failed to enter the study, whereas all 20 outbred families were recruited successfully. During the study we recorded a low incidence of deformity (< 1% lordosis, scoliosis) and there were no apparent differences in specific growth rates between any of the treatments and their respective controls. However, sex ratios in experimental tanks housing separate family lines were skewed significantly towards males following high-level (10 mg l<sup>-1</sup>) exposure to clotrimazole and/or elevated temperature (33°C). Male-skew was greater in inbred treatments compared with equivalent outbred treatments and was also significant in inbreds (but not outbreds) following low-level clotrimazole exposure (2 mg l<sup>-1</sup>) in combination with elevated temperature (33°C). We explored the possibility that the small minority of fish remaining female in the male-skewed treatments was related to aromatase gene expression in ovarian tissue. Ultimately we attempt to assess the potential consequences of sex ratio skew within each of our experimental treatments using a stochastic population dynamics model parameterised for both inbred and outbred zebrafish populations.

#### **TU004 Criteria for bioaccumulation and toxicity to non-aquatic organisms within the framework of PBT/vPvB assessment**

**N.B. Hartmann**, S. Gottardo, B. Sokull-Kluettgen, European Commission - Joint Research Centre / Nanobiosciences Unit - Institute for Health and Consumer Protection. Current international and European regulatory environmental criteria for PBT assessment are based on toxicity and bioaccumulation in aquatic organisms. The regulatory frameworks therefore potentially neglects a group of substances which are bioaccumulative and/or toxic in non-aquatic organisms food chains (exposed through soil and food), but not in aquatic. We have investigated this issue through a review of the legislative status and scientific knowledge on toxicity and bioaccumulation assessment for non-aquatic organisms at international and European level. The review reveals that for bioaccumulation several persistent, low hydrophobic and poorly metabolised organic chemicals can biomagnify in terrestrial food webs and bioaccumulate in human blood and tissues, but not in aquatic environments [1][2]. Hence, these substances may not be classified through current regulatory criteria, which are based on the use of the octanol-water partitioning coefficient (K<sub>ow</sub>) and the BioConcentration (BCF) or BioAccumulation Factor (BAF). Both K<sub>ow</sub> and estimated/measured BCF values reflect the equilibrium partitioning of organic compounds between water and biota, hence applying to aquatic organisms and not taking into account exposure route through the diet. With regards to toxicity, data from the aquatic system are generally considered to result in a conservative classification but still there are exceptions where higher toxicity is observed for the terrestrial system [3]. For example, effects of substances with low water solubility may not be detectable through acute aquatic toxicity tests and may instead be identified through tests with terrestrial organisms exposed through soil or food. Accordingly, we present an overview of state-of-the-art scientific knowledge in this field and provide some preliminary views on possible incorporation of non-aquatic criteria in the regulatory identification of PBT substances. **References:** [1] Gobas FAPC; de Wolf W, Burkhard LP, Verbruggen E, Plotzke K. 2009. Revisiting Bioaccumulation Criteria for POPs and PBT Assessments. Integrated Environmental Assessment and Management 5:624-637. [2] Tonnelier A, Coecke S, Zaldivar JM. 2011. Screening of chemicals for human bioaccumulation potential with a physiologically based toxicokinetic model. Archives of Toxicology 86:393-403. [3] Renaud FG, BoxallABA, Toy R, Robertson S. 2004. Evaluation of approaches for terrestrial hazard classification. Chemosphere 57:1697-1706.

**TU005 Development and optimisation of an aquatic laboratory microcosm** **C. BERNARD**, ENTPELEHNAIPE / UMR CNRS 5023 LEHNA; G. TRIFFAUL-BOUCHET, Centre d'expertise en analyse environnementale du Québec MEDP / Division Ecotoxicologie et

Evaluation du risque; H. DELHAYE, Université de Lyon ENTPE / UMR CNRS 5023 LEHNA. Ecotoxicological assessment of chemical substances is mainly based on standard bioassays, most often single-species tests covering the range of acute and chronic toxicity. In our laboratory we have been developing since 1997 a protocol of ecotoxicological bioassay in 2-L laboratory microcosms and applied it to the study of various pollutants and scenarios of ecotoxicological risk assessment in the field of urban facilities and transport infrastructures. Effects are assessed on five different organisms (micro-algae, duckweeds, daphnids, amphipods, chironomids) using endpoints such as growth, emergence (chironomids), reproduction (daphnids), survival, with a duration exposure of 3-4 weeks. For specific studies, some variants of the 2-L protocol have been developed in higher volumes (up to 100 L) with increasing biological diversity and different hydraulic conditions. More recently, a flow-through microcosm assay (dynamic assay) was developed for the 2-L protocol to improve conditions inside the microcosms, resulting in stabilisation of physico-chemical parameters, increase of organisms fitness and reduction of variability. This communication will present the various protocols, their application to different ecotoxicological risk assessment studies, their limitations (variability and sensitivity, ecological relevance, organism fitness, ...), and the improvement of the 2-L protocol through continuous water renewal. Finally, recent works and perspectives in modelling will be presented.

**TU006 Implications of food quantity and quality on bioassays: a case study with *Chironomus tepperi*** V. Colombo, CAPIM; L.A. Golding, CAPIM Bio Institute University of Melbourne; A.A. Hoffmann, BIO21 - The University of Melbourne; V.J. Pettigrove, The University of Melbourne / Zoology. *Chironomus* species are commonly used in standardised laboratory tests to predict or measure the effect of chemicals on ecosystems. The selected conditions have to represent a physiologically optimal environment for the test organism, be cost effective and not labour intensive for the researchers. The determination of an optimal feeding regime is one essential factor to be considered. It is well known that food quantity and quality affect life-cycle parameters (e.g. growth and emergence time). Food may also alter the sensitivity of the test organism toward the tested substance, especially in sub-lethal ranges. For the Australian species *Chironomus tepperi* the optimal feeding regime has not yet been established: this was the aim of the first part of this study. We compared two commonly used food types, TetraMin and SeraMicron which possess different biochemical features. SeraMicron is a high nutritional quality food, with a high protein content. The first results indicated that larvae achieved good performance when fed with small amount of TetraMin (0.25 - 0.5 mg/larva/day). However, when the amount of food was doubled, the chironomids performed better with SeraMicron. Therefore the suitability of each food for one of the two different larval phases (the somatic phase, corresponding to the first three larval stages and the sexual phase, corresponding to the 4<sup>th</sup> larval stage) was also tested. In the second part of the study we tested whether the paternal nutritional condition (food abundance or restriction) affects the sensitivity of the progeny toward a toxicant. Whilst feeding regimes during the tests are experimentally determined, little attention has been paid to a possible carryover of effects across generations. In fact, cultures of test organisms tend to be fed abundantly, both to reach maximum survival and fecundity and also to avoid starvation, since the effective number of chironomid larvae in a culture can only be estimated. Well-fed animals are usually less susceptible to toxicants than food-limited ones. The questions we aim to answer are: can well fed parents pass this advantage to the offspring? Are offspring of nutrient-restricted parents more or less susceptible to chemical stress? And finally, is the nutritional status of both parents important for the offspring or is only the maternal status important? This study will provide relevant information about the test organism, which in turn will decrease the gaps between lab and field bioassays.

**TU007 Evaluating ecotoxicity of Alkyl Sulfates which have various alkyl chain length by using a flask-sized microcosm** H. Usui, Lion corp / Human and Environmental Evaluation Center; Y. Kousuke, Lion

Corporation Research Development / Research & Development; F. Harada, Lion Corporation. Single species tests are widely used for evaluating aquatic ecotoxicity of a chemical substance but it is possible that the ecotoxicity changes by the presence or absence of interactions between species. On the contrary, ecosystem models are useful methods for evaluating the ecotoxicity in a realistic environmental condition because they consist of multi-species. However, they are used only in a higher tier testing in consequence of their high expense and complexity. A method with flask-sized microcosm solves above problems by using amounts of photosynthesis and respiration as endpoints. This flask-sized microcosm is an experimental ecosystem model which consists of phytoplankton as producer, zooplankton as consumer and bacteria as decomposer with interactions between the species. Because the system is small and closed, the amounts of photosynthesis and respiration can be calculated from a dissolved oxygen concentration, which is fluctuated by a light/dark cycle. Photosynthesis and respiration respectively represent energy production and energy consumption which accompany dynamic equilibrium of the ecosystem. Consequently, they indicate a momentum of the ecosystem and simplify to evaluating the effects of chemical substances. In this study, effects of various Alkyl Sulfates to the flask-sized microcosm were evaluated. Alkyl Sulfates which have various chain lengths ranging from C8 to C16 were used as test substances. As a result the ecotoxicity of Alkyl Sulfates became stronger with increasing in length of alkyl chain. This tendency is known in a case of single species tests. This result suggests that same tendency is observed in the ecosystem with multi-species. Subsequently counting the number of organisms was performed to check whether the shift of ecotoxicity derived from alterations of the species affected or not. And it was showed that non-observed effect concentration (NOEC) of C12 Alkyl Sulfate to the flask-sized microcosm fitted already reported correlation between NOEC in flask-sized microcosm and that in mesocosm. When accumulating the data of wide range chemical substances refines correlation equation and extends the range of application, it is possible that the flask-sized microcosm become a powerful tool for evaluating the effects of chemical substances to the real ecosystem.

**TU008 Using microcosms experiments to evaluate the combined effects of temperature and pesticide exposure on different freshwater species** V. Silva, University of Aveiro / Department of Environment & CESAM; C. Marques, University of Aveiro / department of Biology & CESAM; J. Keizer, University of Aveiro / Department of Environment & CESAM; F. Goncalves, University of Aveiro / department of Biology & CESAM; N. Abrantes, University of Aveiro / CESAMDAO. Diffuse pollution resulting from agricultural activities is one of the main environmental problems contributing to poor water quality. Although climate change is expected to constitute an additional threat to aquatic resources, the combined effects of climate change and diffuse pollution from agriculture are very poorly studied. According to the Fourth Assessment Report of the IPCC the impacts of climate change on freshwater systems and their management are mainly due to the observed and projected increases in temperature, sea level and precipitation variability (very high confidence). Thereby, the present work aims to use microcosm experiments to study the combined effects of temperature and pesticide on several freshwater species under more realistic exposure scenarios. The microcosm experiments consist of artificial tanks of 80L filled with freshwater and sediment collected in a reference site. Two temperature regimes (15°C and 25°C) and two pesticides (Copper sulphate or Tebuconazole; both detected in water bodies nearby vineyard areas) were selected to carry out the experiments. Different bioassays are being conducted at the same time, including: growth rate inhibition tests using the green algae *Pseudokirchneriella subcapitata* and the macrophyte *Lemna minor*; leaf decomposition assays using chestnuts leaves (*Castanea sativa*); and feeding inhibition assays using the trichoptera *Schizopelex* sp.. Water in each tank is also monitored every other day for basic parameters (dissolved oxygen, pH, conductivity and temperature), nutrient concentrations (nitrates, nitrites, ammonium and orthophosphates) and pesticide concentration. Based on previous works, we expect an

increment in the effects of species when exposed to combined effects of pesticide and high temperature as a result of increasing metabolic rates that exacerbate the exposition to the contaminants. The results of these experiments, using organisms from different trophic levels and the combination of two stressors, will provide a more realistic overview of the impacts on the aquatic ecosystem functioning. This information is of crucial importance to anticipate the effects of chemical pollution in the rapidly changing environment. Moreover, considering that the Water Framework Directive is in the implementation phase, it is imperative to assess the impacts of the climate change on aquatic resources impacted by agricultural diffuse pollution contributing therefore for an effective environmental management strategy.

**TU009 Assessment of the toxicity of a post-fire runoff on aquatic ecosystems** V. Silva, University of Aveiro / Department of Environment & CESAM; N. Abrantes, University of Aveiro / CESAMDAO; J. Pereira, University of Aveiro / department of Biology & CESAM; I. Campos, J. Keizer, University of Aveiro / Department of Environment & CESAM; F. Goncalves, University of Aveiro / department of Biology & CESAM. Every year large Mediterranean forest areas are burnt. Wildfires can cause serious impacts on ecosystems and an increasing interest has been devoted to their effects on water chemistry and aquatic biota. To simulate the runoff from a burnt area, ashes were collected immediately after a forest fire in Vale de Cambra (Aveiro, Portugal) and composite samples were used to prepare standard aqueous extracts. A vast group of metals (Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, V, Ti, Tl and Zn) and the sixteen prioritized polycyclic aromatic hydrocarbons (PAHs) were analytically quantified in the aqueous extracts of ashes (AEA). The results identified Ca, S, Mg, K and Na as the major metals in such extracts and only the low molecular weight PAHs Phenanthrene and Naphthalene were quantified. In parallel, an ecotoxicological screening of these AEA was performed with four aquatic species representing different functional groups and trophic levels. AEA induced a decrease in the growth of the primary producers *Pseudokirchneriella subcapitata* and *Lemna minor* (a statistically significant effect was observed at the highest tested concentrations), and inhibited the *Vibrio fischeri* luminescence. No significant *Daphnia magna* immobilization was observed, so that short-term toxicity is not expected for higher trophic levels. The results emphasize the need of additional research to understand the complexity of the potentially deleterious ecological effects of wildfires on aquatic communities due to the exportation and input of post-fire contaminants.

**TU010 The biofiltration potential of *C. fluminea* in the mitigation of post-fire runoff impacts on aquatic ecosystems** V. Silva, University of Aveiro / Department of Environment & CESAM; N. Abrantes, University of Aveiro / CESAMDAO; J. Pereira, F. Goncalves, University of Aveiro / department of Biology & CESAM. Addressed to a growing concern about the effects of wildfires on water quality and on the aquatic biota, an evaluation of the risks of aqueous extracts of ashes was conducted. The results reaffirmed wildfires as a diffuse source of contaminants (metals and PAHs) as well as showed different toxic effects of pyrolytic inputs of aquatic organisms. Based on the reported deleterious effects of wildfires on aquatic ecosystems, the biofiltration potential of the Asian clam *Corbicula fluminea* was tested in a 7-day experiment, using the aqueous extracts of ashes as a model challenge. This approach constitutes a preliminary assessment on whether this species may somehow contribute to the mitigation of potentially deleterious effects of wildfires. The contaminants in biofiltered water were monitored and compared to the variation in contaminant levels in clams (soft tissues and shells). At the end of the biofiltration period, there was a clear reduction of contaminants in the water samples: a removal of more than 70% was achieved for Mn, Ni and Zn while in organic contaminants, NAP was reduced in 50%. Compared to the unfiltered samples, the bio-filtered samples revealed lower environmental toxicity, particularly evident as a smaller inhibition on the growth of the primary producers (statistical significant in both growth rate and yield of *Lemna minor*), and a much lower luminescence

inhibition of the bacteria *Vibrio fischeri* (or even luminescence stimulation of several tested concentrations). The results of this study evidenced that the negative ecological effects attributed to the invasive bivalve can eventually be compensated by additional services provided to the ecosystem. Notwithstanding, additional research is necessary to fully understand the potential and limitations of *C. fluminea* as a bioremediation option.

**TU011 Understanding the role of DOM on metal bioavailability - a prerequisite for the narrowing the gap in lab-to-field extrapolation** V.I. Slaveykova, I.A. Worms, G. Cheloni, University of Geneva / Institute Forel, Earth and Environmental Sciences. Dissolved organic matter (DOM), a major component found in natural waters is considered to protect the aquatic biota from toxic metal stress by decreasing free metal ion concentration and thus reducing their bioavailability (and detrimental effects). Therefore any environmental change (e.g. changes in the light and UV-irradiation regimes) that could affect DOM concentration, structure and reactivity might expect to alter (e.g. decrease) the capacity of DOM to bind toxic metals and reduced their toxicity to the aquatic organisms. Nonetheless very few or controversial information is available on the role of the sunlight regime on the toxic metal bioavailability. The present study aims therefore to determine to what extent the alteration of the natural and waste water treatment plants (WWTP) effluent organic matter (EfOM) by the sunlight will affect their capacity to complex lead and decrease biological availability of this toxic metal to freshwater microalgae. More specifically, the free lead ion concentrations and intracellular Cd, Cu and Pb content in algae *Chlorella kesslerii* and *Chlamydomonas reinhardtii* were determined in the presence of the DOM of different origin, irradiated with sunlight of increasing intensity. In addition, the alteration of selected OM characteristics including, organic carbon content, absorbing and fluorescence properties were determined. The obtained results indicate that increased sunlight intensity altered DOM quantity and quality. The alteration involves photo-bleaching, photo-degradation of fluorophores, while no photo-mineralisation was observed. The sunlight irradiation affected Pb bioavailability to *Chlorella kesslerii* not only by increasing the free lead ion concentrations, but also by influencing the direct interaction of DOM with algal cells and molar mass distribution of Pb-DOM complexes. **Acknowledgement** - The authors thank Swiss National Science Foundation project 200021-134627 and for the financial support.

**TU012 Comparison of Intermittent and Continuous Exposures to Inorganic Mercury in Mussel, *Mytilus edulis*: Accumulation and Sub-lethal Physiological Effects** D. Amachree, University / School of Biomedical and Biological Sciences; J. Moody, University; R. Handy, University of Plymouth / School of Biomedical and Biological Sciences. Intermittent and variable exposure to pollution is the most likely way aquatic organisms are exposed to chemicals, including metals. However, little is known about the bioaccumulation responses of shellfish to metals during intermittent compared to the continuous exposure. This study aimed to determine whether the mercury (Hg) accumulation pattern and physiological responses of *Mytilus edulis* would be different in each exposure mode. *M. edulis* were exposed using a semi-static and triplicated design to either control (no added Hg) or 50 µg l<sup>-1</sup> Hg as HgCl<sub>2</sub> in continuous or intermittent mode (2 day exposure, 2 days in clean seawater alternately) for 14 days. Tissues exposed to either continuous or intermittent mode accumulated more Hg than the unexposed control (ANOVA or Kruskal-Wallis, P < 0.05). The pattern of Hg accumulation in the continuous exposure showed a time-dependent increase, while the intermittent treatment showed step-wise changes in some tissues (e.g., gill). At the end of the experiment, Hg body burden was significantly increased in the continuous compared to the intermittent for digestive gland (5 fold), gonad and remaining soft tissue (>2 fold). However, there were no significant differences between the continuous and the intermittent exposures for Hg body burden in the gill and adductor mussel (ANOVA or Kruskal-Wallis, P < 0.05). There were no differences in tissue thiobarbituric acid reactive substances (TBARS) concentrations at the end of the experiment. There were no overall differences in the tissue and haemolymph Na<sup>+</sup> and K<sup>+</sup>



concentrations as well as osmolarity between the two exposure modes. The neutral red retention ability of the haemocytes was unaltered, but total haemocyte counts were significantly decreased (ANOVA,  $P < 0.05$ ) in the intermittent compared to the continuous exposure. Histopathological examinations showed significantly less pathology in the gill, and high inflammation in the digestive gland of the intermittent mode compared to the continuous exposure (Kruskal-Wallis,  $P < 0.05$ ). In conclusion, these data show that the accumulation patterns for Hg are different between intermittent and continuous exposure, and the intermittent produces toxicity which are similar, less or more than the continuous counterparts, depending on the end point measured.

**TU013 Competition study with the invasive tiger mosquito *Aedes albopictus* and the native Northern house mosquito *Culex pipiens f. molestus*** L. Wiesner, Goethe University Frankfurt / Department Aquatic Ecotoxicology; A. Kress, Goethe University Frankfurt; R. Mueller, LOEWE Biodiversity and Climate Research Centre / Aquatic Organisms and Ecosystems; U. Kuch, Biodiversity and Climate Research Centre Frankfurt/Main, LOEWE / Medical Biodiversity and Parasitology; J. Oehlmann, Johann Wolfgang Goethe-Universität Frankfurt am / Aquatic Ecotoxicology. Over the last 30 years, the Asian tiger mosquito *Aedes albopictus* turned out to be one of the most invasive and dangerous mosquito species in the world. For this effective transmission vector for dangerous tropical diseases like dengue fever or chikungunya fever, the interspecific competition with indigenous species like the common house mosquito *Culex pipiens* plays a key role regarding colonization of new habitats. First observations showed clearly that tiger mosquitos outcompete local mosquito species. To prevent further spreading of the invasive tiger mosquito, synthetic insecticides like the pyrethroid  $\gamma$ -cyhalothrin are used for area wide control. In this study we investigated if this application also affects indigenous mosquito species sharing similar habitats as the tiger mosquito. As test species, *A. albopictus* and *C. pipiens* were provided by own breeding. The effects of  $\gamma$ -cyhalothrin ( $EC_{50}$ :  $100\text{ngL}^{-1}$ ) on the development and mortality ratio of both mosquito species and their interactions were investigated. Therefore, a response surface design with different larval densities (0, 5, 10, 20 and 40 larvae per plastic beaker and species) was chosen. This design tests for the interactions of multiple stressors, i.e. intraspecific competition, interspecific competition and insecticide susceptibility. The results confirm the expected domination of the invasive mosquito species, in particular after insecticide treatment.  $\gamma$ -cyhalothrin caused minor effects on *A. albopictus*. Comparing only the interspecific and intraspecific competition between both species, the invasive species *A. albopictus* shows a clear domination. Interspecific competition leads to a significant higher mortality in both species but particularly in *C. pipiens*. The local species *C. pipiens* is by far more affected by the application of insecticides. Especially female larvae of *C. pipiens* are susceptible. The results indicate that the use of insecticides for the control of the Asian tiger mosquito may support the establishment of the invasive species in new habitats due to the death of *C. pipiens* and thereby nascent resources. This may lead to the suppression of the common house mosquito in known habitats.

**TU014 Fate of ingested gamma-hexabromocyclododecane in laying hens** C. Jondreville, URAFA; A. Fournier, Université de Lorraine / URAFA; A. Venisseau, ONIRIS / LABERCA; A. Travel, ITAVI; P. Marchand, ONIRIS / LABERCA. Although hexabromocyclododecane (HBCD) is rarely detected in food items originating from terrestrial animals, concentrations exceeding 3000 ng HBCD/g lipid were recorded in hen's egg samples collected in the frame of the French monitoring plans. Neither the sources of animals' exposure nor the fate of HBCD in laying hens are known, although they are key elements for an efficient prevention of food contamination. Therefore, an experiment was conducted in order to better understand the fate of ingested  $\gamma$ -HBCD in laying hens.  $\gamma$ -HBCD being the main component of the commercial mixture, our hypothesis was that hens may have ingested materials treated with this brominated flame retardant. Forty-eight laying hens aged 43 weeks and weighing  $1.77 \pm 0.16$  kg were assigned to four blocks

of 12 hens each on the basis of body weight (BW). Of the 12 hens in each block, 6 were assigned to a diet containing  $0.1 \mu\text{g } \gamma\text{-HBCD/g}$  and 6 to a diet containing  $10 \mu\text{g } \gamma\text{-HBCD/g}$ . Diets were obtained by incorporating 0.5% of spiked oil (1,2,5,6,9,10-Hexabromocyclododecane, Sigma Aldrich) into a commercial diet analysed to contain  $0.038 \text{ ng HBCD/g}$ . The experiment lasted 70 days during which feed allowance was adjusted to 6.1% of BW within each block (i.e.  $6.1$  and  $610 \mu\text{g } \gamma\text{-HBCD/kg BW daily}$ ). In each block, 2 hens (one assigned to each diet) were slaughtered after 1, 12, 21, 36, 54 and 70 days of exposure.  $\gamma$ -,  $\alpha$ - and  $\beta$ -HBCD were extracted from egg yolk, liver and abdominal fat by ASE using toluene/acetone 70:30 (v/v) as solvent and analysed by LC-MS/MS. The concentrations of the three isomers in all matrices increased along with the exposure duration. Whatever the date of slaughter and the dietary concentration of  $\gamma$ -HBCD, the isomer pattern in the assayed tissues was clearly dominated by  $\gamma$ -HBCD, followed by  $\alpha$ - and  $\beta$ -HBCD (92-97%, 3-7% and less than 1% of the sum of the three isomers, respectively). After 70 days of exposure, the ratio of HBCD present in tissue (ng/g lipids) to the dietary concentration of  $\gamma$ -HBCD (ng/g feed) was 0.50, 0.37 and 0.84 in egg yolk, in liver and in abdominal fat, respectively. These figures were independent of the dietary contamination. This study confirms the rapid isomerisation of  $\gamma$ -HBCD into  $\alpha$ - and  $\beta$ -HBCD and its low transfer to animal tissues, probably due to metabolism into hydroxymetabolites and debrominated compounds.

**TU015 Can biochar be used for remediation of agricultural soils contaminated with pesticide mixtures?** A. Amaro, University of Aveiro / Department of Biology and CESAM; M. Santos, CESAM DeptBiology / Department of Biology and CESAM; S. Loureiro, Universidade de Aveiro / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; A. Bastos, University of Aveiro / Department of Biology and CESAM. Biochar has been a matter of great interest in recent decades, mainly due to its physical and chemical characteristics, which upon application to soil, contribute to increase soil productivity and improve carbon sequestration. Further, biochar's high sorption capacity (as a result of its large and highly reactive surface), may decrease mobility and bioavailability of soil contaminants and is of great environmental, ecological and societal interest. Contaminants in agricultural soils, such as pesticides, are mostly present in the form of mixtures. Nevertheless, the application of biochar for immobilization of mixtures of pesticides has not been yet fully tested. Further, both factors intrinsic to the biochar (e.g. biomass source, pyrolysis temperature) and the environment on-site (e.g. soil moisture, soil temperature, pH) condition the immobilization of these compounds, as well as the effects on soil biota. However, before biochar can be considered a soil remediation option, its interactions with soil fauna and flora need to be carefully evaluated. Up to now, very few studies exist on the effects of biochar application to soil on soil organisms (*Eisenia fetida* is an exception) or soil function and often, their results are not conclusive or based on realistic scenarios. Having this into account, a microcosms experiment was developed using small-scale terrestrial ecosystems (STEM), in which agricultural field conditions were simulated. The objectives of this study were to test: i) the remediation capacity of biochar (at  $100 \text{ ton ha}^{-1}$ ) in agricultural soils containing pesticide mixtures, at recommended rates; ii) its effects on the representative organisms *E. fetida* and *Brassica rapa*, from two different trophic levels, scavengers and producers respectively, at two soil wetting rates (40 and 60% WHC), during 28 days. By analyzing various complementary parameters (i.e. fresh weight of earthworms and distribution along the soil column; growth, fresh weight and changes in morphology of the plants; soil respiration and feeding activity) it is possible to obtain solid results that help to better understand, both the way biochar can be used to remediate mixtures of pesticides and the local interactions with soil biota.

**TU016 New Standards, Old Methods? A Review of Practical Metal Speciation Methods for Implementing New Standards in Saline Waters** H.B. Pearson, Plymouth University / Geography, Earth and

Environmental Sciences; S. Comber, Environmental Science; C. Braungardt, P. Worsfold, Plymouth University / Geography, Earth and Environmental Sciences. The proposed new European environmental quality standards (EQS) for fresh and saline waters drive considerable interest in maintaining or achieving acceptable quality in UK waters with respect to metal contamination and other toxic substances. The speciation of the element in question, and hence its bioavailability and toxicity, is governed by complexation with both organic and inorganic ligands within the water body, such as dissolved organic carbon (DOC), CO<sub>2</sub>, Na<sup>+</sup>, H<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup>. The overall aims of this research are to investigate the complexing capacity of DOC and its constituent organic ligands for metals (in particular Cu and Zn) in estuaries, and to link the chemical and biological processes resulting in observed metal ecotoxicity. Because estuarine mixing complicates the physico-chemical processes influencing speciation by the alteration of parameters, such as ligand concentrations, pH and ionic strength, the study of estuarine environments will help clarify the appropriateness of the new EQS. In addition, the data generated will contribute to improving analytical methodologies and current models used for predicting metal speciation in saline waters. This poster presentation reviews established and emerging methodologies for measuring speciation in estuarine waters, and proposes the most appropriate for use in this research, as well as for use by regulatory bodies undertaking routine monitoring.

**TU017 The effects of salinity environment on freshwater fish *Tilapia zillii*** H. jenjan, N. salman, F. El- Tumi, Benghazi University. Effects of abrupt and gradual transfer to saltwater environment on the freshwater fish *Tilapia zillii* are presented. Survival rates at different salinity media were recorded. Gradual transfer appeared to be beneficial in this deference of water of full strength (37 g/l salinity) using this method. However, full strength seawater appeared lethal to 26.7 % of fish upon abrupt exposure. Under abrupt and gradual exposure conditions, Na and K rise sharply in high salinity media. *Tilapia zillii* shows freshwater fish characters as appeared from the sudden rise in plasma ions in response to changes in external environmental salinity. Rising ions of plasma in response to high exposure of salinity is the first sign of osmoregulatory stress in this species.

**TU018 Analysis and Assessment of Organotins in Sediments from Scottish Harbours and Sea Disposal Sites** S. Devalla, Marine Scotland Science; C. Robinson, Marine Scotland - Science / Marine Laboratory; C. Megginson, Marine Planning and Policy; L. Webster, Marine Scotland Science. The toxic effects of organotins to non-target organisms even at very low concentrations is well established. The most relevant source of tributyl tin (TBT) in harbour sediments is from its use of as an antifouling agent in paints applied to marine vessels. The UK government in 1987 imposed a restriction on the use of TBT. Being persistent and highly toxic such as causing endocrine disruption, continuous monitoring of TBT is of vital importance to safeguard the marine environment. Under the Marine (Scotland) Act 2010 a marine licence is required for the disposal of dredged material to a registered sea disposal site. This is issued by the Marine Licensing Operations Team (MS-LOT) on behalf of the Scottish Ministers. As part of the marine licensing process Marine Scotland Science monitors the concentrations of different pollutants in dredged sediments and also analyses sediments collected, on a rolling programme, from sea disposal sites. The criteria for disposal of dredged sediments are based on certain guideline Action level values - AL1 (100 µg/kg) and AL2 (500 µg/kg) for TBT. These UK based Action level values are not statutory contaminant concentrations but are guideline values that are used in conjunction with other assessment methods. This will be discussed in the presentation. The analytical method for the analysis of TBT and dibutyl tin (DBT) involves an overnight extraction of organotins from homogenised dried sediments in an acidic medium followed by derivatisation using sodium tetraethylborate. Analysis is performed by Capillary Gas Chromatography- Mass Spectrometry. Details of the various aspects of the methodology such as extraction, derivatisation, clean-up, separation and detection will be discussed. For the harbour sediments collected since 2002, highest TBT concentrations were found from highly

industrialised areas with intensive shipping activities such as Clyde, Firth of Forth, Aberdeen and Peterhead.. Amongst these, Peterhead showed a significant number of samples with concentrations of TBT above the action levels. Amongst the Firth of Forth harbours, Leith showed much higher concentrations of TBT compared to Grangemouth or Rosyth. For the sea disposal sites Peterhead, Cloch Point and Ullapool showed relatively higher concentrations of TBT (> 50 µg/kg) compared to the rest. All sites with the exception of Peterhead were however well below the action limit values.

**TU019 QC, QA and Accreditation in ecotoxicology and environmental monitoring: an example using the EROD assay for cytochrome P4501A activity** K. MacNeish, C.D. Robinson, Marine Scotland Science; C. Askem, Cefas; M. McKenzie, M. Gubbins, Marine Scotland Science. The UK has long been committed to monitoring and assessing the quality of the marine environment, and has a vision for its seas to be Clean, Safe, Healthy, Biologically Diverse, and Productive. Along with other countries, the UK reports monitoring data to international bodies such as the International Council for the Exploration of the Sea (ICES) and the OSPAR Convention for the Protection of the NE Atlantic. In future, monitoring data for the Marine Strategy Framework Directive (MSFD) will be reported to the EU. Under OSPAR and the MSFD, status assessments are conducted at the regional sea scale which requires data comparability between countries. For chemical monitoring, comparability is ensured through the use of standard methods, proficiency testing, ISO17025 accreditation and data quality filters on national and international databases. Similar approaches to quality assurance can also be applied to ecotoxicological monitoring, as we demonstrate using the EROD assay. The EROD (ethoxyresorufin O-deethylase) assay is a fluorometric determination of cytochrome P450 1A enzyme activity, commonly measured in fish liver as an indicator of exposure to planar organic compounds such as polycyclic aromatic hydrocarbons. Marine Scotland Science has held ISO17025 accreditation for the assay since 2000. Accreditation requires that the method is well described, the laboratory demonstrates good repeatability (short and long term), good accuracy, and participation in interlaboratory comparison exercises. The ICES Working Group on Biological Effects of Contaminants (WGBEC) has produced a detailed method protocol, from which controlled document laboratory protocols have been derived. Laboratory Reference Materials (LRMs) were produced by homogenising and aliquoting flatfish livers in which CYP1A activity was induced, either artificially (by injection) or came from animals exposed in the field. An LRM is analysed daily to produce quality control charts and demonstrate accuracy; inter-laboratory ring trials have been conducted that have helped to improve standardisation of the methodology. Key steps in ensuring comparability of the assay include ensuring the quality of the light-sensitive resorufin calibration standard and consistency in the choice of protein assay and standards. This poster describes how we have satisfied the requirements of accreditation and presents data validating the methodology.

**TU020 Accumulation of chemicals in caged molluscs (*Unio pictorum*) and evaluation of their effects using a multilevel biomarkers approach** A.G. Viarengo, Università del Piemonte Orientale / Department of Science and Technological Innovation; M. Boeri, D. Governa, S. Olivieri, University of Piemonte Orientale; A. Oldani, F. Vago, R. Borrelli, S. Chiaberge, T. Fiorani, P. Cesti, eni S.p.A-Direzione Ricerca ed Innovazione; L. Zaninetta, Syndial; S. Sforzini, University of Piemonte Orientale. An increasing number of contaminants of anthropogenic origin is introduced in the aquatic environment, causing, in some case, an alteration of the biodiversity. Freshwater mussels, including *Unio sp.*, are widely used as sentinel organisms for evaluating the quality of lotic and lentic waters. In this study, molluscs *Unio pictorum* were caged for 28 days in 4 sites of a lentic water body. The organisms were used to determine the bioaccumulation and the consequent biological effects of organic and inorganic contaminants (such as PAHs, PCBs, DDT, Hg, Cu, Zn, etc.) present in the waters (and in the surface sediments). A battery of biomarkers evaluated in different cells and tissues (i.e. gills, digestive

gland and haemocytes) has been recently developed on *U. pictorum*. The set of physiological parameters used in this study includes both biomarkers of stress (health status indicators and oxidative stress), and biomarkers of exposure to a specific class of pollutants (such as heavy metals and pesticides). No effect was found on the survival of the mussels from test sites after 28 days. However, stress biomarkers showed that there were harmful sublethal effects in mussels from contaminated sites. Specifically, there was a significant reduction of lysosomal membrane stability in hemocytes, while, in some sites (2, 3), there was also an increase in lysosomal content of lipofuscin and neutral lipid (indicating oxidative stress and perturbation of lipid metabolism). No oxidative injury effects (MDA and protein carbonyls) were found in the digestive gland indicating different effects of the chemicals in the diverse tissues. Impairment of lysosomal function was also evident from the increase in lysosomal/cytoplasmic ratio in the digestive gland of animals from the contaminated sites. Biomarkers of exposure did not show any significant changes. Overall, when the results were integrated using a previously developed expert system, they indicated that the bioavailable contaminants at the test sites provoked a moderate stress syndrome featuring sublethal perturbations at cellular and tissue levels, reaching a higher value at the most polluted site (site 2).

**TU021 Does seawater filtration affect metals concentrations in toxicity tests? Or: To filter or not to filter, that is the question.**

**Anastasi**, CQUniversity Australia; S. Wilson, CQ University. In standard toxicity tests, filtered seawater or an equivalent filtered synthetic seawater is usually the diluent of choice. However, when testing the toxicity of metals, it is seeming now that the rate of oxidation of the metal in question may also need to be taken into consideration when choosing an appropriate diluent. Laboratory based manganese oxidation trials have shown that Mn(II) oxidized at a much higher rate in whole unfiltered seawater than it did in seawater filtered to  $< 5\mu\text{m}$ . It is apparent that this higher rate of oxidation in unfiltered seawater may be due to the metal's affinity for binding with particulate matter in the water. The use of filtered seawater in toxicity tests may be of particular benefit in longer term chronic tests where it is preferable for the metal concentration to remain static for longer periods of time, thus potentially reducing the number of water changes required in static systems. The use of filtered water also reduces the likelihood of the metal in question forming complexes with particulates in the seawater. Seawater intended for use in toxicity testing may be either filtered or unfiltered, with filtered seawater generally being the diluent of preference. However, if the aim of the testing is to gain results that are of particular environmental relevance, then the use of unfiltered seawater may well be warranted, particularly in metals toxicity testing. Oxidation rates of manganese (II) in filtered and whole unfiltered seawater will be discussed, in relation to the applicability of the use of filtered and unfiltered seawaters in toxicity testing.

**TU022 Expanded polystyrene buoys as a source of hexabromocyclodecanes in the marine environment of South Korea**

**S. Hong**, Korea Institute of Ocean Science and Technology / Oil and POPs research group; N. Al-Odaini, G. Han, W. Shim, M. Rani, M. Jang, N. Heo, Y. Song, Korea Institute of Ocean Science and Technology.

**TU023 Toxicity of two marine algal toxins to blue mussel and brine shrimp larvae** **M. De Rijcke**, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; M.B. Vandegehuchte, Ghent University / Applied Ecology & Environment Bio; J. Vanden Bussche, L. Vanhaecke, Ghent University / Laboratory of Chemical Analysis; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology. To meet the present and future global protein demand, aquaculture production is growing rapidly. Simultaneously the occurrence of harmful algal blooms (HABs) is steadily increasing in frequency, intensity and geographical scale because of overfishing, pollution, climate change and the introduction of alien species. Over the past decades, numerous reports of disease outbreaks following the consumption of cultured organisms which were

contaminated with toxins have clearly underlined the co-occurrence of HABs and the aquaculture industry. As the aquaculture sector continues to expand, it increasingly requires the availability of high quality larvae for fish feed or shellfish production. However, to date, knowledge concerning the possible effects of HAB derived toxins on larviculture is limited. This research therefore aims to investigate the effects of two well-known phycotoxins, i.e. domoic acid and okadaic acid, on larvae of two key aquaculture species: the blue mussel *Mytilus edulis* and the brine shrimp *Artemia franciscana*. Larvae were exposed to concentration series of either toxin as well as bloom concentrations of their respective producers *Pseudo-nitzschia multiseries* and *Prorocentrum lima*. Mortality of *A. franciscana* nauplii and larval development of *M. edulis* larvae were carefully monitored after 48 hours of exposure. In addition, effects on the innate immune response of both species were assessed by measuring the phenoloxidase activity. Our results show that the currently reported natural levels of these marine toxins do not detrimentally influence the larvae of either aquaculture species. Concentrations of live harmful algae exceeding reported bloom densities were not found to affect the development of *M. edulis* either. However, even at the lowest applied concentration of  $50 \text{ cells.ml}^{-1}$  of the dinoflagellate *Prorocentrum lima*, nauplii of the brine shrimp *A. franciscana* exhibited  $85.6 \pm 5.5\%$  mortality after 48 hours of exposure. Additionally, the phenoloxidase innate immune response of *A. franciscana* nauplii appeared to be affected by domoic acid. This last observation may imply that the nauplii are more susceptible to bacterial infections which have typically plagued the aquaculture industry for decades. Using *Vibrio campbellii* as a model species this last hypothesis was tested.

**TU024 Towards new assessment criteria for monitoring of priority pollutants in the marine ecosystem - research project "MERIT-MSFD"**

**U. Kammann**, Thünen Institute / Institute of Fisheries Ecology; N. Keddig, Institute of Fisheries Ecology; N. Michel, Thünen Institute / Institute of Fisheries Ecology; S. Schubert, Institute of Fisheries Ecology; N. Theobald, German Federal Maritime and Hydrographic Agency (BSH); W. Wosniok, University of Bremen / Institute of Statistics; M. Haarich, Thünen Institute / Institute of Fisheries Ecology. The research project MERIT-MSFD "Methods for detection and assessment of risks for the marine ecosystem due to toxic contaminants in relation to implementation of the European Marine Strategy Framework Directive" (EU MSFD) aims for the experimental and mathematical investigation of science-based threshold levels for the assessment of pollutant concentrations in the marine environment. These threshold levels are of special importance when monitoring results, e.g. the concentration of a priority pollutant in fish or water samples, shall be evaluated in the light of the requirements of the EU MSFD and/or international guidelines e.g. from OSPAR and HELCOM. Currently the determination and application of threshold levels such as "environmental assessment criteria" is under vivid international discussion. The methodic and experimental setup for the project comprises two test systems: Microinjection into zebrafish embryos and toxicity-directed bacterial detection after chromatographic separation on one hand and a mathematical part for experimental planning and logistic dose-response calculation the other hand. The combination of experimental and mathematical approach is the starting point for the development of a general concept for new threshold levels in marine monitoring. The methodological concept and first results are presented.

**TU025 Gene expression responses of the oceanic ubiquitous cyanobacterium Prochlorococcus due to exposure to organic pollutants**

**M. Fernández-Pinos**, M. Casado, Environmental Chemistry; B. Pina, J. Dachs, IDAEACSC / Environmental Chemistry. Semivolatile persistent organic pollutants can be introduced to oceans by atmospheric transport and deposition, with the subsequent bioconcentration in phytoplankton. Previous studies proved that organic pollutants found in seawater affect abundance and growth rate of phytoplankton, suggesting that mixtures of contaminants may influence ecosystems functions, like oceanic carbon fixation or productivity. Phytoplankton sensitivity to these compounds depends on cell size,

being smaller organisms more sensitive. *Prochlorococcus* is the the smallest known photosynthetic organism, its tiny size and its spherical shape provide a high surface to volume ratio, making it very sensitive to contaminants. This genus numerically dominates the photosynthetic community in the tropical and subtropical regions of the world's oceans, so its contribution to primary production is significant. During the Malaspina circumnavigation cruise from December 2010 to July 2011, an ambitious project was initiated to study the impact of organic pollutants to phytoplankton. Oceanic samples were collected to study the presence of some families of organic pollutants in both seawater and plankton. Concurrently, samples to assess the gene expression of *Prochlorococcus* were collected at three depths along its vertical distribution. In addition, experiments were performed adding organic pollutants mixtures to natural phytoplanktonic populations in the Indian, Pacific and Atlantic oceans. This study is part of a large project to evaluate the implications of organic pollutants in the oceanic ecosystem functions, especially on primary production. The goal here is to quantify the expression of genes responsible of photosynthesis in natural populations and to study their connection with several environmental variables including organic pollutant concentrations. For this purpose *rbcL* (RuBisCO), *psbA* (D1 protein) and *rnpB* (reference gene) were assessed by qRT-PCR technique. Preliminary results show a different pattern for the two main clades of *Prochlorococcus* relative to *rbcL* expression. High-light *rbcL* expression seems to be maximal at surface samples, whereas its low-light counterpart was maximal at DCM+40. This is consistent with the expected physiology of both groups. Whereas, there is no pattern observed for *psbA* mRNA, suggesting that the effect may be specific for the CO<sub>2</sub>-fixing complex. Key words: organic pollutants, *Prochlorococcus*,<sup>2</sup>oceanic primary productivity.

**TU026 The combined effects of DOC and salinity on the accumulation and toxicity of copper in mussel larvae**<sup>D. Deruyter</sup>, University Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; M.B. Vandegehuchte, Ghent University / Applied Ecology & Environment Bio; J. Vergucht, E. Vergucht, Ghent University / X-ray Microspectroscopy and Imaging; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology; K. De Schamphelaere, Ghent University UGent / Environmental Toxicology and Aquatic Ecology; L. Vincze, Ghent University / X-ray Microspectroscopy and Imaging; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology. The biotic ligand model (BLM) is widely used to predict the toxicity of metals. There are two main principles underlying this model: 1) the concentration at the biotic ligand (BL) is directly related to the adverse effects and 2) the concentration at the BL depends on the dissolved metal concentration and the water chemistry. The latter determines the complexation of the metals with dissolved organic carbon (DOC), anions or other ligands and the competition of the metals with cations at the BL. However, recent studies indicate that in marine or estuarine environments, a changing salinity (ion concentration) can also affect metal toxicity by influencing the physiology of organisms. The goal of the present study was 1) assess the combined effects of DOC and salinity on the accumulation and toxicity of Cu in mussel larvae and 2) evaluate if these results are in agreement with the BLM principles. Mussel embryos were exposed to 9 different DOC/salinity combinations. For each combination a Cu concentration response curve was constructed and an EC50 for normal development was calculated. The internal Cu concentration and distribution in a subset of larvae from each DOC/salinity treatment was determined by synchrotron radiation X-ray fluorescence spectroscopy. Cu in the larvae was homogeneously distributed. Both DOC concentration and salinity had a significant effect on the toxicity of Cu. The toxicity and accumulation of Cu decreased significantly when DOC concentrations increased. Salinity had a non linear effect on the Cu EC50 with a maximum EC50 at 28psu, while Cu accumulation increased with increasing salinity. This indicates that salinity does not only influence the water chemistry, but may also influence the physiology of the larvae. When relating the internal Cu concentration directly to the toxicity, no significant influence of DOC concentration nor salinity was detected. This result indicates that the

critical body burden (CBB) concept might be a useful basis to predict copper toxicity, i.e. that the whole larval body could be considered as the BL. Concentration response analysis resulted in a CBB EC50 of 10.5 µg Cu/g larvae. In conclusion, a larva can be regarded as a BL, in which the Cu concentration is directly related to toxic effects. In agreement with the BLM principles DOC reduces the accumulation and the adverse effect. However salinity influences both accumulation and toxic effects in a way that is not accounted for in classical BLM type models.

**TU027 Different sensitivity of benthic microalgae to chlorine using variable chlorophyll fluorescence** <sup>M. vannoni</sup>, V. Creach, D. Sheahan, Cefas. Chlorination is one of the most widely used antifouling methods. In marine and estuarine system chlorine reacts with bromine in seawater to form hypobromous acid and hypobromite. The chlorine residuals in seawater that give rise to toxicity are termed Total Residual Oxidants (TRO). Further reactions could also lead to the formation of chlorination by-products (CBPs), that may be more persistent and toxic than the initial chlorine produced TRO. This study evaluated the toxicity of chlorine produced TRO and CBPs on three different species of benthic diatoms (*Achnanthes* spp., *Amphora* spp. and *Navicula pelliculosa*). A flow-through system was used to maintain the TRO concentration constant. To evaluate the growth inhibition rates over a 72 hours period, algae were immobilized in alginate beads and exposed to different nominal TRO concentrations (0, 0.03, 0.06, 0.12, 0.35 and 0.8 mg/ as Cl<sub>2</sub> l). Growth rates and physiological condition of the microalgae were evaluated using a Fast Repetition Rate fluorometer (FRRf), which allowed both the measurement of variable chlorophyll *a* parameters (ETRmax and Fv/Fm) and chlorophyll *a* content. Measurements were taken after 1 and 5 hours to evaluate short term effects on the photosystem II efficiency as well as after 24 hours and 72 hours for inhibition of growth. Results showed different sensitivity of the three tested algae to the combined effect of TRO and CBPs. *Achnanthes* was the most sensitive species while *Navicula* the most resistant. All the species tested bleached after 72 hours when exposed to the highest TRO concentrations. ETRmax and Fv/Fm did not show significant differences between treatments after the first 24 hours. At the highest TRO concentration, no chlorophyll *a* parameters were detected after 72 hours because of bleaching. ETRmax and Fv/Fm are used to estimate the photosynthetic efficiency of plants, seagrasses and microalgae. Our study showed a low impact of sodium hypochlorite and CBPs on the photosystem II *but a significant effect on the growth*. Algal immobilization in alginate beads could lead to underestimation of this effect and thus, EC<sub>10</sub> and EC<sub>50</sub> and this is being further assessed, but at the same time it is a good method to evaluate the sensitivity of different species to chemicals with low stability in seawater. Further studies are necessary to assess the impact of chlorination on different taxonomic groups and the significance at the community level.

**TU028 Toxicity-directed identification and assessment of hazardous substances in the marine environment- MERIT-MSFD project**<sup>Michel</sup>, U. Kammann, Thünen Institute of Fisheries Ecology; N. Theobald, W. Gerwinski, Federal Maritime and Hydrographic Agency; M. Haarich, Thünen Institute of Fisheries Ecology. <sup>Michel</sup>, <sup>Natascha</sup>, <sup>Thünen Institute of Fisheries Ecology, Hamburg, Germany</sup> <sup>Kammann</sup>, <sup>Ulrike</sup>, <sup>Thünen Institute of Fisheries Ecology, Hamburg, Germany</sup> <sup>Theobald</sup>, <sup>Norbert</sup>, <sup>Federal Maritime and Hydrographic Agency, Hamburg, Germany</sup> <sup>Gerwinski</sup>, <sup>Wolfgang</sup>, <sup>Federal Maritime and Hydrographic Agency, Hamburg, Germany</sup> <sup>Haarich</sup>, <sup>Michael</sup>, <sup>Thünen Institute of Fisheries Ecology, Hamburg, Germany</sup> **Keywords:** Thin Layer Chromatography, *Vibrio fischeri*, Toxicity testing, EU MSFD According to the *European Marine Strategy Framework Directive (EU MSFD)* a "good environmental status" based on 11 descriptors has to be achieved until 2020. Descriptor 8 requires that "Concentrations of contaminants are at levels not giving rise to pollution effects". As a consequence assessment criteria concerning the effects of hazardous substances have to be developed, which is one of the main tasks within the MERIT-MSFD Project (Methods for detection and assessment of risks for the marine ecosystem due to toxic contaminants in relation to implementation of the European Marine Strategy Framework Directive).

Within this project the method of combining Thin Layer Chromatography (TLC) and detection of the bioluminescence inhibition of the marine bacteria *Vibrio fischeri* is used as it provides an excellent tool for detecting and assessing toxic substances in the marine environment meaning that toxic substances are directly linked to their effects on a marine organism. After a sample (e.g. from water or biota) is being separated by TLC, the TLC-plate is dipped into a suspension of *Vibrio fischeri*. Afterwards the inhibition of bioluminescence can be detected using a CCD camera. Zones showing inhibiting effects can be eluted and the toxic compounds can be identified using HPLC-MS/MS or GC-MS. Effect concentrations (EC-values) in terms of dose-response-relationships can be determined by applying defined concentrations of substances on the TLC-plate followed by quantitative evaluation of bioluminescence inhibition corresponding to a certain substance concentration, respectively. [natascha.michel@vti.bund.de](mailto:natascha.michel@vti.bund.de)

**TU030 Possible pollutant-induced changes in freshwater infochemical or allelochemical interactions** E.M. Gross, University of Lorraine / Laboratoire interdisciplinaire des environnements continentaux (LIEC), CNRS UMR 7360. Biotic interactions are more affected by infochemicals or allelochemicals than generally appreciated. This is not only the case in terrestrial habitats, but also in freshwater and marine systems. Nevertheless, the types of habitats, the physico-chemical properties of the environment (air, soil, water), and the organisms involved in competitive or trophic interactions vary among these systems. Freshwater habitats are in a sense unique as they include habitats along a gradient from almost dry to completely wet, and are inhabited by relevant classes of organisms found also in marine or terrestrial habitats. Open water communities represent trophic links between plankton and fish, while benthic communities may be dominated by higher aquatic plants, which are all secondarily aquatic, i.e. have terrestrial ancestors), and are often grazed by secondarily aquatic herbivorous insects such as larvae and/or adults of Lepidoptera and Coleoptera. Allelochemically or infochemically driven interactions in freshwater systems have been described for plant-plant, plant-herbivore and predator-prey interactions. Given that freshwater habitats of any size and shape are important ecosystem components, we should be aware of their proper functioning to warrant future advantages from the ecological functions and services they provide. This conceptual paper will highlight the importance of infochemicals and allelochemicals in benthic and pelagic freshwater systems. Pollutants are very likely to interfere with the activity of info- or allelochemicals in freshwater systems. So far, we have only scattered evidence for the role of different pollutants (fragrances, pesticides, heavy metals) on info- or allelochemically mediated interactions in lakes, rivers or wetlands. We not only need to search for pollutants that mimic or modify infochemicals, but we should also be aware that pollutants may change quantitatively and qualitatively the production and mode of action of allelochemicals. A concerted action of ecologists, chemical ecologists and ecotoxicologists would be needed to tackle the complex processes involved in the interference of pollutants with “normal” info- or allelochemical interactions. Even if we consider general ecotoxicological (endpoint) studies highly relevant in finding priority pollutants, we should be aware of the strong impact at the sublethal level of many pollutants, and that such effects may well disturb community interactions and ecosystem processes.

**TU031 When are Communication Problems fatal? – Relevance of the Infochemical Effect for Aquatic Ecosystems** U. Klaschka, University of Applied Sciences. Most organisms live in an “odor environment” and recognize their biotic and abiotic environment via sophisticated, specific and dynamic blends of odorants, called *infochemicals*. The complexity and sophistication of the chemical communication systems are difficult to imagine from a human perspective. The *Infochemical Effect* describes that anthropogenic substances can influence organisms so that they perceive their chemical environments differently. Anthropogenic infochemicals could interfere at several sites in the reaction cascade of the chemical communication. Communication problems are not fatal in cases where environmental

organisms can cope with disturbances of their chemical communication. They might be able to actively avoid the anthropogenic infochemicals or use physiological stress compensation mechanisms. Furthermore, it is possible that organisms could learn to interpret a changed composition of scents and filter the specific signals. Infochemicals play a role in life history, habitat search, food related aspects and survival which shows that disturbed communication could affect population vulnerability at various decisive points. Various examples from the literature will be compiled in the presentation to illustrate these effects. Disturbed communication could be an additional stressor on top of these which has not been considered so far and which could be fatal for species who suffer already from various stressors in their natural environments. The description of natural infochemicals in the aquatic ecosystem is far from being completely elucidated. Therefore, it is presently not possible to evaluate the implications of anthropogenic chemicals in this subtle communication system. The potential fatal effects described here are so relevant for the vulnerability of populations that it can be considered as imperative to have a closer look at the infochemical effect. A systematic analysis is needed to answer the question whether and to what extent anthropogenic chemicals interfere with the chemical communication in natural ecosystems.

**TU032 Infochemical effects of repellents (PT 19 biocides) in surface waters: suitable test substances for proof of concept** M. Nendza, Analytisches Laboratorium; U. Klaschka, University of Applied Sciences; R. Berghahn, Federal Environmental Agency / Field Station Marienfelde. The experimental testing of the infochemical effect is a challenge. This project strives to test the influence of anthropogenic substances on chemical communication in aquatic systems, an effect that has mainly been described theoretically so far. For an experimental systematic analysis it is crucial to select promising test substances. This first step is described here. Repellents (PT 19 biocides) and odorants may affect the behaviour of aquatic populations and communities. The mostly polar and stable compounds could disturb the chemical communication between organisms and cause organismic effects like drift (downstream dislocation of e.g. crustacean and insect larvae in streams). Most emissions of repellents occur indirectly via waste waters and sewage treatment plants or directly in bathing water (washing off skin and clothes). In this literature study (project part 1) suitable chemicals and endpoints are selected for confirmatory assessments by laboratory tests on infochemical effects in project part 2 (which is currently in progress). The use pattern and physico-chemical properties of the substances, in combination with limited biological degradability, indicate potential aquatic relevance with possible chronic impacts. After due consideration of advantages and limitations, three PT 19 repellents have been considered suitable test compounds for proof of concept in the experimental project part 2: DEET (CAS 134-62-3), Icaridin (CAS 119515-38-7) and EBAAP (CAS 52304-36-6). An alternative option is isophorone (CAS 78-59-1), a natural attractant and an anthropogenic HPV-chemical. The candidate chemicals are being tested in project part 2 in established behavioural assays addressing vertical migration of daphnids, aggregation of aquatic pulmonates and organismic drift of insect larvae in artificial streams. The experimental results will contribute to answer the relevant questions about infochemical effects of PT 19 biocides and other odorants in aquatic ecosystems. *Acknowledgement* – This study was financed by the German Federal Environment Agency (FKZ 3712 67 417.1).

**TU033 ?Influence of nano-sized TiO2 materials on the fate and bioavailability of triclocarban in terrestrial systems** A.J. Schneider, RWTH Aachen University / Institute for Environmental Research; R. Conrad, RWTH Aachen University / Dpt of Ecosystem Analysis; M. Gauer, RWTH Aachen University / Institute for Environmental Research; A. Meister-Werner, R. Petto, IBACON GmbH; A. Dybowska, Natural History Museum London / Mineralogy; S. Strekopytov, J. Najorka, B. Smith, Natural History Museum London; M. Heggen, Forschungszentrum Jülich GmbH / Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons; H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V);

A. Schaeffer, RWTH Aachen University / Chair of Environmental Biology and Chemodynamics; H.M. Maes, RWTH Aachen / Institute for Environmental Research. We investigated the influence of different sized TiO<sub>2</sub> materials (7-200 nm) on the fate and bioavailability of triclocarban (TCC) in terrestrial systems. Therefore, we performed leaching experiments with radiolabelled TCC (<sup>14</sup>C-TCC), acute and chronic toxicity tests with *Eisenia fetida* and bioaccumulation studies using <sup>14</sup>C-TCC. In leaching experiments (OECD 312), the upper segment of soil columns (~ 1 cm) was spiked with 2 mg <sup>14</sup>C-TCC/kg and with or without nano-TiO<sub>2</sub>. <sup>14</sup>C-TCC was quantified by scintillation counting (LSC) in combusted samples from different segments of the soil. In acute and chronic toxicity tests the TiO<sub>2</sub> materials (0.4 and 1 g/kg) and TCC (0.04-0.68 g/kg) were tested individually as well as in mixtures of TCC and one of the TiO<sub>2</sub> materials (TCC 0.04-0.68 g/kg together with either 0.4 or 1 g TiO<sub>2</sub>/kg; OECD 207 and 222). In the bioaccumulation experiments <sup>14</sup>C-TCC (2 mg/kg) was applied to the system by either spiking <sup>14</sup>C-TCC to the soil or to the food, additionally either 0 or 1 g nano-TiO<sub>2</sub>/kg was spiked to the soil. <sup>14</sup>C-TCC was determined in soil and worm samples after 8 d with LSC after biological combustion. Addition of nano-TiO<sub>2</sub> to the soil reduced the mobility of TCC about half, keeping it in the upper soil layer where soil organisms dwell. Results of acute toxicity tests showed that the mixture of TCC and the smallest sized TiO<sub>2</sub> material led to mortality (40%) comparable with exposure to TCC alone (33%), whereas the two larger TiO<sub>2</sub> particles seem to hinder mortality (7%; at 675 mg TCC/kg). However, bioaccumulation factors determined in the bioaccumulation study did not differ between each other indicating no influence of the nano-TiO<sub>2</sub> material on the bioavailability of TCC for *E. fetida*. Although coexposure of TCC with lower TiO<sub>2</sub> concentrations of the larger material caused statistically significant lower chronic toxicity (EC50 n.d.), coexposure with higher TiO<sub>2</sub> material concentrations induced higher chronic TCC toxicity (EC50 693 mg/kg) than exposure to TCC alone (EC50 956 mg/kg). Results for the chronic toxicity tests with the other TiO<sub>2</sub> materials will be discussed in the presentation. In conclusion, toxicity tests showed that the toxicity of TCC was influenced by the TiO<sub>2</sub> materials in a size-, mechanism- and concentration-dependent manner. Furthermore, our study indicates the importance of testing combinations of organic contaminants and nanomaterials as well as testing of different sizes and concentrations of one and the same material.

**TU034 Aquatic exposure models for engineered nanomaterials. Role of heteroaggregation.** J. Quik, RIVM / Aquatic Ecology and Water Quality Management Group; J. de Klein, Wageningen University / Aquatic Ecology and Water Quality Management Group; A.A. Koelmans, Wageningen University / Environment. Recent literature identified several nano-specific fate processes affecting their transformation and transport in the aquatic environment. The main removal processes are sedimentation and transformation processes such as changes in coating and heteroaggregation with natural colloids. This interaction with natural colloids (NCs) is assumed to dominate the fate of nanomaterials in aquatic systems. Here we studied heteroaggregation in concurrence with homoaggregation as precursor to sedimentation of ENMs. This is done using a Smoluchowski-Stokes (SS) mechanistical model, for two reasons. First to evaluate a pseudo first order model describing ENM sedimentation in natural waters in order to understand the conditions under which such a simplified modelling approach is valid. Secondly, we use the mechanistical model to test the experimental method for quantifying heteroaggregation through estimation of the attachment efficiency. In combination with previously reported experimentally obtained attachment efficiencies this yields ranges of attachment efficiencies for different water and ENM types. First a scenario without spatially explicit variation is investigated. Second, a spatially explicit scenario for the small river Dommel (NL) is studied, which includes waste water treatment plants, sediment catches and weirs. These system model simulations will be discussed, accounting for different regimes of heteroaggregation and sedimentation. Simulations show that initial ENM concentration is most important for the influence of homoaggregation on sedimentation. Low initial ENM concentrations

result in marginal sedimentation of ENMs. In case of a low initial particle concentration heteroaggregation is shown to have a large effect on the sedimentation. For the circumstances where heteroaggregation plays a large role in sedimentation of ENMs from the water phase, the empirical pseudo first order model fits well to the calculated ENM concentration data. This shows that a simplified modelling solution can be used for practical exposure modelling of ENMs. The system model simulations using Duflo show the large effect resuspension of sediment has on the removal of ENMs from the water phase combined with subsequent sedimentation.

**TU035 Bioaccumulation, subcellular localization and cell and tissue alterations in mussels exposed to different Ag forms (nanoparticles, bulk and aqueous)** A. Jimeno, University of the Basque country UPVEHU; D. Berhanu, E. Valsami-Jones, Natural History Museum; D. Gilliland, Joint Research Centre; M. Cajarville, University of the Basque country (UPV/EHU); A. Warley, King's College London / Centre for Ultrastructural Imaging; I. Marigomez, M. Soto, University of the Basque country (UPV/EHU). Mussels *Mytilus galloprovincialis* have been used in the present work to assess the effects of Ag nanoparticles (NPs) in comparison to bulk Ag and aqueous Ag. Mussels were exposed during 21 d to 0.75 µg/L nominal concentrations in the form of Ag NPs, bulk Ag and aqueous Ag. A net Ag accumulation in soft tissues (ICP-MS), was observed for the three Ag forms after 1 d of exposure compared to control; after 21d Ag accumulation decreased but still was significantly higher than in controls. Intralysosomal metal accumulation, measured as volume density of black silver deposits (V<sub>v</sub><sup>BSD</sup>) in sections of the digestive gland, was dependent on the exposure concentration for the three silver forms but it was higher after exposure to AgNP. TEM observations and EDX spectra revealed that Ag was mainly localized within residual bodies and lysosomes of digestive cells. Exposure to AgNPs provoked a stress response reflected as a significant alteration in the lysosomal membrane stability (reduced labilization period); and as a significant increase in the relative proportion of basophilic cells in the digestive tubule epithelium. These responses were not so evident for exposures to bulk forms, suggesting that NPs exerted a more marked toxicity and at shorter times of exposure than the rest of the silver forms. Acknowledgements: TEM and EDX were carried out at the Centre for Ultrastructural Imaging, King's College London. Work funded by the EU (FP7/2007-2013, grant agreement nCP-FP 214478-2), the Basque Government (GIC07/26-IT-393-07) and the Univ. Basque Country -UPV/EHU- (UFI 11/37). A.J-R is recipient of a pre-doctoral fellowship from the UPV/EHU.

**TU036 Influence of carbon nanotubes (CNT) on the bioavailability of 17β-ethinylestradiol (EE2) for zebrafish (*Danio rerio*)** S. Rhiem, Institute for Environmental Research Biology V / Institute for Environmental Research; L. Prodoehl, RWTH Aachen University / Institute for Environmental Research; B.M. Liebeck, C. Popescu, DWI at RWTH Aachen e.V / Interactive Materials Research; A. Schaeffer, H.M. Maes, RWTH Aachen University / Institute for Environmental Research. The aim of this work was to identify the fate of 17β-ethinylestradiol (EE2) in the presence of carbon nanotubes (CNT). EE2 tends to accumulate in biota and to sorb to organic material like CNT. Since, CNT material itself showed no toxicity to zebrafish (*Danio rerio*), a combination of both substances might result in differed bioavailability and effect of EE2 to fish. At first, interactions between CNT and EE2 were investigated. After incubating vessels containing different amounts of dispersed CNT and EE2 material for different time periods, samples were taken and transferred to a thermo gravimetric analyses (TGA) device. Furthermore, vessels containing <sup>14</sup>C-labelled EE2 and dispersed CNT material were filtered (glass filter paper) to separate freely dissolved EE2 from the fraction bound to CNT. Subsequently, the radioactivity on the filter and in the filtrate was determined. To analyze, the bioavailability of EE2 for zebrafish, in a second stage, fish experiments according to OECD 305 were performed. Individual male fish were either exposed to 1 mg CNT/L and 1 µg <sup>14</sup>C-EE2/L or <sup>14</sup>C-EE2 only for different time periods up to 168 h. Fish samples were dried for 48 h (60 °C) ground in ethyl acetate and the

associated radioactivity was quantified. The third experimental stage aimed at evaluating the influence of CNT on the effects of EE2 to male zebrafish. Fish were exposed to EE2 (1 µg/L) and EE2 plus CNT (1 mg/L CNT) for 3 days. Afterwards, the fish blood was collected and the vitellogenin (egg yolk precursor protein, Vtg) content was determined by means of an enzyme-linked immunosorbent assay (ELISA). The TGA and the filter experiments showed EE2 to interact with the CNT material over time. Fish experiments outlined a difference in the bioavailability of EE2 for zebrafish between treatments containing CNT plus EE2 and EE2 only after exposing them for 24 and 48 h. The EE2 content in the fish body was significantly lower in presence of CNT, which can be explained by the interaction of EE2 and CNT in the medium. After 72 h, EE2 uptake by the fish of both setups could not be statistically distinguished. Therefore, it is possible that the presence of CNT does not only influence the EE2 uptake, but also its elimination. The Vtg experiments showed a lower blood Vtg content in zebrafish when CNT were present in the medium. To sum up, it can be concluded that CNT reduce the bioavailability of EE2 in zebrafish and the associated effects.

#### **TU037 Nanoparticle Exposure Alters Pesticide Residue**

**Accumulation by Agricultural Plants** J.C. White, R. De La Torre Roche, J. Hawthorne, C. Musante, Connecticut Agricultural Experiment Station / Department of Analytical Chemistry. Although nanomaterial use (NM) has increased dramatically, the risks posed by this emerging class of contaminants have been investigated only recently. A number of studies evaluating NM exposure to a range of receptors have been published in the last few years but there is little information on agricultural plants. Importantly, the interaction between NM and co-contaminants in agricultural systems remains unknown. Results from experiments investigating NM-organic contaminant interactions using model (vermiculite) and soil-based exposure scenarios with plant and worm species will be shown. In a study recently published, C<sub>60</sub> fullerenes (1000 mg/L) increased DDE accumulation by zucchini, soybean, and tomato grown in vermiculite by 30-65%. In addition, fullerenes were detected in over half the zucchini shoots. A follow up study of similar design was initiated where soybean and zucchini were exposed to DDE in the presence of nanoparticle (NP) or bulk Ag at 500 and 2000 mg/L or ionic Ag at 5 and 20 mg/L. Total shoot DDE levels in non-Ag exposed soybean and zucchini were 500 and 970 ng, respectively; the root DDE content was 13,700 and 20,300 ng, respectively. Ag decreased the DDE content of soybean by up to 40%, with NP exposure resulting in less contaminant uptake than bulk Ag. Bulk and NP Ag at 500 mg/L suppressed DDE uptake by zucchini by 21-29%, although Ag exposure at 2000 mg/L had no impact on contaminant uptake. Total Ag plant content ranged from 50.5-373 µg; NP-exposed plants had 1.2-2.2 times greater overall element than bulk particle treatments and also greater relative Ag transport to shoot tissues. A series of additional experiments are currently being conducted in soils contaminated with weathered DDE and chlordane and data will be presented. In a preliminary experiment, fullerene presence in the soil had no impact on weathered DDE uptake under single species exposure conditions but when crop (pumpkin) and worm (red worm) species were co-exposed, C<sub>60</sub> decreased the shoot contaminant content of the plants by 29%. Collectively, these findings show that nanomaterials may significantly alter co-contaminant bioaccumulation in agricultural systems but that realistic exposure scenarios may produce markedly different findings than that of model systems. The importance of a mechanistic understanding of the chemical interactions, as well as the implications of these findings for accurate exposure assessment and for food safety, will be discussed.

#### **TU038 Physiologically based pharmacokinetic modelling of polyethylene glycol-coated polyacrylamide nanoparticle in rat**

Q. Li, University of Michigan Ann Arbor / Department of Environmental Health Sciences; G. Johanson, Karolinska Institutet / Institute of Environmental Medicine; C. Emond, BioSimulation Consulting Inc.; U. Carlander, Karolinska Institutet; O. Jolliet, University of Michigan / School of Public Health. Though the use of engineered nanoparticles

has increased exponentially, main factors affecting nanoparticle bioavailability and biodistribution are still poorly understood. This study develops a physiologically based pharmacokinetic (PBPK) model to explore the biodistribution of intravenously injected polyethylene glycol-coated polyacrylamide nanoparticles in rats in order to improve the understanding of nanoparticle behaviour in the body. The model consists of 9 compartments (blood, liver, kidneys, lungs, heart, brain, lymphatic nodes and bone marrow, spleen, and rest of the body) interconnected via the systemic circulation and was able to represent 99.4% of the variability of measured nanoparticle concentrations across the different organs (an R<sup>2</sup> of 0.994). Each compartment includes a sub-compartment of macrophages which may phagocytise nanoparticles in a saturable process. According to both PBPK model, and supported by the experimental data, the nanoparticles are quickly captured by the macrophages, whereafter the amount of nanoparticles approaches in organs saturated at a relatively constant level in the different organs, as the macrophages become saturated. The macrophages thus serve as the major reservoir of nanoparticles in fast perfused organs (liver, kidneys, lungs, heart, brain, lymphatic nodes and bone marrow, spleen), storing 87.2% of the total nanoparticles in these organs after 120 h according to the model. This model offers an appropriate framework for modelling of biodistribution and produces good fits when adapted to other nanoparticles, yielding R<sup>2</sup> ranging from 0.863 to 0.950.

#### **TU039 Time of aging as an important factor triggering the combined toxicity of titanium dioxide nanoparticles and heavy metals**

R.R. Rosenfeldt, University of KoblenzLandau Institute for Environmental Sciences / Institute for Environmental Sciences; F. Seitz, Inst for Environmental Sciences / Institute for Environmental Sciences; K. Schmuecking, R. Schulz, University of KoblenzLandau / Institute for Environmental Sciences; M. Bundschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment. As an ingredient in many consumer products, e.g. paints and sunscreens, titanium dioxide nanoparticles (nTiO<sub>2</sub>) may enter aquatic ecosystems via various pathways. In surface water bodies nTiO<sub>2</sub> probably co-occur with other stressors, such as heavy metals. Their interaction may reduce heavy metal toxicity, due to the removal of free ions from the water phase by adsorption onto nTiO<sub>2</sub> surfaces. However, given the ionic strength in natural waters, nTiO<sub>2</sub> agglomerate and sediment. Meanwhile, those agglomerates – together with the adsorbed metal ions – may be actively ingested by organisms, such as *Daphnia magna*, enhancing overall toxicity. Therefore, the present study investigated shifts in copper toxicity in the presence or absence of nTiO<sub>2</sub> considering ionic strength and time of interaction prior introduction in the acute toxicity test (=aging) as variables. Thus, juvenile *D. magna* were exposed for 96 h to the combinations of 0 or 2 mg/L nTiO<sub>2</sub> with seven concentrations of copper, which were previously aged for 0, 24 and 72 h in Milli-Q water (ionic strength: I<sup>MO</sup> 70 µM) or test medium (ionic strength: I<sup>TM</sup> 210 µM). The results were interpreted relative to parallel experiments<sup>TM</sup>, which investigated the toxicity of an unaged mixture. The presence of nTiO<sub>2</sub> reduced copper toxicity in the Milli-Q water aged mixture to an extent comparable to the unaged mixture. Contrary, 72-h aging in test medium lead to a 40% enhanced copper toxicity following 48 h of exposure in presence compared to absence of nTiO<sub>2</sub>. Moreover, an aging in test medium reduced the copper concentration in the aqueous phase, regardless of nTiO<sub>2</sub>, while the copper concentration remained stable during an aging in Milli-Q water until test initiation. After 96 h test duration, the copper concentration in the aqueous phase was in all treatments reduced by 50-80%, except for copper aged in absence of nTiO<sub>2</sub> in Milli-Q water. This suggests that the test organisms finally ingest nTiO<sub>2</sub> agglomerates together with the absorbed copper ions. Hence, nanoparticle agglomerates may contribute meaningfully to the uptake and effects of other chemical stressors.

**TU040 Alkoxide-free sol-gel synthesis of Mg-Al Layered Double Hydroxide, a novel (an)ion exchange adsorbent** N. Chubar, Glasgow Caledonian University / School of Engineering and Built Environment. Inorganic ion exchangers is a class of the adsorptive



materials which are capable of lowering the maximum (permissible) contaminant level (MCL) to very low concentrations. Having the initial goal to develop a competitive anion exchanger of the complex structure (such as mixed/double (against individual) hydrous oxides) the authors developed a green alkoxide-free sol-gel synthesis of Mg-Al-CO Layered Double Hydroxide (LDH) which showed the highest ever adsorptive capacity/affinity toward arsenate and high(er) removal capacity to the several other target (for water industries) anions. This synthesis method will be shown in the presenting paper along with surface, structure and adsorptive properties characterisation of the developed material. Comparison to Mg-Al LDHs produced by the other two syntheses approaches demonstrating the advantageous of this green synthesis method will be given.

**TU041 Characterisation of functionalised and non-functionalised carbon nanotubes and their dispersion behaviour in synthetic freshwater** B. Glomstad, Norwegian University of Science and Technology; L. Sorensen, SINTEF Materials and Chemistry; M. Ramzan, Norwegian University of Science and Technology; C. Marioara, H. Johnsen, SINTEF Materials and Chemistry; B.M. Jenssen, Norwegian University of Science and Technology; A. Booth, SINTEF Materials and Chemistry. Carbon nanotubes (CNTs) represent an important group of nanoparticles (NPs) engineered for application in new and improved technologies. CNTs are unusual among NPs due to their high aspect ratio (length in  $\mu\text{m}$  scale). However, relatively little is known about their fate and behaviour in the environment, and concern for their possible toxicological effects towards humans and organisms is increasing. The hydrophobic nature of CNTs means they readily interact with organic substances (natural and anthropogenic) when dispersed in water, but are prone to aggregation and settling. However, it has been shown that surfactants, such as natural organic matter (NOM), and hydrophilic surface chemistry may aid in stabilising free dispersed CNTs in the water column. Knowledge of how the physical and chemical properties of CNTs (e.g. size, surface area, functionalisation) can impact their behaviour in freshwater (agglomeration, water column residence time, interaction with other substances) is important for understanding their environmental impact. This study investigates the behaviour of seven commercially available CNTs dispersed in synthetic freshwater containing NOM. The study includes one single-walled and six multi-walled CNTs. Three of the multi-walled CNTs are non-functionalised CNTs of different sizes, while the others are functionalised with  $-\text{NH}_2$ ,  $-\text{COOH}$  and  $-\text{OH}$  groups. Prior to experiments, dry CNTs are characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to confirm their physical properties. Media is prepared by dissolving Suwannee River NOM (20 mg/L) in moderately hard reconstituted water (MHRW). Pre-weighed CNTs are added to the media in concentrations of 1-50 mg/L and the effect of shaking and sonication as dispersion methods on the stability of the dispersions are investigated. Zeta potential for the different dispersions are measured. The size of CNT aggregates in dispersion are estimated by laser diffraction measurements (using a Malvern Mastersizer 0.02-2000  $\mu\text{m}$ ). A method for determining the concentration of freely dispersed CNTs in NOM-MHRW solutions by UV-VIS spectroscopy is established to monitor the stability of the different dispersions. This study highlights the importance of adequately characterising CNT dispersion behaviour and concentrations in aqueous environments prior to conducting relevant effects studies.

**TU042 Determination of ENMs in biological samples by microwave-assisted digestion and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)** A. Brunelli; G. Pojana, F. Visin, G. Rampazzo, A. Marcomini, University Ca' Foscari. The extensive usage of engineered nanomaterials (ENMs) in both industrial and consumer products is triggering a growing attention on the side of the potential risk posed to humans and the environment by these new materials. Although many studies are recently being performed with the aim to elucidate the behavior and effects of ENMs, only few of them are actually focusing on the quantitative uptake and biodistribution in cells from *in vitro* experiments, and in tissues from *in vivo* (eco)toxicological

studies. This work, developed within EU-FP7 (ENPRA) and national (Toxicological and environmental behaviour of nano-sized titanium dioxide) projects activities, investigated the development of analytical protocols for the quantitative determination of selected ENMs of wide consumption and concern, such as n-TiO<sub>2</sub>, n-ZnO, n-Ag and carbon nanotubes (CNTs), into biological tissues of mice, rat and mussels, i.e. in organisms used for (eco)toxicological testing. A tailored digestion protocol, depending on the different physico-chemical properties and functionalization, as well as on the considered biological matrix, has been developed and applied for each ENM type. The digestion procedure was carried out by employing microwave assisted digestion, properly adapting the acid mixture, operating time for each ENM and matrix tested, and was followed by quantitative detection of dissolved "marker" elements by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The whole procedure and the applications are described.

**TU043 Development of a method for the analysis of fullerene derivatives in water using HPLC-UV** A. Carboni; E. Emke, KWR Watercycle Research Institute; J. Parsons, University of Amsterdam; P. de Voogt, KWR Watercycle Research Institute; K. Kalbitz, University of Amsterdam. The possibility to functionalize the closed cage structure of fullerenes led to the synthesis of a class of compounds with increased solubility and enhanced properties that can be used in many fields, from industrial to household applications. In the last decade a number of functionalized fullerenes are being produced but still few studies addressed the need for specific analytical methods for the analysis of these compounds. In the present work, several fullerenes were selected as representative of this new class and a method was developed with the use of normal phase liquid chromatography and UV detection. The method allows the separation ( $R_s > 1$ ) of very similar structures and the detection of the compounds with a LoD in the range of  $\mu\text{g/L}$  in water that can be further improved with the use of mass spectrometry. The method was later applied for the analysis of water samples after the addition of all the compounds under investigation. Water samples at different humic acids contents were spiked with either fullerenes dissolved in toluene or fullerene aqueous solution in order to compare the effects of the two spiking procedures and the humic acids concentration on the recovery of fullerenes.

**TU044 Extraction and Analysis of Silver and Gold Nanoparticles From Organism Tissues Using Tetramethylammonium Hydroxide and Single Particle ICP-MS Analysis** E.P. Gray, Colorado School of Mines / Environmental Science and Engineering; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; J. Coleman, US Army Corps of Engineers; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory; C.P. Higgins, Colorado School of Mines / Civil & Environmental Engineering. The use of ENPs in consumer products will inevitably lead to environmental exposures through the disposal of wastes, creating exposure potential for aquatic and terrestrial organisms. Probabilistic flow modeling suggests that terrestrial exposures may be of higher concern due to ENP the application of biosolids to crops as fertilizer and atmospheric deposition of ENPs from incinerated sewage sludge. Most of the established analytical methodology is incapable of accurately quantifying ENP size, mass and particle number distributions in complex environmental matrices. This work applies the recently developed, and highly sensitive, analytical detection technique of spICP-MS to tissue extraction to quantitatively describe ENPs in environmental systems. The tissue extraction method is a simple alkaline digestion using the organic base, tetramethylammonium hydroxide (TMAH). Method development was performed using ground beef and has been verified in, *Lumbriculus variegatus*. ENPs investigated include 100 and 60 nm Au and Ag NPs stabilized by PVP. Spike recovery experiments showed high Au and Ag NP recovery values from all tissues tested were high, ranging from 95% to 110%. Recovery values were determined by comparison of both mass and particle number to particle standards in water. Additional spike recovery experiments proved that particle mixtures (60 and 100 nm) could be extracted, and dissolved Ag spiked into tissues did not form particles though the extraction process. ENPs

were also extracted and sized from exposed organism tissues.

#### **TU045 Fullerenes' partitioning between sediment and water:**

**Sediment is not their final sink** K. Pakarinen; J. Akkanen, University of Eastern Finland / Department of Biology; M.T. Leppanen, Finnish Environment Institute / Department of Biology; J.V. Kukkonen, University of Jyväskylä / Biological and Environmental Science.

Carbon nanoparticle fullerene-C<sub>60</sub> is widely investigated for various applications. Some applications<sup>60</sup> offer fullerenes a straight route to the aquatic environment via waste waters. In aquatic environment fullerenes form water-stable agglomerates. Water-stability predicts fullerenes to be mobile and available for aquatic organisms and enhances possibility of fullerenes to become transformed by sunlight as well. In natural conditions fullerenes are likely to interact with the other abundant particles, colloids and ions which can lead fullerenes' settling to the bottom sediments. Thus fullerenes' environmental fate is complicated and their ecological risks may reach also benthic species along with pelagic ones. Nevertheless, sediment is not a final sink for many traditional chemicals, and it cannot be assumed that for fullerenes, either. Especially surface of sediment is susceptible to mixing by water flows and bioturbation by benthic species. Thus fullerenes in sediment could become re-suspended to water and remain mobile and available for aquatic species. Interaction of colloidal fullerene agglomerates with sediment particles was investigated and fullerenes' targeting on the zone between sediment and water in three freshwater-sediment systems representing oligo-, meso-, and poly-humic lakes was assessed. Water suspended fullerenes were allowed to interact with natural freshwater sediments by slow continuous mixing which mimicked natural turbations on sediment-water interface. A part of fullerenes released from sediment back to water stable agglomerates relatively quickly. The most effective release, almost 10% of spiked fullerenes, took place from the sediment having highest amount of amorphous matter and aliphatic character. Instead, about 5 % of fullerenes' released from aromatic sediments. This indicates that sediments are not the final sink for fullerenes: although the most of the water suspended fullerenes were found in the sediment, a portion can become available to aquatic species and be susceptible for transporting or transforming.

#### **TU046 How aging affects the sorption behavior of nC60**

T. Huffer, University of Duisburg-Essen / Instrumental Analytical Chemistry; M. Kah, University of Vienna / Department of Environmental Geosciences; T. Hofmann, University of Vienna; T. Schmidt, University of Duisburg-Essen. Research on carbon-based nanomaterials (CNM) has been increasing over the past decade due to their unique physico-chemical properties. Even though there is consensus that CNM will eventually reach the environment, their environmental behavior and impact on the fate of other pollutants remains controversial. Numerous studies have shown that environmental factors may influence the surface properties and dispersion state of aqueous fullerenes (nC60). These obvious effects of aging on their properties will inevitably influence the environmental behavior of nC60. The consequences of these changes in surface chemistry on their sorption behavior are yet poorly understood. The very limited information on the sorption behavior of partially/fully dispersed nC60 systems might be explained by difficulties associated with the generally-applied batch sorption test set-ups, in which solid and liquid phases are separated by centrifugation or filtration. However, a fraction of dispersed sorbent may remain in solution and cause an underestimation of determined distribution coefficients. We thus used a passive sampling technique to accurately measure sorption coefficients applying polyoxymethylene (POM-SPE). To probe the impact of changes in surface properties of dispersed nC60 on their sorption behavior due to irradiation and presence of oxygen, five different scenarios were considered. Sorption isotherms of polycyclic aromatic hydrocarbons by nC60 were determined over a range of environmentally relevant concentrations applying POM-SPE. Both the presence of oxygen and irradiation significantly decreased the sorption affinity and capacity of nC60, while commercially available polyhydroxy fullerenes had the smallest sorption. Isotherms were best fit with the Dubinin-Ashthakov model, suggesting that conceptual pore-filling and sorption

to flat surfaces are the major sorption processes. In addition, multiple sorbate experiments with four polycyclic aromatic hydrocarbons suggested no competition for sorption sites. A strong relationship between sorption coefficients and hydrophobic properties of sorbates suggests that hydrophobic interactions are of major importance. The results emphasize that aging processes are essential to account for when evaluating the fate of nC60 in the environment. Aging processes significantly affect the properties and consequently the reactivity of nC60.

#### **TU047 How suitable are standard test protocols for the assessment of effects of nanomaterials?**

J. Kinross, HeriotWatt University / School of Life Sciences; J. Mullinger, P. Fuke, J. Curry, Heriot-Watt University; T.F. Fernandes, HeriotWatt University / School of Life Sciences. Nanomaterials are coming into widespread use in consumer products and the logical consequence of this is that they will appear in environmental compartments as the products are used and reach the end of their life and are disposed of. It is important to be able to assess the potential impact of these materials on the ecology of the receiving compartments, using standardised toxicity tests. Standard (e.g.OECD) ecotoxicity test protocols were designed for use with soluble materials, or materials that can be dispersed using solvents. Adaptation these protocols is therefore necessary to enable them to be used with materials in suspension such as nanomaterials. We have investigated modifications necessary to the standard Algal Growth Inhibition Test (OECD 201). Titanium dioxide presents a particularly extreme example of the difficulties presented by NMs when using standard protocols, because it is of low toxicity to algae. The high concentrations necessary to detect toxic effects interfere with some of the usual methods for assessing cell growth in algal cultures. We give examples and present data validating a procedure which can bypass some of these interferences via extraction of chlorophyll, and discuss limitations of the test.

#### **TU048 Impact of agglomerated single walled carbon nanotubes on**

**marine green algae *Tetraselmis suecica*** m. alshaeri, HeriotWatt University / Centre for Marine Biodiversity & Biotechnology, School of Life Sciences; M. Hartl, HeriotWatt University / Centre for Marine Biodiversity and Biotechnology, School of Life Sciences; L. Paterson, Heriot-Watt University / School of Engineering and Physical Sciences; P. Cyphus, Heriot-Watt University. Al-Shaeri, M<sup>1,2</sup>. Paterson, L.<sup>3</sup> Cyphus, P.<sup>4</sup> Hartl, M. G. J.<sup>1,2</sup> <sup>1</sup>Heriot-Watt University, Centre for Marine Biodiversity & Biotechnology, School of Life Sciences, Riccarton, Edinburgh EH14 4AS, Scotland, UK. <sup>2</sup>Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Saudi Arabia, P.O Box 80082 Jeddah 21589<sup>3</sup>**School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, Scotland, UK(n\*)** **correspondence: m.hartl@hw.ac.uk**

**<strong>Abstract** The present study investigated the impact of various nominal concentrations of agglomerated single walled carbon nanotubes (SWCNTs) on the growth rate of the marine algae *Tetraselmis suecica*. *T. suecica* was exposed in triplicate to 5µg L<sup>-1</sup>, 10µg L<sup>-1</sup>, 50µg L<sup>-1</sup>, 100µg L<sup>-1</sup> and 500µg L<sup>-1</sup> SWCNTs for 8 days. Light microscopy, Raman spectroscopy and SEM were used to confirm SWCNT-algal interaction, whilst flow cytometry was used to assess the cell viability. Growth rates were determined by flow cytometry and confirmed using an improved Neubauer haemocytometer. Raman shift peaks at 1500 cm<sup>-1</sup> as well as SEM images showed agglomerated SWCNTs adhered to the external surface of *T.suecica*. The observed growth rates at the two highest exposure concentrations were significantly lower compared to the control (P< 0.001). Our observations suggest thatthe growth rate inhibition may in part be the result of a shading effect, that was accompanied by a significant decrease (P=0.031) in the cell viability of *T. suecica* by day 7 at concentrations of 500µg L<sup>-1</sup>. The significance of this as well as the possibility that direct toxicological effects may be contributing to the observed growth inhibition, as well as the probability that SWCNT-algal interaction observed in the present experiment may facilitate the trophic transfer of SWCNTs are currently being investigated in our lab.

**TU049 Influence of Environmental Conditions on the Acute Toxicity of Ag Nanoparticles to *Daphnia magna*** A. Gibson,

Western Washington University / Environmental Science; R.M. Sofield, Western Washington University / Huxley College of Environment. Materials containing silver nanoparticle (AgNP) is on the rise and present in everyday life with the potential to be released into the environment. Although no substantial toxicity has been recorded towards humans it has been shown that silver does have toxicity to aquatic organisms. This study aims to determine acute toxicity to *Daphnia magna* (*D. magna*) using AgNO<sub>3</sub> and silver citrate nanoparticles (AgNP-Cit) under varying environmental conditions. A modified version of the ASTM E729-07 method was used for acute toxicity testing, with no chloride added to the synthetic hard water solution to avoid precipitating the silver. Characterization of the nanoparticles included digital light scattering (DLS) for size confirmation, ultraviolet-visible spectroscopy (UV-Vis) for the determination of sedimentation, and ion selective electrodes (ISE) to measure the percent of ionic Ag (Ag<sup>+</sup>). Acute exposures were conducted in the presence and absence of Suwannee River fulvic acids (FA) and in 48 hours of light or 48 hours of dark. The results of the acute tests indicate that light and FA decrease the toxicity of AgNP-Cit significantly. Exposure time and FA presence are more important factors contributing to AgNO<sub>3</sub> toxicity than light conditions. Results of this study will help define the contribution of the Ag<sup>+</sup> to the nanoparticle toxicity for acute exposures and clarify the role of potential environmental conditions on toxicity modification.

**TU050 Influence of the use of sewage sludge containing ZnO nanoparticles as soil amendments on earthworm population** Garcia Gomez,

M. Babin, INIA / Dpto. Environment; A. Obrador, J. Alvarez, ETSI Agronomos (UPM); C. del Rio, INIA / Dpto. Environment; J. Pareja, M. Fernandez, INIA. ZnO nanoparticles (ZnONPs) have widespread uses in personal care products among other industrial applications including coating and paints. Manufacturing processes, handling, use and disposal increase their appearance in environmental compartments. Nanoparticles can reach the soil through the application of sewage sludge. This agricultural practice has potential for contaminating not only soils but also waters. Little information is available on the risk of soils contaminated with nanoparticles as result of the addition of contaminated amendments. The objectives of this work were i. to assess the potential effects of sewage sludge contaminated with ZnONPs on earthworms when they are used as amendment of soils. ii. to study the influence of the physicochemical properties of sludge on earthworms toxicity by testing two different sewage sludge samples. iii. to evaluate the role of sludge on the availability of Zn comparing with the effects of a soil directly contaminated with ZnONPs. Two sewage sludge were contaminated with ZnONPs at different concentrations to get a final concentration in soil of 125 to 1000 mg kg<sup>-1</sup> (Zn based). Control soil was amended with the different sludge samples at 5% (w/w) concentration. Purged adult earthworms (*Eisenia fetida*) were placed in the amended soils for a 28 days period. In order to isolate the effects of the amendments on bioavailability of Zn and hence on earthworms toxicity a parallel assay was carried out using control soil without amendment but spiked with ZnONPs at the same concentrations. The endpoints included adult mortality, body weight growth and change in enzymatic activities such as catalase (CAT), glutathione s-transferase (GST) and superoxide dismutase (SOD). Effects on reproduction focus on fecundity (number of cocoons laid per adult), fertility (egg yield) and delay in hatch time. The bioaccumulation of Zn by earthworms under the presence of amendment at different ZnONPs concentration was also determined. Total concentration of Zn in soil and earthworms were determined by atomic absorption spectrometry. The available concentration in soils was determined using different extractants. This study was supported by the Spanish projects RTA2010-00018-00-00 and EIADES S2009/AMB1478.

**TU051 Influence of titanium dioxide nanomaterial on the mobility of copper or triclocarban in soil columns** C. Nickel, Institute of

Energy and Environmental Technology eV IUTA / Air Quality & Sustainable Nanotechnology; B. Hellack, Institute of Energy and Environmental Technology e.V. - IUTA / Air Quality & Sustainable Nanotechnology; H.M. Maes, A. Schaeffer, RWTH Aachen / Institute for Environmental Research (Biology V); T.A. Kuhlbusch, Institute of Energy and Environmental Technology e.V. - IUTA / Air Quality & Sustainable Nanotechnology. Environmental pollutants can reach soil ecosystems directly via application of e. g. agrochemicals, indirectly via sewage sludge or aeolian transport, and via wet / dry deposition. Little research is available on their mobility and movement into deeper soil layers in the presence of nanomaterials like TiO<sub>2</sub>. In this study, the possible effect of TiO<sub>2</sub> (P25) nanomaterials onto the copper sulphate (CuSO<sub>4</sub>) or <sup>14</sup>C-labelled triclocarban (TCC) transport in different soils was investigated. This is an important topic since all substances may have the same entry path into the environment, e.g. through biosolid amendment on soils. Based on OECD guideline 312 (2004), glass columns with an inner diameter of 12 cm and a height of 20 cm were filled with an 11 cm column of three different air-dried, sterilised natural soils. The pollutants and the nanomaterial were separately applied to the soil column to avoid any prior adsorption onto the P25 surface. The upper 1 cm of the soils were spiked with 750 mg CuSO<sub>4</sub> or with 200 µg TCC. Deionised water and the P25 suspension were added to the TCC reference and the test system, respectively. Afterwards a 0.01M CaCl<sub>2</sub> solution was sprayed to the top of the columns for 48h. Both pollutants showed low mobility in all soil types. Nevertheless, an expected soil type dependency of both copper and TCC transport was observed with the highest mobility in the soil with low pH and higher sand content. For P25 only a low mobility of isolated agglomerates was observed. The low mobility of the positively charged nanomaterial was most likely due to heteroaggregation with natural colloids and deposition to soil surfaces. In the presence of P25, a tendency to even lower mobilities of TCC and copper was observed for all soil types. Nevertheless, it was still possible to observe the same soil type transport dependency. We hypothesize that the tendency to a lower mobility of copper as well as TCC can be explained by the adsorption onto the surface of P25. This indicates that the nanomaterial and other pollutants may accumulate in the top soil layer, ultimately leading to higher exposure concentrations for plants and animals of that habitat. *Acknowledgement: This work was sponsored by the Federal Environment Agency of Germany (UBA).*

**TU052 Investigation of the Factors Responsible for the Ecotoxic Effects of Nano Titanium Dioxide on Aquatic Organisms** A. Nakamura,

Graduate School of Frontier Sciences; M. Yamamuro, The University of Tokyo / Graduate School of Frontier Science; S. Hirano, National Institute for Environmental Studies / Center for Environmental Risk Research; N. Tatarazako, National Institute for Environmental Studies / Environmental Risk. Manufactured nanomaterials are being utilized in various fields due to their superior characteristics, but at the same time, adverse effects on humans and wildlife have also been reported. Since the 2000s, plenty studies have been reported about the ecotoxic effects of industrial nanomaterials for aquatic organisms in terms of quantity. In recent years, the trend of this branch is shifting to more advanced approach such as combined effects with other elements or transitions and concentration in the food web. However, it essential question has not been considered sufficiently that what is the critical toxic factor. This is because existing researches did not grasp and control behavior of nanomaterials in water. It is an urgent issue to clarify the factors of impact in order to evaluate ecotoxic effects of nanomaterials for aquatic organisms property. Therefore, we investigated factors responsible for the ecotoxic effects of nanomaterials on aquatic organisms by sensitivity analysis approach using titanium dioxide that regarded chemically stable and non-toxic as a bulk material. First, dispersions were prepared that controlled their conditions in regularly. Target factors are as follows: dispersion concentration, particle size distribution, specific surface area, and particle number. Then, eggs of zebrafish were exposed to above dispersions in compliance with OECD guidelines for the testing of chemicals No.212: fish, short-term toxicity test on embryo and sac-fry stages. On the occasion of the exposure, light condition was also set intentionally.

Before and during exposure, condition of titanium dioxide and its dispersions were measured by various methods such as dynamic light scattering method, scanning electron microscope, Brunauer-Emmett-Teller Method. After the exposure, effects of each case were analyzed from endpoints such as hatchability, survival rate, and etcetera. On it, change of degrees of effects accompanying with each factor were analyzed one by one. From the above relationships between the values of each element and degrees of effects were investigated.

**TU053 Nanoparticle weathering in the presence of extracellular polymeric substances of freshwater biofilms** A. Kroll, Environmental Toxicology; R. Behra, Eawag / Department of Environmental Toxicology; L. Sigg, Eawag / Environmental Toxicology. River biofilms, or periphyton, are the main producers of biomass and O<sub>2</sub> in river ecosystems and have been shown to be a sink for nanoparticles (NP). Interactions of deposited NP with extracellular polymeric substances (EPS) of biofilms is expected to alter their properties before interaction with periphytic organisms. In this work we focus on the effects of EPS on Ag and CeO<sub>2</sub> NP as well as formation of from AgNO<sub>3</sub>. Periphyton was colonized on glass slides under controlled conditions in a flow-through system with river water (Chriesbach, CH). EPS was extracted from 3 week old periphyton and analyzed for cell lysis, protein content, DOC size distribution. Experiments were performed with EPS obtained at 5 different times of the year. AgNP (CO<sub>2</sub>-stabilized), AgNO<sub>3</sub>, and CeO<sub>2</sub> NP were diluted in 2 mM NaHCO<sub>3</sub> with 10mg DOC/L EPS and stirred in the dark or light for 2 weeks. Exposure conditions mimicked periphyton growth conditions (+/- light, varying pH). Nominal Ag and Ce concentrations were 0.5 mg/L and 5 mg/L. Sample characterization included size (DLS, NTA, EM), stability (electrophoretic mobility), Plasmon resonance (UV-VIS), dissolution (ICP-MS), chemical composition (XPS). AgNP and CeO<sub>2</sub> NP remained stable in NaHCO<sub>3</sub> at pH 6-8.6 for 2 weeks in light and dark. AgNP exposed to all 5 EPS extracts lead to pH-dependent size increase over time. AgNP size increase showed slower kinetics in one EPS (April 2012) extract displaying a different DOC size distribution and biopolymer-composition. Increase in AgNP size was due to Ag<sup>+</sup> photoreduction on existing NP. Particle formation was detected upon exposure of AgNO<sub>3</sub> in EPS under light. Kinetics of NP formation was slower in the April 2012 extract. Dissolution of AgNP did not change over time. Free Ag<sup>+</sup> decreased in AgNO<sub>3</sub> solutions corresponding to the formation of AgNP. CeO<sub>2</sub> NP remained unaffected by pH, light, and EPS. Dissolution slightly increased over time in all samples and was especially pronounced at pH6 in EPS-free samples. EPS prevented an increase in dissolution of CeO<sub>2</sub> NP in the light but not in the dark. Conclusions: (a) AgNP and CeO<sub>2</sub> NP may remain stable in the presence of EPS; AgNP may be affected by EPS in the light. (b) AgNP may continuously form from Ag<sup>+</sup> under natural conditions. (c) Depending on the time of day and year, NP properties will change leading to variable exposure of periphytic organisms.

**TU054 Silver nanoparticle bioaccumulation kinetics in the terrestrial isopod *Porcellionides pruinosus*** P.d. Tourinho, University of Aveiro / department of Biology & CESAM; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; S. Loureiro, Universidade de Aveiro / Biology. Silver nanoparticles (Ag NPs) are widely used in the nanotechnology industry and consumer products. Due to their release from Ag NP-containing products, nanoparticles may enter the soil compartment. Terrestrial isopods are considered suitable indicators for assessing the ecotoxicological risk of metals in soil due to their capacity to accumulate metals from the environment in their body (i.e., hepatopancreas). However, the processes of uptake and elimination for metal-based nanoparticles may be different. The objective of this study is to determine the accumulation kinetics for Ag NPs in the isopod *Porcellionides pruinosus* exposed to contaminated soil or food. To enable distinguishing uptake of particles or the dissolved free metal ions, similar experiments were also conducted with an ionic form of silver (AgNO<sub>3</sub>). Adult isopods (males and non-gravid females) were

exposed individually in plastic boxes containing dosed soil or food. For soil contamination, Lufa 2.2 soil was spiked with Ag NPs or AgNO<sub>3</sub> as aqueous solution and moistened to 45% of the maximum water holding capacity (WHC). To obtain dosed food, alder leaves (*Alnus glutinosa*) were soaked into Ag NPs or AgNO<sub>3</sub> solutions and shaken for four days. The leaves were left to dry for one day before being offered as food *ad libitum*. Isopods were exposed to contaminated soil or food for 21 days during the uptake phase. Then, the animals were transferred to a clean medium (soil exposure) or offered clean leaves (food exposure) during the elimination phase. At different times during the uptake and elimination phases, three isopods were sacrificed for Ag analysis. Total Ag concentrations in isopods, soil and food were measured by Atomic Absorption Spectrometer (AAS). Kinetics of Ag internal concentrations in isopods were calculated by fitting a one-compartment model simultaneously to the uptake and elimination data. Preliminary results showed that after 14 days, isopods accumulated up to 346 µg Ag/g when exposed to AgNO<sub>3</sub> via food. Bioaccumulation was observed upon soil exposures as well, but data have not been analysed yet. The present study is the first to report on the uptake and elimination kinetics of Ag NPs in a terrestrial isopod.

**TU055 Sorption behavior of carbon nanotubes: importance of dispersion and surface chemistry** X. Zhang, University of Vienna; M. Kah, University of Vienna / Department of Environmental Geosciences; T. Hofmann, University of Vienna. Carbon nanotubes (CNTs) are one type of widely produced engineered nanoparticles. CNTs have strong affinity toward organic contaminants and have been proposed as superior sorbents for remediation applications. Understanding the interactions between organic contaminants and CNTs is therefore essential for evaluating the materials' potential environmental impact as well as the potential efficiency as superior sorbent. Even though a great deal of work was carried out, data are still limited due to limitations associated with the generally-applied sorption set ups. We applied a passive sampling method to gain a better understanding of the interactions between polycyclic aromatic hydrocarbons (PAHs) and CNTs over a wide range of conditions. Sorption data and extensive characterization of the CNTs systems were combined in order to support mechanistic interpretations of the results. The main results are summarized below: (i) Conversely to previous studies carried out in the high concentration range, sorption isotherms in the low concentration range (pg-ng/L) indicate that sorption can be described using single sorption coefficients.[1] (ii) Sorption coefficients for 13 PAHs showed that no competition occurred in the low concentration range and sorption affinity was directly related to the solubility of the subcooled liquid of the compounds.[1] (iii) Conversely to previous observations restricted to large aggregates, our study highlights the importance of considering both the size and structure of sorbent aggregates.[2] (iv) To date, limited published data generally suggested that the presence of functional groups on the CNTs decrease the sorption of nonpolar compounds. We analyzed differences due to the nature of the functionalization and demonstrated that the impact on sorption behavior greatly depends on the CNT dispersion status. The suppression of sorption by natural dispersants greatly depends on the CNTs surface chemistry. Overall, this study demonstrates that aggregation/dispersion significantly affects the sorption behavior of CNTs. Both the nature (e.g., sonication, presence of dispersants or functionalization) and the chronological sequence of the dispersion events are essential in determining the extent and irreversibility of the effects on sorption behavior of CNTs. [1] Kah M, Zhang XR, Jonker MTO., Hofmann T. 2011. Environ Sci Technol 45: 6011-6017 [2] Zhang XR, Kah M, Jonker MTO., Hofmann T. 2012. Environ Sci Technol 46: 7166-7173

**TU056 Stability of citrate-capped silver nanoparticles in exposure media and their effects on the development of embryonic zebrafish (*Danio rerio*)** K. Park, College of Pharmacy; S.L. Harper, G. Tuttle, F. Sinche, Oregon State University. The stability of citrate-capped silver nanoparticles (AgNPs) and the embryonic developmental toxicity were evaluated in the fish test water. Serious aggregation of AgNPs was

observed in undiluted fish water (DM-100) in which high concentration of ionic salts exist. However, AgNPs were found to be stable for 7 days in DM-10, prepared by diluting the original fish water (DM-100) with deionized water to 10%. The normal physiology of zebrafish embryos were evaluated in DM-10 to see if DM-10 can be used as a control vehicle for the embryonic fish toxicity test. As results, DM-10 without AgNPs did not induce any significant adverse effects on embryonic development of zebrafish determined by mortality, hatching, malformations and heart rate. When embryonic toxicity of AgNPs was tested in both DM-10 and in DM-100, AgNPs showed higher toxicity in DM-10 than in DM-100. This means that the big-sized aggregates of AgNPs were low toxic compared to the nano-sized AgNPs. AgNPs induced delayed hatching, decreased heart rate, pericardial edema, and embryo death. Accumulation of AgNPs in the embryo bodies was also observed. Based on this study, citrate-capped AgNPs are not aggregated in DM-10 and it can be used as a control vehicle in the toxicity test of fish embryonic development.

**TU057 Study on the degradation of fullerenes by fungi** A. Carboni, J. Parsons, University of Amsterdam / IBED. Fullerenes are carbon-based nanoparticles considered as some of the most promising materials in emerging nanotechnology. Their current use vary from industrial to household applications and the expected increase in production is likely to mean the release of such materials into the environment. Their environmental fate and behavior are, however, still largely unknown and few studies have directly addressed the possible degradation of these compounds by biota in soil and water. Transformation by fungi could be a potentially important contribution to the degradation of fullerenes. In this work we incubated strains of fungi in the presence of fullerenes (C60 and C70) and functionalized fullerenes ([60]PCBM and [70]PCBM). Fungi cultures were grown in liquid media and then spiked with aqueous solutions of fullerenes. Each strain of fungi was exposed to only one fullerene at the time at different concentrations. Samples were collected at specific time intervals and underwent liquid-liquid extraction with toluene before being analyzed with HPLC-UV and HPLC-MS for the assessment of degradation rates and the identification of transformation products.

**TU058 The gastrointestinal uptake of Titanium Dioxide nanoparticles and associated toxicity** C. Gitrowski, University of Plymouth / School of Biomedical and Biological Sciences; A. Al-Jubory, University of Plymouth; R. Handy, University of Plymouth / School of Biomedical and Biological Sciences. The mechanisms of gastrointestinal uptake and toxicity of different TiO<sub>2</sub> nanoparticle (NP) crystal types was investigated using intestinal cell culture (Caco-2), electron microscopy, ICP-OES and pharmacological agents. Caco-2 monolayers exhibited time dependent, saturable uptake of TiO<sub>2</sub> at a concentration of 1 mg l<sup>-1</sup> over 24 h which was influenced by crystal type. Initial uptake rates of TiO<sub>2</sub> NPs ranged between 3.7- 5.3 nmol mg<sup>-1</sup> protein hr<sup>-1</sup>. The Bulk form (primary particle size > 100 nm) of TiO<sub>2</sub> exhibited the greatest accumulation at 24 h (14.1 nmol mg<sup>-1</sup> protein),<sup>2</sup> whilst nano Rutile TiO<sub>2</sub> exhibited the least (6.93 nmol mg<sup>-1</sup> protein). All exposures showed significant Ti metal accumulation in the cells relative to the control (ANOVA  $P < 0.05$ ). TEM and SEM micrographs of the Caco-2 monolayer showed 1 mg l<sup>-1</sup> exposures of NPs and Bulk TiO<sub>2</sub> accumulated below the apical membrane inside vesicles within cells. X-ray microanalysis was used to confirm the presence of TiO<sub>2</sub>. Incubating cells with 120 IU nystatin (putative cholesterol binding agent) caused an increase in intracellular TiO<sub>2</sub> for all crystal types relative to no drug controls (ANOVA  $P < 0.05$ )<sup>2</sup> indicating that cholesterol plays a role in exocytic pathways. Vanadate (ATPase inhibitor, and apparent tyrosine phosphatase inhibitor) incubation (100 μmol l<sup>-1</sup>) produced a very large increase in TiO<sub>2</sub> accumulation (for all crystal types except rutile) relative to controls (ANOVA  $P < 0.05$ ). Incubating cells with 90 μmol l<sup>-1</sup> genistein (tyrosine kinase inhibitor) or 27 μmol l<sup>-1</sup> chlorpromazine (Clathrin mediated endocytosis inhibitor) caused a large decrease in intracellular TiO<sub>2</sub> relative to controls (ANOVA  $P < 0.05$ ). Cell viability measures were generally good (low LDH leak, normal cell morphology), but there

were some changes in intracellular electrolyte composition (K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) in exposed cells relative to controls. A rise in total intracellular calcium was noted for all TiO<sub>2</sub> crystal type exposures, with the greatest rise seen in the P25 NP trials (28.0 ± 12.4 nmol Ca mg<sup>-1</sup> protein) relative to the control (10.6 ± 1.2 nmol Ca mg<sup>-1</sup> protein) (ANOVA  $P < 0.05$ ). Furthermore, P25 exposed cells appeared to show decreased microvilli density relative to control cells. Calcium levels are thought to play an important role in cytoskeletal changes. Overall, the data shows that TiO<sub>2</sub> accumulation by Caco-2 cells is crystal structure-dependent, and that the mechanism involves endocytosis of intact particles.

**TU059 Toxicity of copper oxide nanoparticles- influences of pH change, humic acid and citric acid additions on *Daphnia magna*** K.D. Huggins, Technical University of Denmark / Department of Environmental Engineering; A. Thit, Roskilde University / Environmental, Social and Spatial Change; C. Engelbrekt, Technical University of Denmark / Department of Chemistry; A. Baun, Technical University of Denmark / Department of Environmental Engineering. The potential ecotoxicity of CuO nanoparticles (NPs) is poorly known, however recent studies have speculated that the toxicity of these nanoparticles in an aquatic environment may be due the release of the metal ion dissolved from the NPs. In this study, to assess the toxic effects of CuO NPs, *Daphnia* 48h mobility inhibition tests were performed with two different types of CuO NPs: polydispersed CuO NPs (100nm, poly-CuO) and 6nm CuO. In order to disclose whether the toxicity of CuO nanoparticles followed ionic behaviour, changes in pH, the addition of organic matter and a chelator was studied. The changes in speciation of CuSO<sub>4</sub> were modeled with MINTEQ under the varying conditions in the test media. ICP-OES, DLS, NTA and TEM were used to characterize the CuO NPs before and after 48hrs incubation in test media. The results demonstrated that the toxicity of CuSO<sub>4</sub> and the both CuO NPs increased when the pH changed from 7.8 to 6.5 but also revealed the Cu ion in CuSO<sub>4</sub> to be the more toxic one. Contrarily, the addition of humic acid and citric acid caused a drastic decrease in toxicity especially in the poly-CuO NPs. These outcomes were in agreement with the predictions made by MINTEQ successfully indicating ionic behavior for the copper nanoparticles toxicity. This indicates that consideration of the ionic form may be important when assessing the risks of CuO NPs in the aquatic environment.

**TU060 Toxicity of nanomaterials and preliminar synergistic-antagonistic studies between nanomaterials and organic pollutants** J. Sanchis, IDAEACSIC / Environmental Chemistry; M. Farre, IDAEACSIC; D. Barcelo, IIQABCSIC. Due to the increase in the production of nanomaterials, their emission to the environment has been intensified during the last years, as it has been reported in several works [1,2]. In addition, the possible harmful effects associated to their emission have been cause of concern during the last few years and it has driven a relevant amount of research to assess their toxicological behavior in the environment. Nevertheless, this research is in its early steps of development, in particular in evaluating the possible interaction between nanomaterials and other organic contaminants in real scenario conditions. In the present work, aqueous suspensions of polyamidoamine dendrimers, multiwall carbon nanotubes, graphene nanopowder, gold nanoparticles, silver nanoparticles, fullerene soot and functionalized fullerenes (fullerol and several engineered fullerene derivatives such as phenyl-C<sub>60</sub>-butyric acid methyl ester, phenyl-C<sub>60</sub>-butyric acid methyl ester and thienyl-C<sub>60</sub>-butyric acid methyl ester)<sup>71</sup> have been prepared. Different real scenarios have been reproduced, such as estuarine water, under different situations of salinity, pH and organic matter contents. The water matrices characterization was carried out by means of Transmission Electron Microscopy (TEM) and their acute toxicity was assessed using the standard test based on the immobilization of *Daphnia magna* and the bioluminescence inhibition of the marine bacteria *Vibrio fischeri*. Binary mixtures of nanomaterials and organic pollutants (nonylphenol, diuron malathion, glyphosate, triclosan) were studied. The synergistic/additive/antagonistic relationships between both substances were assessed according to a

simple additive model to the two studied standardized methods. Our results show that the presence of nanomaterials in aquatic media could vary the bioavailability of some organic pollutants modulating their biological effects. [1] M.Farré *et al.* "First determination of C60 and C70 fullerenes and N-methylfulleropyrrolidine C60 on the suspended material of wastewater effluents by liquid chromatography hybrid quadrupole linear ion trap tandem mass spectrometry" *Journal of Hydrology*, 2010, 383(1-2), pp.44-51. [2] J.Sanchís *et al.* "Occurrence of Aerosol-Bound Fullerenes in the Mediterranean Sea Atmosphere", *Environmental Science and Technology*, 2012, 46 (3), pp 1335–1343.

**TU061 Toxicity of Silver Nanoparticles with varying coatings to *Daphnia magna*** K. Paul, J. Kinross, V. Stone, Heriot Watt University; T.F. Fernandes, Heriot Watt University / School of Life Sciences. Silver nanoparticles (Ag NPs) have become of growing interest due to their wide ranging applications and proliferation of use in industrial, medical and consumer products. It is thought that silver nanoparticle production accounts for over 30% of all nanoparticle technology output. Furthermore due to their small size, high reactivity, varying sizes, coatings and complexation in the industry and environment predicting the toxicity or impact of the release of Ag NPs is a challenging task. There has been, therefore, an increasing concern regarding environment and health risks following exposures to Ag NPs. As such as part of the NanoBEE consortium our lab has based environmentally relevant exposures of *D. magna* to Ag-NPs. Of importance to the consortium is the elucidation of common themes of toxicity and also in refining methodologies to allow quick and appropriate policies, risk assessment and mitigation strategies. This poster reports on work carried out as part of consortium NanoBEE (Consortium for Manufactured Nanomaterial Bioavailability & Environmental Exposure). The effects of three types of silver nanoparticles (Ag NPs) with different coatings were assessed using *Daphnia magna* in 48-hour static non-renewal immobility tests. The particles studied were 10 nm PVP-AgNPs, 10 nm PEG-AgNPs and 7 nm citrate coated-AgNPs. Along with these tests SEM imaging was carried out to qualitatively assess if there was any difference to exposed and non-exposed organisms, furthermore it afforded the possibility of seeing if there was any surface attached Ag NPs which may cause irritation or mechanical stress such as reduced feeding ability or movement. Further investigations will assess any differences in uptake, depuration and assimilation efficiencies of the different Ag NPs and how this might affect their overall toxicity. Results of this work will contribute to the overall aim of the project NanoBEE which is the development of models focused on exposure to nanomaterials and their bioavailability in the environment in order to improve our understanding of fate, effects and management of nano-enabled products. **Keywords:** Nanotoxicology, *Daphnia magna*, silver nanoparticles, coatings

**TU062 Xanthyletin in Biodegradable Polymeric Nanoparticles for controlling the interactions of leaf cutting ants and its symbiotic fungus** J.B. Fernandes, Universidade Federal de São Carlos / Química; C.d. Cazal, Goiano Federal Institute of Education, Science and Technology / Chemistry; M.R. Forim, Universidade Federal de São Carlos / Chemistry; O.C. Bueno, Universidade Estadual Paulista / Centro de Estudos de Insetos Sociais; M.N. Fernandes, Universidade Federal de São Carlos / Physiological Science; M.G. da Silva, P.C. Vieira, Universidade Federal de São Carlos / Chemistry. Nano and microencapsulation of natural compound may intensify its activity as the encapsulation may increase the stability of the compound and release it in the active center, allowing determining the mechanism of action and the interaction between insects and fungus. Since there is a symbiosis between the fungus and leaf-cutting ants, one of the mechanism for ants control is to eliminate the fungus. Xanthyletin is one coumarin which is used as growth inhibitor of the symbiotic fungus (*Leucoagaricus gongylophorus*) of the leaf-cutting ant (*Atta sexdens rubropilosa*), one of the most important agricultural plague insects and it do not present toxic action to aquatic organism such as *Brachidanio rerio*. The administration of encapsulated fungicide will permit studies of biotransformation of the fungicide by the fungus and the action mechanism. The incorporation of

xanthyletin in nanoparticles is a promising approach to effectively control leaf-cutting ants. The development and validation of a specific analytical method using HPLC for quantification of the xanthyletin in biodegradable polymeric nanoparticles is presented here. The polymeric matrix applied was poly- $\epsilon$ -caprolactone (PCL). The validation was performed by using a reverse-phase fenil-hexil column, a mobile phase consisting of acetonitrile/water 60:40 (v/v), flow rate of 1.0 mL min<sup>-1</sup> and UV-vis detector at 263 nm. The method has been applied in quality control program to develop new selective nanoformulations loaded of xanthyletin, which should be used in environmental friendly program to control leaf-cutting ants. The higher encapsulation efficiency found in nanocapsules and nanospheres were 98.5% and 92.3%, respectively. The absolute recovery of xanthyletin in colloidal suspensions was nearly 100%. \nFAPESP, CNPq, CAPES, INCT-CBIP\n\n**Keywords:** Xanthyletin; Polymeric Nanoparticles; Poly- $\epsilon$ -caprolactone; Mechanism for leaf-cutting ants control.\n\n

**TU063 Potential artifacts in nanoecotoxicology testing** E.J. Petersen, National Institute of Standards Technology. Engineered nanoparticles are a novel technology that are expected to be incorporated into increasing numbers of consumer products given the unique properties observed with particles that possess nano-scale (1 nm to 100 nm) dimensions. However, one potential barrier to the widespread commercialization of products containing nanoparticles is uncertainty regarding the potential toxic effects that these particles could pose. Assessing such risks is hindered by uncertainty regarding the adequacy of current standard methods for assessing the ecotoxicological risks of these particles given substantial differences in behaviors between nanoparticles and traditional environmental pollutants. Public sensitivity to the potential effects of nanoparticles and the risk that miscommunication of these risks could bias the public against nanomaterials make it critical that robust nanoecotoxicology tests are developed and their results are reported accurately. One of the most important factors that can influence the accuracy of nanoecotoxicology measurements is the potential for nanoparticles to cause artifacts during testing. Artifacts due to nanoparticles have been identified during all stages of the test setup including preparation of the nanoparticle dispersion and quantification of changes in biomarkers after nanoparticle exposure. However, these artifacts have not yet been systematically reviewed. Some of the artifacts that will be discussed during this presentation include interferences with reagents during toxicity assays, toxic byproducts produced during nanoparticle synthesis or dispersion techniques such as sonication, misinterpretation of nanoparticle distributions in tissues during electron microscopy analysis, lack of a mechanism for nanoparticle transit to organs where toxic effects are reported, and endotoxin contamination in nanoparticles tested.

**TU064 Method development to visualize nanoparticle uptake in phytoplankton and higher plants** F. Schwab, Duke University / Civil Environmental Engineering CEINT. The study of ENM uptake mechanisms and modes of action into phytoplankton and plant tissue is often hampered by the lack of validated, easily accessible high-resolution visualization techniques [1]. The objective of this work was therefore to develop and set of complementary visualization techniques applicable to different phytoplankton and plant species exposed to nano-Au particles (nAu). Experiments were performed using axenic cultures of *Pseudokirchneriella subcapitata*, *Anabaena Flos-aquae*, and *Navicula pelliculosa*, and as a representative species for higher plants, *Egeria densa*. The phytoplankton and *E. densa* were exposed to citrate coated nano-Au particles (15 nm) at standard conditions [OECD]. Protocols for three complementary visualization techniques using TEM, confocal microscopy, and the novel technique hyperspectral imaging were developed and tested. TEM imaging carried out at the outer cells of *E. densa* revealed heavy accumulation of the nAu in the cuticle of the cell. The nAu particles were identified by highly resolved imaging of the cuticle tissue, and the X-ray pattern of Au. Preliminary results of the HSI and confocal microscopy imaging will be presented at the conference. The size and shape of the pores across the cell wall of *E. densa* was quantified. The implications on the currently discussed

hypotheses on uptake mechanisms will be discussed. [1] Kahru, A, Dubourguier, HC. 2010. From ecotoxicology to nanoeotoxicology. *Toxicology* 269:105-119. [OECD] Organisation for Economic Co-operation and Development. 2006. Freshwater alga and cyanobacteria, growth inhibition test, Nr. 201. In: OECD Guidelines for the Testing of Chemicals. Paris, F. *Acknowledgement* - The authors thank the Swiss National Science Foundation, the National Science Foundation (NSF), the Environmental Protection Agency (EPA, EF-0830093), the Center for the Environmental Implications of NanoTechnology (CEINT), and TINE for the funding of the study. Any opinions, findings, conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF or the EPA. This work has not been subjected to EPA review and no official endorsement should be inferred.

**TU065 Effect of changing environmental conditions on silver nanoparticle aggregation and dissolution** C. Walters, CSIR / Natural Resources and the Environment. The release of metal nanoparticles (NPs) into the environment is on an increase due to their increased use in several consumer products. Once released into the aquatic environment, NPs tend to form aggregates, or are associated with suspended solids which may be accumulated by aquatic organisms thereby entering the aquatic food chain. These fate processes are largely dependent on both the characteristics of the NP and that of the environment. Of all the metal NPs, silver NPs (AgNPs) are the most widely used, making up approximately 55% of nano-containing consumer products. As such, there is a high potential for the release of AgNPs into the aquatic environment. To better understand their fate and toxicity in aquatic environments, this study investigated the aggregation and dissolution behaviour of AgNPs in freshwater microcosms under simulated environmental conditions. Specifically, this study aimed to assess whether AgNPs will be present as aggregates or isolated particles in aqueous solutions. Dry AgNPs, purchased from the supplier, was characterized via scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive x-ray (EDX), powder x-ray diffraction (PXRD), Brunauer, Emmet and Teller (BET) and ultraviolet-visible (UV-vis) spectroscopy methods. TEM was used to investigate the aggregation potential of aqueous AgNPs under various environmental conditions. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to investigate dissolution potential by assessing the concentrations of Ag in aqueous media. The results obtained showed that at the low temperature regime, higher Ag concentrations were measured when compared to the high temperature regime. The smaller aggregates and lower Ag concentrations measured in T3 when compared to T2 possibly reflects resorption of Ag to the particle surface. The TEM images obtained for primary AgNP suspensions in the flood regime showed micrometer-size aggregates of varying densities. This study proved necessary in order to predict the potential environmental fate, behaviour, bioavailability and toxicity of NPs in the natural environment.

**TU066 Considerations and Recommendations to Standard Testing with *Daphnia magna*.** D. Cupi, DTU Technical University of Denmark / Department of Environmental Engineering. Standard testing procedures for aquatic organisms, as developed by OECD and ISO, have been developed for chemicals that generally dissolve in aqueous solution. However, many nanomaterials will not dissolve in media and incubation durations used in standard ecotoxicity tests. Therefore, modifications of current testing procedures may be warranted. Here, we focus on highlighting limitations to *Daphnia magna* immobilization testing, and proposing modifications to ensure the procedures are fit to the type of testing being conducted. In the current study, six reference nanoparticles [TiO<sub>2</sub> (NM-104), Ag (NM-300K), CeO<sub>2</sub> (NM-212), ZnO (NM-110), ZnO (NM-111), and SiO<sub>2</sub> (NM-200)] were employed to test acute toxicity on freshwater crustacean *Daphnia magna*. It was seen from the results that the toxicity of nanoparticles to *Daphnia magna* ranked in the following order Ag > ZnO (NM-110) > ZnO (NM-111) > CeO<sub>2</sub> > TiO<sub>2</sub> > SiO<sub>2</sub>. In general, nanoparticles in the powdered form were more difficult to suspend in MilliQ water, especially those present

in a hydrophobic state, which had a tendency to float. For the latter, 0.1% ethanol was used to create a wetting effect and achieve a paste form, prior to suspension. It was noticed that the physico-chemical properties/size distribution of the stock suspensions changed over time, therefore it is recommended that stock suspensions are prepared fresh shortly before testing. Another issue that affected size distribution was the presence of ions in media (ionic strength), and sonication procedure (bath vs. probe sonicator). Therefore, dispersion of different types of nanoparticles should be considered on case-by-case basis. Additions of environmentally relevant substances such as natural organic matter (NOM) to different concentrations of Ag NPs decreased toxicity. This effect was seen starting from 10 mg/L humic acid, and more efficiently at concentrations of 50 mg/L and 100 mg/L. While addition of NOM may assist in more controlled dispersions with less degree of agglomeration, it may also influence the sensitivity of the test systems. Hence, further research is needed to disclose the influence of allowing addition of NOM in standard *daphnia* tests.

**TU067 Mercury induced physiological alterations in *Helix aperta* granulocytes** L. Alessandro, UNIVERISTY OF SALENTO / Department of Environmental Sciences and Technologies; M. LIONETTO, UNIVERISTY OF SALENTO / DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES AND TECHNOLOGIES; T. Schettino, Università del Salento; A. Calisi, University of Salento. Among terrestrial invertebrates the gastropods *Helix spp* are able to accumulate several classes of chemical pollutants. Recently, increasing interest has been placed on the use of these organisms as bioindicator species for monitoring trace metals, agrochemicals, and urban pollution. Snails hemolymph is particularly interesting from a toxicological perspective for the development of novel cellular biomarkers of pollutant exposure. It can transport pollutants throughout the exposed organism and its cells (hemocytes) are involved in the internal defence system. Two cell types were observed in *Helix aperta* hemolymph: leukocytes type I (ialinocytes) and II (granulocytes). The aim of the present work was to investigate possible mercury induced alterations in snail hemocytes in view of future application as sensitive biomarker for soil monitoring and assessment. Morphometric alterations were determined by image analysis on Diff-Quick® stained cells. A considerable enlargement of granulocytes was observed in mercury exposed snails with respect to control group. On the other hand, the other cell type did not show any changes in the cell size. The enlargement was quantified by measuring the area of 2D digitalised granulocyte images. Moreover, in mercury exposed animals the increase of the granulocyte dimension was accompanied by cell rounding with loss of pseudopods. This effect could be ascribed to mercury-induced alteration of the actine cytoskeleton of the cells. Further, granulocyte enlargement was paralleled by a decreased of lysosomal membrane stability in snails granulocytes. Due to the important immunological role of granulocytes, the observed adverse effects on these cells may increase the susceptibility of animals to diseases and reduce their survival ability. Therefore, early subtle alterations in some of the components of the immune system can be used as early indicators of altered organism health. In this work heavy metals induced morphometric alterations in snails granulocytes have been demonstrated and hence their possible applications as sensitive, simple, and quick biomarker for monitoring and soil risk assessment is suggested.

**TU068 Study of thermal stress in soil health assessment: effects of temperature in cell biomarkers, acute toxicity and reproduction of *Eisenia fetida*** N. Garcia, UPVEHU / Department of Zoology and Animal Cell Biology; A. Irizar Loibide, University of the Basque country UPVEHU / Department of Zoology and Animal Cell Biology; I. Marigomez, University of Basque Country UPV/EHU; M. Soto, University of Basque Country / Zoology and Animal Cell Dynamic. The Intergovernmental panel on climate change (IPCC) forecasts future scenarios in which terrestrial ecosystems are subjected to multiple environmental aggressions due to climate change and to an increase in the synthesis and release of chemical pollutants. The most feasible



effects to occur in soils are those related to temperature raises, changes in moisture content, acidification and hypoxia phenomena. Hence is important to assess the effects of these environmental conditions as they could affect the responses to environmental pollutants. Early changes occurring at cell and tissue levels in key organisms can be used for soil health assessment. Earthworm species like *Eisenia fetida* has been widely used in standard toxicity tests (OECD, ISO) due to its sensitivity and easy maintenance. The aim of this work is to understand the effects produced by thermal stress and how these effects can affect soil health assessment. Adult *E. fetida* earthworms were maintained in LUFA soil (standard soil no. 2.3, Speyer, Germany) and subjected to thermal stress according to historical data series of maximum temperatures in the zone (19°C - 26°C) for short and long time periods (3, 28 and 56 days). A battery of standardised tests including acute Toxicity Test (ATT) and earthworm Reproduction Test (eRT) (OECD, 2004) were performed together with other biomarkers measured *in vitro* (riboflavin content and neutral red uptake, NRU). Flow cytometric probes were carried out to address changes in coelomocyte subpopulations ratios. Antioxidant enzyme activity (catalase and SOD) and histochemical (lysosomal marker enzymes) measurements were also carried out. Thermal stress produced a severe weight loss (>20%) and extreme reduction in juveniles and cocoon production suggesting affection in the reproductive output. Riboflavin content, NRU and changes in amoebocyte/eleocyte ratios exhibited a high variability not solely related to thermal stress. It can be concluded that thermal stress affects the significance of (stress and exposure) biomarkers of pollution and therefore the use of these biomarkers in a scenario with the co-occurrence of thermal and chemical stresses should be reviewed to fulfil an accurate assessment of soil health.

**TU069 Biomass estimation of the terrestrial ecotoxicological species *Folsomia candida* (Collembola) using real-time PCRL.** Hou, Y. Yanagisawa, Yokohama National University, Japan; S. Yachi, National Institute for AgroEnvironmental Sciences; N. Kaneko, Yokohama national university; T. Nakamori, Yokohama National University, Japan. The abundance and growth of the soil arthropod *Folsomia candida* has been used widely to assess the environmental impact of a range of soil pollutants, and increasing concerns about environmental pollution call for advanced and rapid methods in estimating ecological toxicity. Here, we developed a quantitative polymerase chain reaction (qPCR)-based assay for determining the biomass of soil animals and used it to conduct ecotoxicity tests of pollutions in a soil ecosystem. Prior to DNA extraction, the appropriate concentration and amount of artificial sequence was spiked into the test samples, which were used for normalization and allowed us to assess the assay's extraction efficiency. We designed primers based on the sequencing information of the cytochrome oxidase subunit I (COI) and RNA polymerase II (Pol II) genes of *F. candida*. The assay was first performed on different numbers of collembolans that were 13 and 34 days old (0, 1, 5, 10, 20, 30, 40 and 50 individuals). We found a linear relationship between DNA quantity and the number of collembolans. Then the assay was used to quantify growth of collembolans that were 0–3, 6, 13, 20, 27 and 34 days old. A high positive correlation was observed between real-time qPCR and body length (observed via microscopy). We believe that this technique could be used to detect and quantify all soil animals and would thus improve ecotoxicological testing.

**TU070 Higher plants to assess the genotoxicity of soil** A. Deram, University of Lille / LSVF/ILIS University of Lille 2; F. Bernard, University of Lille 1; S. Dumez, University of Lille 2 / LSVF; C. Lanier, University of Lille 2; S. Lemièr, University of Lille 1 / LENE; A. Platel, Institut Pasteur de Lille / Laboratoire de Toxicologie; F. Nesslany, Institut Pasteur de Lille; F. Vandenbulcke, University of Lille 1; D. Cuny, University of Lille 2. Many environmental pollutants ending up in the soil have a significant genotoxic potential. They can induce mutagenicity in living organisms which results in DNA damage. Although often evoked, the genotoxic risk is difficult to estimate. However, higher plants are known to enable the detection and monitoring of environmental contaminants that could cause damage at

the genetic level. Among the tests developed to assess the genotoxicity, the Comet assay is a sensitive technique to appreciate DNA damages in individual cells. This technique, primarily applied to animal cells, has been adapted to higher plant tissues which has significantly extends the utility of plants for environmental mutagenesis researches. Therefore, our objective was to develop biological tools able to detect genotoxic hazards to better take them into account in environmental and health risk assessment. Two complementary species, commonly used in ecotoxicology, have been investigated: *T. repens* (White clover) and *B. oleracea* (Cabbage). Work was conducted in 3 phases: 1- exposure of plants to a range of soil artificially contaminated with cadmium (known to be genotoxic) or lead (not genotoxic), 2- co-exposure to cadmium and lead in mixture, and 3- exposure to field collected urban soils. This third phase is a multi-contaminant exposure, close to real conditions, which aims to address the expectations of risk managers. Results focus mainly on the results of the comet assay (2 species, 5 ranges from contaminated soil, 3 times of exposure) complemented by an analysis of the bioaccumulation of the tested species. To go beyond the measure of DNA breaks, related results will be discussed. They deal with the analysis of the relationship between metal genotoxicity and oxidative stress. More exactly, a protocol for detection of oxidized DNA using DNA repair enzymes (FPG) was tested. Recently used for animal models, this approach is innovative in plants.

**TU071 Environmental genotoxicity of cadmium and/or lead in natural soil, for the earthworm, *Eisenia fetida*.** S. Lemièr, University of Lille / Nord; M. Delattre, PRES Univ Lille Nord de France / Univ Lille1; J. Leclercq, PRES Univ Lille Nord de France / LGCgE Univ Lille1; F. Bernard, PRES Univ Lille Nord de France / LGCgE Univ Lille1 and LSVF Univ Lille2; S. Dumez, PRES Univ Lille Nord de France / LSVF Univ Lille2; F. Nesslany, A. Platel, PRES Univ Lille Nord de France / Toxicology Lab - Pasteur Institute of Lille; D. Cuny, PRES Univ Lille Nord de France / LSVF Univ Lille2; A. Deram, University of Lille / LSVF/ILIS University of Lille 2; F. Vandenbulcke, PRES Univ Lille Nord de France / LGCgE Univ Lille1. In genetic toxicology, cadmium was a well-known genotoxic and mechanisms explaining its clastogenicity were mainly indirect: induction of ROS and/or inhibition of DNA repair mechanisms. For Lead, all published works were unclear or even contradictory. Concerning its sometimes reported clastogenic and mutagenic effects, its toxic action mechanisms seemed unclear and probably indirect. In environmental toxicology, for contaminants, study of their genotoxic character, alone or in combination, in field soils, was even more complex, since we had to consider: (1) the notions of environmental availability, environmental and toxicological bioavailabilities, (2) their concomitant presences in contaminated/polluted sites and soils, (3) and then their potential toxic interactions. The comet assay was a technique of microelectrophoresis of single isolated cell nuclei which allowed the evaluation of DNA damage (single and double strand breaks, alkali-labile sites) in a cellular population. This assay was developed and validated on coelomocytes of our biological model, largely used in soil ecotoxicology, *Eisenia fetida*. In this work, we conducted *in vivo* short-term exposures (3 and 10 days) using a well-characterised urban-surrounding soil spiked with cadmium or/and lead at environmental concentrations. The main observed results, detailed on this poster, are the following: (1) Significant DNA damage was observed after single Cd exposures without time-dependent and/or concentration-dependent relationships; (2) after single lead exposures, a weak increase of DNA damage was observed but never significant; lead appeared no-clastogenic for our worm after *in vivo* exposures; (3) observed DNA damage after Cd/Pb mixture exposure were intermediate compared to after single metal exposures. Several hypotheses could explain our results in case of combined metal exposures: low bioavailability of lead; other DNA damage induced by lead rather than DNA breaks; involvement of lead specific defence mechanisms for example. Complementary experiments have been conducted to test them and are detailed on the poster.

**TU073 Effects of copper and nickel on earthworm coelomocytes** J. Kwak, Konkuk University; Y. An, Konkuk University / Department of

Environmental Sciences. Earthworms are representative test species in soil toxicity and their coelomocytes are widely used in cytotoxicity test. In this study, *in vivo* and *in vitro* toxicities of copper and nickel on earthworm coelomocytes were tested using *Eisenia andrei*. After exposure, Neutral red retention assay (NRR assay) and flow cytometry after calcein AM staining were conducted to assess cytotoxicity of test chemicals. Mortality, abnormality (mucous excretion, bleeding, swelling, thinning, and fragmentation), bioaccumulation, avoidance, and skin irritation were also observed. As results, lysosomal stability (NRR assay), coelomocytes viability, mortality, abnormality, avoidance, and skin irritation were depended on exposure concentration. We observed that calcein AM staining to coelomocytes and skin irritation was sensitive endpoints. *This subject is supported by Korea Ministry of Environment as the GAIA project (2012000540011).*

**TU074 The fate of arsenic in sludge-amended soils** B. Mařáková, Faculty of Science, RECETOX; J. Kuta, J. Hofman, J. Vasickova, M. Svobodova, M. Sudoma, Masaryk University / Faculty of Science, RECETOX. Sludge amended to soil allows better conditions for plant growth, however, it also increase the risk of chemical compounds leaching to the soil solution. A pot experiments were conducted to examine the changes in the phytotoxicity of soils amended with industrial sludge (in excess of 254 mg/kg of As) with relation to phytoextraction of arsenic with the use of *Sinapis alba*. The aim of this study was to illustrate that arsenic behaviour in soils and plant uptake is strongly dependent on the nature of the metal, sludge, soil properties and crop. The study was realised in the system of a pot experiment for a period of three months. Four different dose of sludge was added to two kinds of soils, with varying properties, and white mustard (*Sinapis alba* L.) was used as a test plant. Solution of 0.1M NH COOCH<sup>4</sup> was employed to investigate the easily mobile fraction<sup>4</sup> and dynamic of As speciation at different phases of treatments. This study extended results from the composting and vermicomposting experiments of this industrial sludge.

**TU075 Potentially bioavailable metals and PAHs in Lufa 2.2: back to basics on contextualisation and implications for use as a natural reference soil** A.C. Bastos, M. Prodana, University of Aveiro / Biology; J. Oliveira, University of Aveiro; C. Calhoa, CESAM Centro de Estudos do Ambiente e do Mar / Biology; M. Santos, CESAM DeptBiology / Department of Biology and CESAM; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; S. Loureiro, Universidade de Aveiro / Biology. Natural reference soils such as Lufa 2.2 (Speyer, Germany) have been widely employed for assessment of contaminated soils. In this context, aquatic bioassays have proven effective for screening of water-soluble soil contaminants, in relation to soil filter and buffer functions, as well as to bioavailability to aquatic and soil organisms. This study has revisited the issue of potentially bioavailable priority contaminant classes (metals, metalloids and 16 U.S. EPA PAHs) in Lufa 2.2 soil and associated implications of its use as reference substrate in ecological risk assessment, in view of the 'applied' bioavailability concepts. Consecutive soil wetting-drying coupled to mild water-extraction (1:10 mass:volume, 150 rpm, 2h, 20°C) was used to estimate water-extractable fractions of such contaminants in Lufa 2.2, while a battery of standard aquatic bioassays was employed to assess the potential acute toxicity of its aqueous extracts on a range of representative organisms (*Vibrio fischeri*, *Pseudokirchneriella subcapitata* and *Daphnia magna*). Aqueous contaminant concentrations (sum) ranged between 177.9-889 ng l<sup>-1</sup> for metals and metalloids and 63.8-72.1 ng l<sup>-1</sup> for the 16 PAHs, dependently on the number of wetting-drying cycles (i.e. 18.1 and 19.3% of their estimated concentrations in bulk soil, respectively). While individual and total contents were generally below (often within the same magnitude order) the corresponding Environmental Quality Standards for surface waters under the 2008/105/EC Directive, the sum benzo(g,h,i)perylene + indeno(1,2,3)pyrene surpassed by >50% that stated in the Directive. When exposed to Lufa extracts, all test organisms showed compromised performance, on the short term. Responses were the most pronounced

after 6 soil wet-dry cycles, with *V. fischeri*, *P. subcapitata* and *D. magna* showing, respectively < 80% luminescence reduction (EC50 of 25.25 and 39.6% extract; 5 and 15 min), < 40% growth inhibition (EC50 of 56% extract) and < 20% immobilization (EC50 below toxicity threshold), at 100% extract concentrations. Results suggest that despite aqueous concentrations of priority contaminants in Lufa soil being below the available benchmarks for water quality and aquatic life functioning (and acknowledging heterogeneity between Lufa 2.2 batches), its use as reference soil may lead to biased toxicity thresholds for relevant compounds and their mixtures, if not adequately addressed as part of the experimental approach and design.

#### **TU076 Comparison between chemical and biological tools to assess the bioavailability of trace elements in long term contaminated soils**

C. Garcia-Gomez, INIA; B. Sanchez-Pardo, E. Esteban, J. Penalosa, UAM; C. del Rio, J. Pareja, INIA; M.D. Fernández, INIA / Environment. It is widely recognized that toxicity of contaminants in soil is better related to the fraction of contaminants present in bioavailable form than to total concentration. This has special importance in the case of metals, where the bioavailable fraction may be only a small percentage of the total concentration. However, the measurement of bioavailable concentration is not easy since it is highly dependent on the physicochemical characteristics of soil and the physiological characteristics of the specific organism (exposure route, uptake, etc). Biological availability is often thought to be comparable to chemical availability. The use of extractant solutions, pore water concentration, or measure of free metal ion activity are some alternatives to chemically estimate the bioavailable concentration. However, a more direct way to measure bioavailability for an organism is to measure the biological effects and/or accumulation in that organism. The goal of this work was to achieve an assessment of the bioavailability of the contaminants in soil samples combining both chemical (total, available and tissue contaminant concentrations) and biological tools. With this aim, four soils contaminated with essential (Zn and Cu) and non-essential (As and Cd) trace elements were used in the study. Soils were taken from a mining site placed near Madrid. Long-term contaminated soils were preferred rather than soils freshly spiked with chemicals, since such soils have limited relevance to aging contaminated soils. The study of bioconcentration and toxicity was performed in a microcosms in soil columns where three plant species (*Triticum aestivum*, *Raphanus sativus* and *Vicia sativa*) and earthworms (*Eisenia fetida*) were simultaneously exposed during 21 days. Samples were tested at different soil concentrations (12.5, 25, 50 and 100% contaminated soil/soil (w/w)) to study how changes in physicochemical soil properties due to dilution can affect the availability. At the end of the assay, effects on earthworms (survival and weight) and on plants (seedling emergence and growth) and bioconcentration in both organisms were determined. Total concentration of metals in soil, plants and earthworms were determined by atomic absorption spectrometry or atomic fluorescence. The available concentration in soils was determined chemically using a weak extractant solution (ammonium sulphate). This work has been financed by Madrid Community through EIADES Project S-2009/AMB/1478

**TU077 The impact of nano-vesicles of sodium dodecyl sulphate/didodecyl dimethylammonium bromide (SDS/DDAB) on soil invertebrates and plants** V. Teixeira, Department of Biology, Faculty of Sciences, University of Porto; S. Bouguerra, Laboratory of Water, Energy and Environment (3E), Engineering School of Sfax, University of Sfax / Department of Biology, Faculty of Sciences of the University of Porto; P. Ribeiro, Department of Biology, Faculty of Sciences, University of Porto; M. Rasteiro, University of Coimbra / CIEPQPF and Chemical Engineering Department; A. Gomes, T. Rocha-Santos, Instituto Piaget / ISEIT/Viscu; I. Lopes, University of Aveiro / department of Biology & CESAM; F. Antunes, University of Coimbra / Department of Chemistry; A.C. Duarte, University of Aveiro / Department of Chemistry & CESAM; M. Ksibi, Engineering School of Sfax, University of Sfax / Laboratory of Water, Energy and Environment (3E); R. Pereira, University of Aveiro / CESAM, Center of Environmental and Marine Studies, University of Aveiro. The

manufacturing of nanomaterials (NMs) is a broad and evolving field and it encompasses a wide range of scientific areas, including chemistry, physics, materials engineering, biology, medicine and electronics. There are several reviews and studies addressing the ecotoxicological aspects of NMs, however they are mainly focused on a limited number of NMs (e.g. quantum dots, nanogold, buckyballs, carbon nanotubes), only few addressing NM with organic composition. Furthermore, till now little ecotoxicological information exists for terrestrial organisms, especially regarding organic NMs. Vesicles of sodium dodecyl sulphate/didodecyl dimethylammonium bromide (SDS/DDAB) are organic soft nanoparticles with potential for cleaning and pharmaceutical products. To date, no evaluations were made about the real impacts of soil contamination with SDS/DDAB on soil habitat and production functions. In this study, avoidance and reproduction assays with earthworms (*Eisenia andrei*), and collembolans (*Folsomia candida*), and seed germination and growth assays with plants (*Avena sativa*, *Brassica oleracea*, *Zea mays* and *Solanum lycopersicum*) were performed to assess the effects of soil contamination with SDS/DDAB nano vesicles. A wide range of concentrations of SDS/DDAB were tested, following standard protocols, and using the standard OECD soil as test substrate (5% of organic matter). The aqueous suspensions of SDS/DDAB used to spike the soils, with the range of concentrations tested, were characterized by light scattering techniques for hydrodynamic size of the vesicles, aggregation index, polydispersity index, zeta potential and surface charge. The results gathered in this study allowed us to infer about the ecotoxicological effects of SDS/DDAB on soil invertebrates and plants, giving rise to data that could be used in the determination of risk limits for this NM. Further the effect of size *versus* chemical composition in the effects observed for the different species will be discussed, since at higher concentrations the nano-vesicles in the aqueous suspension aggregate being added to the soil in this form.

**TU078 Differential iron and zinc accumulation in common beans and caupi as affected by Cd in a typical soil of Brazil** D.H. Silva, Secretaria da Agricultura e Abastecimento do Estado de São Paulo / Coordenadoria de Assistência Técnica Integral; L.A. Figueiredo, University of São Paulo / Ecotoxicology; M.F. Moraes, Universidade Federal do Paraná / Soil Department; F.C. Villanueva, Center for Nuclear Energy in Agriculture/University of São Paulo / Plant Nutrition; T. Muraoka, Center for Nuclear Energy in Agriculture/University of São Paulo / Soil Fertility. The common bean is the most important grain legume for direct human consumption in the world and still the mainly form for the ingestion of protein and essential minerals, e.g. Iron (Fe) and Zinc (Zn) in Latin America. Cadmium (Cd) is one of the most dangerous heavy metals, considered as an extremely toxic pollutant of the environment resulting from various agricultural, mining and industrial activities. We evaluated the differential Fe, Zn and Cd accumulation in cultivars of common beans (*Phaseolus vulgaris* L.) and caupi beans [*Vigna unguiculata* (L.) Walp.] using an Oxisol contaminated with Cd. An experiment was carried out in greenhouse conditions, arranged in completely randomized design, using plastic pots with limed and fertilized soil, two treatments (0 and + 3 mg kg<sup>-1</sup> Cd) and four replications. Plants were harvested at the end of each cultivar cycle, washed with distilled water and oven-dried at 70°C, weighed and 0.5 g of powdered grain samples were digested in nitric acid (HNO<sub>3</sub>) and perchloric acid (HClO<sub>4</sub>). The digested samples were diluted with distilled/deionized water and filtered for Cd analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Analysis of variance (ANOVA) was performed with a Scott-Knott test to compare means of Cd accumulation in grains of contaminated plants. The cultivars of *Vigna* and *Phaseolus* responded differentially to Cd added to soil and the Cd accumulation in grains varied widely. The accumulation of Zn in grains decrease with cadmium accumulation, but not the same occurs with Fe. Cultivars of *Phaseolus* sp. and *Vigna* sp. showed different responses to Cd contamination and it was antagonistic to Zn accumulation. The genotypic difference in grain Cd accumulation may be attributed to the difference in uptake, transportation or remobilization of this heavy metal to grain.

**TU079 Comparison bioavailability of POPs in earthworms exposed to non-sterilized and sterilized soils** S. Kim, Masaryk University / RECETOX; S. Kim, Global Environmental Regulation and Compliance Center, Korea Institute of Toxicology; K. Smidova, J. Hofman, Research Centre for Toxic Compounds in the Environment (RECETOX) / Faculty of Science, Masaryk University. It is obvious that the fate and bioavailability of organic pollutants to organism in artificial soil are different from in natural soil of real ecosystem and one of important factor which causes these differences is soil organic matter. In our previous study, we assumed the bioavailability of POPs to earthworms in natural soil and artificial soil that have same total organic carbon (TOC) amounts could be similar. However, it showed different result and we concluded that TOC content is not a single factor affecting the bioavailability of organic pollutants. Extrapolation based on only TOC amount can make incorrect result when bioavailability in natural soil and artificial soil are compared. We suspect that the microorganism's activity in organic matter of soil can influence their properties and finally it can affect to the fate and bioavailability for soil-biota. Therefore, in this study, we compared the difference of bioavailability of organic pollutants (phenanthrene, pyrene, PCB153, lindane and 4,4-DDT) to earthworms between natural and artificial soils with 3 different TOC. And we also compared the difference of bioavailability in non-sterilized and sterilized soil to verify sterilization effect for microorganisms activity. The result of this study ascertained that bioavailability was affected by organic matter amounts in soils. Specially, bioavailability in high organic matter amount soil was lower than other soils even though the total concentrations of pollutant in soils were similar. The remarkable tendency of bioavailability was not observed by aging time and only differences were observed between chemical and soil types. This study confirmed that sterilization process causes significant change in chemical fate and bioavailability. Some chemical such as PAHs' concentrations didn't decrease in sterilized soils and the bioavailability changed in sterilized soils in comparison with non-sterilized soils. Probably the sterilization was not effect on microbial activities but microbial effects on POPs bioavailability. In conclusion, the fate and bioavailability is affected by several factors (soil type, organic matter amount, properties of organic matter and pollutant type, aging time of soil etc.) and therefore, should be considered carefully when the risk assessment of pollutant is evaluated.

**TU080 Effects on litter mineralization of biochar application to a corn crop soil** X. Domene, CREA/Universitat Autònoma de Barcelona / Unit of Ecology; S. Mattana, CREA; J. Lehmann, Department of Crop and Soil Sciences, Cornell University; E.A. Marks, CREA. Biochar addition has been shown to increase crop yields, and the increased nutrient availability has been proposed as one of the mechanisms involved. Such increase might be the result of the direct addition of nutrients in fresh chars and the subsequent mineralization of the labile fraction of biochar, the reduced nutrient leaching, or a faster nutrient cycling mediated by the soil biota. In this study we assessed the medium-term potential impact of biochar on nutrient cycling, measured as mineralization rates, in a corn-based agroecosystem located in Aurora (NY, USA). Soil cores from each plot were subdivided into subreplicates, finely ground corn stover was added, and the mineralization products (CO<sub>2</sub> and soluble NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>, and Cl<sup>-</sup>) were assessed after 7, 14 and 28 d of incubation.<sup>4</sup> Mineralization rates for each compound in each plot were estimated from the slope of the linear regression of the mineralization products along the incubation. Significant differences between incubation times were observed for mineralization products, with the exception of PO<sub>4</sub><sup>3-</sup> and Cl<sup>-</sup>. However, no global differences were found between treatments with the exception of Cl<sup>-</sup>. Regarding mineralization rates, both positive and negative values were found, indicating net release or loss of mineralization rate, respectively, irrespective of the initial concentrations of mineralization products. Most of the mineralization rates assessed resulted in negative values, indicating a reduction of the water-soluble ionic content in the incubation period attributable to microbial assimilation, although mineralization rates were generally unaffected by biochar treatments when compared to rates in control

plots. The results of our study suggest that the increase in microbial biomass levels associated with this practice might result in a potential competition of microbial biomass with crops for nutrient assimilation, which might potentially have impacts on crop yield if this effect persists along the growing season.

**TU081 Avoidance response of soil invertebrates for the characterization of biochars** X. Domene, CREA Universitat Autònoma Barcelona / Unit of Ecology; J. Lehmann, Department of Crop and Soil Sciences, Cornell University; E.A. Marks, CREA. Biochar is any material obtained by pyrolysis of biomass used as soil amendment. Biochar increases soil moisture and nutrient retention, and stimulates soil biota. However, biochars obtained from polluted feedstocks or excessive application rates might have unintended effects on soil quality. Avoidance response of soil invertebrates has been used as screening method for the assessment of chemicals, wastes or polluted sites samples. However, its use for polluted organic wastes has been debated as they can act both as a source of pollutants and nourishing substrate. This is why attraction rather than avoidance of such wastes has been occasionally reported in literature. In our study we assessed the avoidance response to biochar the test species *Folsomia candida* (Collembola) and *Enchytraeus crypticus* (Oligochaeta), with special interest in the interference with this response due to the known enhancement of microbial communities provided by most biochars. A corn stover biochar was mixed with an agricultural soil at 0, 0.2, 0.5, 2, 7 and 14% (w/w). Avoidance tests were carried out in these mixtures preincubated at increasing time intervals (2, 17, 31 and 61 days). Each avoidance test replicate consisted of a plastic container filled with two adjacent soil portions (control and biochar-treated soil) each occupying half the container. Age-synchronized individuals were transferred to the centre of the container, and kept in dark at 20°C for 48 h in collembolans and 72 h in enchytraeids. After this period, each soil portion was separately removed and individuals were counted. Additional replicates were prepared for the assessment of microbial properties (respiration and biomass), pH, electrical conductivity and soluble ionic contents ( $\text{PO}_4$ ,  $\text{Cl}$ ,  $\text{NO}_3$ ,  $\text{Br}$ ,  $\text{NO}_2$ ,  $\text{NH}_4$  and  $\text{SO}_4$ ). No clear avoidance behavior to biochar was observed with increasing concentrations, and attraction responses were observed more often, although these were not clearly linked to any particular biochar concentration or incubation time. Models were constructed for the behavior of each species using biochar concentration, soil chemical, and microbial properties as explanatory variables. For both species, the resulting models discarded biochar concentration as a significant variable contributing to animal behavior, mainly related to the particular changes in chemical and microbial properties induced by biochar at each concentration and incubation time.

**TU082 Joint toxicity of four different PAH compounds to selected plant species** A. Klimkowicz-Pawlas, Institute of Soil Science and Plant Cultivation - State Research Institute / Department of Soil Science Erosion and Land Protection; B. Maliszewska-Kordybach, B. Smreczak, Institute of Soil Science and Plant Cultivation – State Research Institute. Polycyclic aromatic hydrocarbons (PAHs) represent the group of persistent organic pollutants and may cause adverse effects to the biotic elements of the soil environment. Assessment of the toxicity and the type of pollutants interaction in the mixture is a very important issue since soils are seldom contaminated with individual compounds. For non-ionic organic chemicals such as PAHs, characterised by narcosis as a mode of toxic action, the additivity in the mixture toxicity should be observed. The aim of the study was to evaluate the phytotoxicity of four model PAHs compounds (anthracene, phenanthrene, pyrene and chrysene) applied to the soils as individual hydrocarbons and as a mixture of pollutants. PAHs characterised with various physicochemical properties (water solubility  $2 - 1300 \text{ } \mu\text{g dm}^{-3}$ ,  $\log K_{ow}$  4.54 – 5.86) and diverse toxic activity. Three soils spiked with solution of individual PAHs as well as with their mixture at the levels of 1 – 1000  $\text{mg kg}^{-1}$  were applied in the study. Three plants species – wheat (*Triticum aestivum* L.), tomato (*Lycopersicon esculentum* Miller) and rape (*Brassica napus* L.) – were used as target organisms. The root and stem length at initial period

of plant growth were selected as phytotoxicity endpoints. Plants were growing in laboratory conditions at room temperature ( $20 \pm 2^\circ\text{C}$ ) under natural lighting for the period of 7-14 days. Contamination of soils with PAHs caused adverse effects on the plants growth in the initial period of their development. The response of tested plants was affected by the concentrations and the properties of pollutants and the type of species tested. The first significant effects were noticed at the level of 10  $\text{mg kg}^{-1}$ . The sensitivity of plant species was as follows: tomato > wheat > rape. The inhibition of plant growth increased with the parameters governing PAHs bioavailability (higher water solubility, lower sorption affinity). Relatively stronger toxic effects of phenanthrene and pyrene, as compared to anthracene and chrysene were observed. The data did not confirm the hypothesis about additivity: in most of the cases the phytotoxicity of 4PAHs mixture was significantly lower than the sum of toxic effects of individual hydrocarbons suggesting rather antagonistic type of interaction.

**TU083 Toxicity interaction between chlorpyrifos, mancozeb and soil moisture to the terrestrial isopod *Porcellionides pruinosus*** R. Morgado, P. Gomes, University of Aveiro / Department of Biology and CESAM; N.G. Ferreira, CESAM Universidade de Aveiro / Departamento De Biologia & CESAM; M. Santos, CESAM DeptBiology / Department of Biology and CESAM; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; S. Loureiro, Universidade de Aveiro / Biology. The wide recognition that different natural and chemical stressors can interact influencing each other's toxicity to soil biota, has been pushing researchers to assess increasingly complex scenarios. These studies become particularly important in agricultural fields, given the likelihood of organisms to be simultaneously exposed to a wide range of pesticides while coincidentally experiencing harsh environmental conditions. However, the way that these natural conditions influence the behavior and toxicity of pesticides in a mixture is still poorly understood, and is worth of a special attention. In this work, we investigated the joint effects of two widely used pesticides (the insecticide chlorpyrifos and the fungicide mancozeb) to the terrestrial isopod *Porcellionides pruinosus* under different soil moisture conditions. A full factorial design with three treatments of each pesticide (plus an unexposed control) and three soil moistures (25%, 50%, and 75% of the WHC) was developed. Endpoints included survival, consumption ratio and biomass gain/loss. Feeding parameters and mainly survival of *P. pruinosus* seem to be significantly affected with the interaction of multiple stressors. This emphasizes the importance of assessing the influence of natural stressors on pesticides' toxicity, particularly in case of mixtures.

**TU084 Survey study of heavy metals accumulation in soil and soil organisms (earthworm, isopoda) around Benghazi city** M.H. Haeba, Benghazi University / Zoology; J. Kuta, Masaryk University; R. Gebril, W. Ahmed, Benghazi University. Contamination of soils with heavy metals became a serious problem due to their toxicity, persistence and accumulation potential in soils and biota. Invertebrates form important components in soil functioning, and they play an important role in chemical element transformations in many soils. Soil, Earthworms and Isopoda were sampled in March 2012 within the municipality of Benghazi (Libya) for evaluation of contamination status of soils. Samples were taken from 4 sampling stations (Bouatni, El-Guarsha, Jarotha, and Hawari). Selected heavy metals were determined in biota samples (microwave digestion with nitric acid and hydrogen peroxide) and in soils for "pseudototal" content with aqua-regia extraction (ISO 11466) and bioavailable fraction with 1M ammonium nitrate extraction (ISO 19730) followed by ICP-MS analysis. Total content of Hg in soils and biota was determined by thermo-oxidation solid sampling AAS method. Total concentrations in Bouatni soils were the highest compared to other stations (33.1, 0.39, 0.43, 0.76, 6.73, 321.8, 44.8, 24.6, 9.2, 44, 49.5 and 0.239  $\text{mg/kg}$  for Pb, Sb, Cd, Mo, As, Zn, Cu, Ni, Co, Cr, V and Hg) respectively. Bioavailable concentrations were highest also at Bouatni compared to others (0.008, 0.008, 0.003, 0.05, 0.08, 0.08, 1, 0.2, 0.1, 0.05 and 0.07  $\text{mg/kg}$ ) for Pb, Sb, Cd, Mo,

As, Zn, Cu, Ni, Co, Cr and V. Concentrations of most metals were highest in Bouatni earthworm, namely for Cd, Mo, Zn, Cu, Ni, Cr, V and Hg (6.9, 0.92, 576.5, 14.9, 4.96, 8.65, 9.04 and 0.197 mg/kg). Pb and Sb were highest in El-Guarsh earthworm (4.13 and 0.027 mg/kg). As and Co were highest in Jarotha earthworm (9.94 and Co 6.63 mg/kg). Mo, Zn, Cu and Hg were highest in Bouatni isopoda with 0.55, 462.5, 296 and 0.059 mg/kg respectively. Except Pb 8.21 and As 1.54 in Jarotha isopoda all other 0.027, 0.77, 2.06, 2.56, 3.8, 4.68 (Sb, Cd, Ni, Co, Cr and V) respectively were with highest concentration at Hawari isopoda.

**TU085 Bioaccumulation of heavy metals in the woodlouse *Porcellio laevis* under laboratory conditions** a. mohamed; L. Vasiluk, University of Guelph / School of Environmental Sciences. *Porcellio laevis* and other woodlice clearly contribute to leaf-litter breakdown and are an integral part of decomposition process which recycles essential nutrients and maintains the fertility of soils. On the other hand isopoda responds quickly to environmental contamination and impact with increased mortality, loss of biomass and decrease the species number. Consequently isopods including *P. laevis* have been convincingly useful for monitoring heavy metal pollution in both industrialized and urbanized area. The purpose of this study was a laboratory assessment of the sensitivity of *P. laevis* to cadmium, copper, lead and zinc at moderate concentrations as well as to detect the translocation and accumulation of these metals among the treated leaf-litter. The results indicated low mortality at the concentrations of 100 ppm cadmium, 250 ppm copper, 250 ppm lead and 500 ppm zinc tested. However, the detected cadmium levels in leaf-litter, *P. laevis* and soil were 40.45 µg/g, 22.75 µg/g and 3.43 µg/g respectively. The levels for copper in the same compartments were 15.04 µg/g, 36.04 µg/g and 2.52 µg/g. lead levels were 41.74 µg/g, 7.59 µg/g and 26.61 µg/g for leaf-litter, *P. laevis* and soil respectively, while the levels. For zinc were 20.74 µg/g, 12.95 µg/g and 3.37 µg/g for leaf-litter, *P. laevis* and soil respectively. This study concluded that *P. laevis* can accumulate Cd, Cu, Pb and Zn to an appreciable level which eventually not only have negative effects on the animal itself, but also may extend to their predators. In addition this animal can be endorsed as a biomarker to heavy metals pollutants in their habitat.

**TU086 Soil algae assay with extracts from heavy metal-polluted soils**

S. Nam, Konkuk University; Y. An, Konkuk University / Department of Environmental Sciences. Soil algae are primary producers in soil ecosystem and play an important role as a food source for micro- and meso-fauna. However, there were very limited soil toxicity data of soil algae, and recommended guidelines for the testing of chemicals using soil algae was not developed yet. Here we conducted soil algae toxicity test based on liquid medium and soil extracts. Test species were representative soil alga of *Chlorococcum infusionum* and test chemicals were copper and nickel. In this study, we observed the inhibition of yield for *Chlorococcum infusionum* exposed to copper and nickel in liquid medium and soil extracts. To the best of our knowledge, this is the first result to present toxicity effects of *Chlorococcum infusionum* exposed to copper and nickel in soil extracts. *This subject is supported by Korea Ministry of Environment as the GAIA project (2012000540011).*

**TU087 Comparative avoidance behavior of the earthworms *Eisenia fetida* and *Aporrectodea caliginosa* exposed to the pesticides Salute, Roundup and Rubjan** W.A. Awgie, Benghazi University / Zoology; a. mohamed; M.H. Haeba, Benghazi University / Zoology. The earthworms, *Aporrectodea caliginosa* and *Eisenia fetida* are among the widely used as pollution indicators and monitors in many habitat. Although acute and chronic tests were the common routine tests. However, avoidance behavior tests are now becoming accepted by many researchers. In the present work we compared the response behavior of the two worms species exposed to three different pesticides commonly used in Benghazi agroecosystem. The worm avoidance was performed by using four - chambers plastic containers. *A. caliginosa* results revealed that the Op-salute was the most toxic at all concentrations as compared to control and the two pesticides. The mean  $\pm$  SE of worms

reported in the control were 63.3%, 53.3% and 43.3% compared to that reported in Salute of 0.0, 3.3 and 0.0% in the three concentrations. Rubjan came second to salute in toxicity, where, the mean  $\pm$  SE were 6.7%, 10% and 13.3 %, whereas, Roundup mean  $\pm$  SE were 30, 33,3 and 43.3% in their three concentrations tested. The results in *E.fetida* came similar in both control and salute to that of *A. caliginosa*, where, the mean  $\pm$  SE of worms reported in the control were 57%, 23% and 40% and 0%, 3% and 0% in salute for the three concentrations. However, different responses of the worm were reported toward the Rubjan and the Roundup. *E.fetida* was more sensitive to Roundup than *A. caliginosa*, where the means  $\pm$  SE of both compounds to *E.fetida* were 30%, 47% and 13% for Rubjan compared to 13%, 27%, and 37% for Roundup in their three concentrations. In conclusion the test proved its validity in evaluating toxic substances in a manner similar to acute toxicity except that no mortality would be involved in this test.

**TU088 Enchytraeid Reproduction TestPLUS - Upgrade to the standard with *Enchytraeus crypticus*: hatching and growth parameters and full life cycle test** C. Bicho, M. Goncalves, F. Santos, Universidade de Aveiro; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; M.J. Amorim, Universidade de Aveiro / Department of Biology and CESAM. Soil ecotoxicology standard tests in soil invertebrates are usually limited to the assessment of endpoints like survival and reproduction. This is often related to time constraints and practical limiting issues. Nevertheless, it is recognized of course that adverse effects may occur at other developmental stages. Embryo development (including hatching success) and growth are examples of such endpoints. Moreover, full life cycle tests, which are virtually absent from current test batteries, should be able to cover effects that are at present disregarded. The species *Enchytraeus crypticus* is a model organism in the standard soil ecotoxicology test, where survival and reproduction are assessed starting from adult clitellate animals. In the present study we aimed to improve the present test to a more comprehensive format, to include two more endpoints: hatching success and growth. Further, we propose a full life cycle test. Organisms were grown in synchronized culture plates, allowing both the test start with 1-2 days juveniles or cocoons, easily picked from the agar. This, as opposed to the standard test, where adult organisms with developed clitellum are used independently of age, constitutes a major advantage. The study was performed in a stepwise approach, starting with control conditions to evaluate and define the experimental design: normal time for cocoons to hatch (11 days), juveniles' growth curve (18 days) and time to reach maturity (32 days). The second step consisted on testing a test substance (CdCl<sub>2</sub>) using the refined test design. Results confirmed the potential advantage to include extra endpoints, showing that a limited time effort can provide a much better effect discrimination, e.g., if reproduction effect (no. juveniles), is due to juveniles death or if the effect is actually at the hatching and development in the cocoon. Further, the full life cycle can discriminate between pre- and post-egg formation. Such information level can be very important, e.g. to study the endocrine disrupting potential of compounds which is highly connected to developmental stages.

**TU089 Influence of handling and storage of soil matrix to microbial parameters** L. Sindelarova, Faculty of Science, RECETOX; J. Hofman, Masaryk University / Faculty of Science, RECETOX. Soil conditions (recovery of soil micro flora, activity of micro organisms) or influence of adding dredged sediments to a soil could be evaluated by microbial parameters. That is because microbial communities are very sensitive to different factors. PAO method – (Potential Ammonium Oxidation) was chosen for measuring of state of soils and toxicity of sediments. This work was aimed at elaborating differences between measured data with the same samples and different handling and storage of them which were observed within the experimental work. The samples of soils (3 arable lands) were handled and stocked in different ways (air-dried, wet frozen and wet stored in refrigerator), two sediments were air-dried and stored dry. Tests were done without pre-incubation and with 5-days pre-incubation. Testing by method PAO

should show main differences between particular preparations, which were made with samples. The highest nitrifying activity was measured in variant where the both components were fresh with 5-days pre-incubation. Consequent nitrification activity in most samples was influenced by drying and freezing of soil samples. It is good to pay attention to methods of handling and storage of samples during evaluation of toxicity not only chemicals but also mixtures – e.g. sediments.

**TU090 Effects of nonylphenol on soil bacterial and fungal communities** S. Mattana, CREA; X. Domene, CREA; Universitat Autònoma de Barcelona / Unit of Ecology; S. Chelinho, IMAR CMA / Department of Zoology; J. Sousa, University of Coimbra / IMAR-CMA, Dept. of Life Sciences; E.A. Marks, CREA. Nonylphenol polyethoxylates (NPEOs) are a group of surfactants known to be toxic and able to mimic estrogen compounds and thus interfere with the action of an animal's endogenous hormones. NPEOs are easily biodegraded in the environment, but the last end product, nonylphenol (NP), is the most toxic and recalcitrant form and hence can have a longer half-life in the environment. Despite the fact that most NP is finally degraded, a small fraction may remain in soil for longer periods. The aim of this work was to investigate the effects of different concentrations of NP (0, 10, 30, 90, and 270 mg NP kg<sup>-1</sup>) over time (after 28, 56, and 112 days) on soil bacterial and fungal communities. With this purpose, bacterial and fungal diversity was estimated using the terminal restriction fragment length polymorphism technique (T-RFLP). T-RFLP fingerprinting revealed shifts in microbial bacterial and fungal community structure and diversity related to NP dose applied and sampling time.

**TU091 The *Eisenia fetida* avoidance behaviour test to assess metal-contaminated soil quality recovery after phytostabilisation** S. Demuynck, I. Rosalia Succiu, LGCgE - EA 4515; C. Pernin, LGCgE EA; S. Lemiere, University of Lille / Nord; F. Douay, A. Lepretre, LGCgE - EA 4515. Phytostabilisation aims to establish a vegetation cover to promote *in situ* immobilisation of trace elements by combining the use of metal-tolerant plants and inexpensive mineral or organic soil amendments. Plant cover is known to control erosion and to provide organic matter, improve soil structure and its binding capacity for MTEs. Regarding their efficiency, fly ash amendments were shown to strongly decrease MTE availability to different tree species. However, phytostabilisation and aided-phytostabilisation have to be assessed in terms of soil quality recovery for soil fauna and more particularly for oligochaetes. In such context, an experiment was conducted to evaluate the quality of soils submitted for 10 years to either phytostabilisation using a tree mix or to aided-phytostabilisation. Soils originated from the vicinity of a former lead and zinc smelter plant. The measured parameter was the avoidance behaviour of *Eisenia fetida*. Unplanted soils as well as non-amended soils with similar metal contents were used for comparison. Any significant avoidance for worms exposed to the metal-contaminated soils even to the most contaminated ones, was observed. This is likely to be due to a low availability of the metals present because of soil ageing. In contrast with these findings, *E. fetida* showed the ability to discriminate soils devoid of any pronounced metallic contamination, suggesting that pedological parameters other than the level of contaminants are of importance for earthworms. Regarding the phytostabilisation and the aided-phytostabilisation, it appeared that *E. fetida* showed a preference for soils with tree plantations compared to unplanted soils but significantly avoided fly ash treated soils. This last result indicates that ash-treated soils have limited or reduced habitat function for oligochaetes.

**TU092 Thiophanate-Methyl as an Alternative Toxic Reference in the Earthworm Field Test?** T. Vollmer, O. Klein, Eurofins Agrosience Services EcoChem GmbH; S. Knaebe, EAS EcoChem GmbH. Earthworm field tests are required to assess the effects of pesticides on earthworms. According to the ISO guideline, tests must include a toxic reference. A reduction of the total earthworm population of 50 % is required to validate the study for risk assessment. The recommended active substances benomyl and carbendazim are not

available any more or registrations are running out. The proposed alternative boric acid is not applicable in field trials mainly due to the high concentrations needed to achieve an effect. Thiophanate-methyl (TM) is a registered a.i. that could be an alternative. The active ingredient degrades within several days to the metabolite carbendazim (methyl 2-benzimidazole carbamate, MCB) in soil. In order to prove the suitability of TM as an alternative toxic reference substance, a field trial was performed in late spring 2012 (15/05/2012). Two rates of thiophanate-methyl (10 kg a.i. /ha and 20 kg a.i. /ha) were tested and compared to an untreated control and to carbendazim at a rate of 10 kg a.i. /ha. The earthworm population was assessed 4 and 6 weeks after the application by a combination of hand-sorting four sample areas of 0.25 m<sup>2</sup> to 20 cm depth with subsequent formalin extraction in the excavated holes. The high rate of TM (20 kg a.i. /ha) reduced the earthworm abundance by 70 % at 4 weeks after application and by 60 % at 6 weeks after application, compared to the untreated control, thereby meeting the validity criteria of 50 % reduction. The low rate of TM (10 kg a.i. /ha) clearly failed to produce the required reduction of earthworm abundance (4 weeks: increase of 4 %, 6 weeks: reduction by 8 %). The effect and the suitability of the alternative toxic reference TM are discussed. The results indicate that TM could be used as a possible substitute for MCB/benomyl. Further tests on best rates and application technique should be carried out under different soil conditions (bare soil, grassland, soil texture and organic carbon content).

**TU093 Evaluation of Effects of soil stress environmental factors of metal-contaminated soils using earthworms: Metal accumulation and life history traits.** J.M. Rodrigues, School of Applied Sciences; A.F. Miranda, School of Applied Sciences / School of Applied Sciences; D. Nugegoda, RMIT University / School of Applied Sciences. Annual and seasonal fluctuations in earthworm populations is a natural and normal phenomenon. The amplitude and duration of these fluctuations depend first of all on variation in soil environment conditions such as: soil temperature, pH, salinity, Organic Matter Content (OMC) and Organic Carbon (OC). Heavy metals have also been shown to cause significant reductions in earthworm populations. However, not much studies have emphasized the combined effects of metal pollution and climatic stress factors on earthworms. This study aimed to derive a quality criterion standard in a defined substrate with the eventual aim of protecting earthworms against soil stress factors, and to assess the influence of these factors on partitioning of, uptake in and toxicity of heavy metals to earthworms in separate laboratory trials. To achieve this, five experiments were conducted with specimens of *Eisenia andrei* exposed in the laboratory for 28 days using natural contaminated soil as a substrate. Partitioning of the metals was evaluated with 0.01 M CaCl<sub>2</sub> DTPA. The metal content of worms was determined by acid digestion, while growth, cocoon production, behaviour and mortality were used as endpoints showing toxicity to metals and/or the other five measured parameters. Five different and independent laboratory trials were conducted. In each trial a population of earthworms were exposed to natural contaminated soil with metals having different levels of: salinity, pH, temperature, OMC and OC. Natural contaminated soil was placed in cylindrical plastic vessels of (12cm\*10 Ø) and allowed to equilibrate for five days before earthworms were introduced. Ten earthworms per container were used in each exposure regime and were introduced into the relevant test soil by placing them on the surface and allowing them to burrow in. The experiments were run for 28 days and four replicates were used. This study showed that stress soil environmental factors such as salinity, temperature and soil pH can have detrimental effects on earthworms. Results showed also that metal bioavailability in soils is greatly affected by the soil parameters measured, thus affecting metal accumulation by earthworms. The outcome of this work can help to predict changes in toxicity and bioavailability of trace metals in soils associated with climate change.

**TU094 INFLUENCE OF MICROORGANISMS ACTIVITY IN ROUNDUP® TOXICITY TO TERRESTRIAL NON-TARGET**

J. Bori, Universitat Politècnica de Catalunya UPC; J. Ribo, Technical University of Catalonia UPC; C. Riva, Technical

University of Catalunya (UPC). The herbicide Roundup® and its active ingredient glyphosate are among the most widely used herbicides in the world. Due to its widespread use, numerous studies have focused on the lethal and sub-lethal effects that the use of this herbicide may have on non-target organisms living in soil although there is still controversy on whether those effects are produced by glyphosate itself or by additives in the commercial herbicide. It is also accepted that one of the major mechanisms of glyphosate degradation is due to the activity of microorganisms naturally present in soil. Nevertheless, the way in which the microorganisms may influence the effects that Roundup® application may have on various terrestrial organisms is still poorly studied. The objective of this research work is to study the influence that the presence/absence of microorganisms may have on the potential toxic effects of Roundup® and its active ingredient glyphosate on terrestrial non-target invertebrates. For this purpose, several ecotoxicological tests were performed using natural unsterilized and sterilized soil. Selected species are representative terrestrial invertebrates of the species *Eisenia fetida* and *Folsomia candida* grown under laboratory conditions. Toxic effects were evaluated on the basis of the avoidance test with collembolans (*Folsomia candida*) and earthworms (*Eisenia fetida*). Tests were performed at different times after the application of RoundUp to the unsterilized soil and results were compared with those obtained from sterile soils (i.e. with no degradative action of microorganisms) contaminated with the same herbicide. Data from these ecotoxicological tests was supplemented with the assessment of the effect that exposure to Roundup® has on two earthworm biomarkers. Selected biomarkers include the study of the lysosome membrane stability through the Neutral Red Retention Time Assay and the determination of the Acetylcholinesterase activity.

**TU095 Ecotoxicological evaluation of ashes from biomass combustion in soil organisms** C.M. Pereira, University of Aveiro / Department of Biology; D.F. Silva, L.A. Tarelho, University of Aveiro / Department of Environment and Planning & CESAM; M.J. Amorim, University of Aveiro & CESAM / Department of Biology. The amount of ashes resulting from thermochemical conversion of forest biomass to energy are rapidly increasing in result of biomass to energy policies, and the management of this material is becoming an environmental concern. Research is still lacking in regard to the environmental effects of material valorization these ashes, namely considering the recycling of plant nutrients to agricultural and forestry soils. To ensure a sustainable application, ashes should be characterised prior use, both via a chemical and biological ID. Ecotoxicological evaluation of biomass ashes in soil organisms can be performed following standard procedures as established in the guidelines (OECD, ISO). In the present, two different sources of ashes were studied from two industrial plants, referred to as A and B. Further, each source provided 2 types of ashes: the fly ash (mixture of ashes from heat recovering equipment and electrostatic precipitator) (FA) and bottom ash (from the bottom bed) (BA). The thermal plant A has bubbling fluidized bed technology and B has great furnace technology, and both installations use a mixture of forest biomass residues as fuel. To assess the impact of these biomass ashes, two standard soil organisms were used: *Enchytraeus crypticus* and *Folsomia candida*, assessing avoidance behaviour, survival and reproduction tests. Experiments were performed in the natural standard soil LUFA 2.2, following the standard guideline procedures for avoidance and reproduction test with *F. candida* (ISO, 2011; OECD, 2008) and *E. crypticus* (ISO, 2008, 2005). Dilutions of the test materials were prepared and are expressed as percentage in dry weight. Both species avoided mostly as a concentration-response relationship towards the increasing ash dilution series, with the exception of BA\_B. No reproduction occurred with 20% of FA\_A, BA\_A and FA\_A for *F. candida*. The survival of *F. candida* was affected by 50% at 75 % of FA\_A, 20% of BA\_A and 40 % of FA\_B. For *E. crypticus* the reproduction was totally affected when at 30% of FA\_A, 20% of BA\_A and 40% of FA\_B. Results are further discussed in terms of species sensitivities and ashes characteristics. **Keywords:** biomass ash; collembola; enchytraeids

**TU096 Black soldier fly applications in converting organic waste into feed and fertilizer** B. Mažáková, Faculty of Science, RECETOX; M. Kalova, M. Borkovcova, Mendel University / Department of Zoology, Fisheries, Hydrobiology and Apiculture; J. Hofman, J. Kuta, Masaryk University / Faculty of Science, RECETOX. The aim of this study was to investigate the possibility of using the insect *Hermetia illucens* for treatment of biodegradable municipal waste. The black soldier fly, *Hermetia illucens* (L.), is a nonpest and warm-temperature region insect that is useful for bioconversion of putrescent waste. Lab scale experiments were used for evaluation of potential of the biodegradable municipal waste materials (kitchen residues, grass, sewage sludge, separated solid material from biogas plant) reduction by black soldier fly larvae. We concluded that the use of black soldier fly larvae has a great potential in organic waste management. The chemical analysis was focused mainly on limitations of this treatment, especially the heavy metals contents in the feed material, which negatively influence life traits of the fly population, and could accumulate in the prepupae.

**TU097 Evaluating the toxicity of perfluorooctanoic acid and perfluorooctane sulfonic acid in soil with endogeic geophagus *Aporrectodea caliginosa*** P. Zareitalabad, Department of Soil Science; J. Siemens, University of Bonn; R. Joergensen, University of Kassel; W. Amelung, University of Bonn. To evaluate the ecotoxicity of PFCs on soil organisms, a microcosm experiment was set up with PFOA and PFOS at three concentration levels (1, 100, and 500 mg/kg). The soils were subjected to the activity of endogeic geophagous earthworms of the species *Aporrectodea caliginosa* for 40 days, using labeled with oats straw (*Avena sativa* L.) as carbon source. Microbial biomass C increased in the presence of the PFOA, PFOS in all of the treatments, irrespective of the applied PFOA and PFOS concentrations. In contrast, the basal respiration followed the inverse trend and produced scattered data. Also the fate of the labeled oat carbon source was not significantly influenced by the presence of PFCs, whereas soil  $\delta^{15}\text{N}$  values clearly differed among the treatments. We conclude that PFCs affect the fate of soil N more sensitively than the fate of soil C. Besides, they displayed a significant toxicity on the earthworms itself. The earthworms lost between 29 and 78% of their weight in the soils contaminated with lower PFC concentrations, but in the treatments with the highest concentration of 500mg/kg PFOA or PFOS, no earthworms survived. We conclude that faunal activity may be strongly hampered by PFC contamination, whereas overall microbial activity may even be enhanced, possibly because some organisms benefited from the death of other community members.

**TU098 TOXICOLOGICAL EFFECTS OF IMIDACLOPRID APPLICATION RATES ON NON-TARGET ORGANISMS *Eisenia fetida*, *Daphnia magna* AND *Selenastrum capricornutum*** Bori, Universitat Politècnica de Catalunya UPC; J. Ribo, Technical University of Catalonia UPC; C. Riva, C. Ribalta, Technical University of Catalunya (UPC). The use of phytosanitary products has been increasing for decades due to the need to satisfy the demands of the human population and to solve the stagnation in agricultural production. Among them, the relatively new insecticide Imidacloprid is gaining importance partly because it is seen as a potential candidate to replace organophosphate pesticides like Diazinon. However, despite its apparent selective toxicity for insects and its immobilization in soil, there is still controversy on whether Imidacloprid could affect ecologically important soil organisms or even leach to groundwater and produce detrimental effects on non-target aquatic organisms. Additionally, toxic effects of its transformation products are still poorly studied. This research aims to study the effects that Imidacloprid, in its commercial form Confidor, causes to non-target organisms of two different environmental compartments. For this purpose, the representative terrestrial invertebrate *Eisenia fetida* and aquatic organisms *Daphnia magna* and *Selenastrum capricornutum* grown under laboratory conditions were selected. The experimental procedure consisted in treating natural soil from an uncontaminated area with application rates of Confidor. Toxicity in soil was assessed by means of earthworm avoidance (ISO



17512-1) and acute toxicity (OECD 207) tests whereas aquatic toxicity was evaluated on the basis of *Daphnia magna* acute toxicity test (OECD 202, ISO 6341) and Algal growth inhibition test (OECD 201) using leachates from treated soils.

#### **TU099 Vegetable Uptake and Exposure to DDT in Contaminated Soil from a First Nations Community in Subarctic Canada**

**E.N. Liberta**, Ryerson University / School of Occupational and Public Health; L.J. Tsuji, University of Waterloo / Environmental and Resource Studies. **Introduction** As part of a food security project in the First Nations community of Fort Albany, two agroforestry gardens and an open site garden were prepared to grow vegetables. At the start of the growing season, soil was sampled; while, at the end of the season potatoes and beans were collected and matched to the previous soil samples, and then analyzed for a suite of pesticides including DDT (2,4-DDT, 4,4-DDT, 2,4-DDE, 4,4-DDE, 2,4-DDD, 4,4-DDD) and metals. We used this unique opportunity to assess plant uptake of contaminants which may be present in the soil and to perform an exposure assessment associated with ingestion of potentially contaminated vegetables.

**Methods** Soil and vegetable samples were matched and taken randomly in one of the three plots (Plot A and B were the agroforestry sites, while Plot C was the open site). Soil samples were taken to the depth occupied by the roots. Samples were sent directly to Queen's University, Analytical Services Unit, for organochlorine analysis via GC-MS and metal analysis via ICP-OES. **Conclusion and Discussion** Soil levels of  $\gamma$ -DDT were found to exceed Canadian regulatory guidelines of 0.7 mg/kg for agricultural or residential/parkland use (Table 1) in 1 of the 3 plots (Plot A). Contaminated soil from plot A was correlated with vegetable tissue concentrations ( $R^2 = 0.54$  for potatoes and  $R^2 = 0.16$  for beans respectively). Exposure analyses associated with the ingestion of contaminated vegetables from Plot A were orders of magnitude less than the regulatory guidelines, such as, the acceptable daily intake (ADI) or the reference dose (RfD). While the exposure to the contaminated vegetables may not pose a health risk, the contaminated soil did exceed regulatory guidelines. Table 1. Soil levels of  $\gamma$ -DDT (mg/kg)

Plot	Mean	SD	Min	Max
A	1.12	1.66	0.09	4.19
B	0.09	0.04	0.04	0.19
C	0.01	0.01	0.00	0.03

#### **TU100 Species sensitivity distribution of tributyltin (TBT) on terrestrial organisms**

**P.V. Silva**, department of Biology & CESAM; A.R. Silva, S. Mendo, University of Aveiro / department of Biology & CESAM; S. Loureiro, Universidade de Aveiro / Biology. The contamination of the terrestrial environment by disposal of tributyltin (TBT) contaminated harbour sediments, sewage sludge, and/or biocide products has been raising concerns and it may pose a risk to soil invertebrates and plants. This study aimed to construct Species Sensitivity Distribution (SSD) for TBT in soil aiming at assessing the ecological risk to terrestrial ecosystems. With that purpose, toxicity data was gathered from open literature together with the results obtained from bioassays performed with the species *Porcellionides pruinosus*, *Folsomia candida*, *Brassica rapa* and *Triticum aestivum* in order to construct SSDs and to calculate the hazardous concentration at 5% (HC<sub>5</sub>). SSDs, HC<sub>5</sub> and Predicted No-Effect Concentration (PNEC) values were estimated for all data, for each type of soil and TBT formulation tested. The HC<sub>5</sub> value calculated for TBT in soil was 0.85 mg TBT/Kg soil dw. Few information is available on the concentrations of TBT in soils. The only study available describes TBT concentrations lower than 0.024  $\mu$ g TBT/Kg in wetland soils. In this study the PNEC value determined for all data was 0.03 mg/Kg soil, indicating that the real determined TBT concentrations in this soil represent low risk for environmental effects (ratio between the Predicted Environmental Concentration (PEC) and the PNEC is smaller than 1). In conclusion, the construction of SSDs and the calculation of HC<sub>5</sub> using all the data available showed to be a more suitable method rather than the construction of several SSDs for each soil and TBT types. Further investigations concerning real TBT concentrations and toxicity on soil organisms need to be performed to increase data and improve risk calculations.

**TU101 Effects of composting and vermicomposting on fate of arsenic in industrial sludge** **B. Mažáková**, Faculty of Science, RECETOX; J. Kuta, J. Hofman, J. Vasickova, M. Svobodova, Masaryk University / Faculty of Science, RECETOX; M. Sudoma, Masaryk University / Faculty of Science. A large-scale sludge composting experiment was conducted to study the influence of composting and vermicomposting processes to arsenic chemical speciation and availability from industrial sludge (in excess of 254 mg/kg of As). Multiple ecotoxicological tests were used to evaluate changes in As phytotoxicity and zootoxicity. Composting of sludge, horse manure and grass was performed in compost bin for 90 days. Simultaneously, non-composted and composted material was used for vermicomposting experiments. Solution of 0.1M NH<sub>4</sub>COOH was employed to investigate the easily mobile fraction and dynamic of As speciation at different phases of treatments. The result showed that during composting, (1) the content of total arsenic increase during the first 60 days and levelled off thereafter, (2) mobile As fraction decrease approximately 3.1-fold in the final product. Vermicomposting of non-composted and composted matter showed in both cases, (3) a general increase in the total As concentrations manifested by (4) a decrease in mobile As fractions. Results from this study indicate that composting and vermicomposting of As-rich sludge significantly reduce mobile arsenic fraction. It seems that combination of composting and vermicomposting stages can be an effective step for decreasing arsenic availability by stabilizing and making it less mobile.

**TU102 Comparing different strategies of Vermicomposting** **H. SULEIMAN**, A. RORAT, PRES Lille University North of France / Czestochowa University of Technology / LGCE, University of Lille 1 / Institute of Environmental Engineering; **B. PLYTYCZ**, Jagiellonian University / Institute of Zoology; **M. KACPRZAK**, Czestochowa University of Technology / Institute of Environmental Engineering; **E. VANDENBULCKE**, LGCE Université de Lille / LGCE, University of Lille 1. A significant increase in sewage sludge volume has been observed due to the implementation of the European Directive concerning the treatment of wastewater 91/271/EEC in 2005. In Europe, the total amount of sludge increased from 5.5 million in 1992 to 10 million tons of dry mass in 2007. Usually, sludge may be valued by spreading on agricultural land but other strategies of valorisation do exist. A promising method is *vermicomposting*. It is a simple biotechnological process in which earthworms are used to convert organic waste material into vermicompost which is an excellent organic manure. However, vermicomposting may be affected by the sludge composition itself; the worm species; the presence of added material such as grass, sawdust, municipal solid waste and the duration of the composting period. It is therefore crucial to assess the effectiveness of different vermicomposting strategies applied on different sewage sludge. In this context, a large laboratory experiment was set up, i.e., 1-using metallic trace elements concentration as a guide parameter, 3 different sludge have been selected (a slightly, a moderately and a highly polluted); 2- two different earthworm species (*Eisenia fetida* and *Eisenia andrei*) have been used separately or mixed. Each sewage sludge has been mixed with an added material (organic commercial compost, ratio 1 sludge : 3 organic compost) then monitoring of the process has been done after three time periods (3, 6, 9 weeks). Analysis of the composting process was carried on using biomarkers measurements in the substratum compartment (matrix= compost) and in the biological compartment (worms). All sewage sludge-bulking material mixtures were analyzed before experiment and after all time periods for heavy metal content and microbiological pollution as two main stress factors and for total carbon, total nitrogen and total phosphorus content to assess its value as a fertilizer. Worm viability was monitored and coelomic fluid samples were analysed in respect of coelomocyte numbers and contents of riboflavin and MUG-like fluorophore.

**TU103 Evaluation of artificial soil preparation conditions for toxicity testing of hydrocarbons using exhaustive and equilibrium**

**extractions** M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences, T&ES Division; R. Manning, ExxonMobil Biomedical Sciences; J. Guo, RUTGERS UNIVERSITY; D. Letinski, ExxonMobil Biomedical Sciences; M. Connelly, RUTGERS UNIVERSITY. Numerous spiking procedures and soil conditions for hydrocarbon terrestrial toxicity testing are described in the literature. Conditions applied (i.e. organic matter content, equilibration time) can result in a wide range of variation in test results for specific endpoints. A series of experiments were conducted to evaluate several variables potentially impacting soil hydrocarbon compound concentrations and bioavailability in terrestrial ecotoxicity tests. The variables examined included organic carbon content (0.8% versus 3.0%), mixing times and equilibration times with and without hydration, in order to assess the pre-treatment and mixing conditions for optimal analytical recovery and toxicity test system preparation. To assess these variables, conventional exhaustive solvent extraction and passive sampling using polydimethylsiloxane (PDMS) solid-phase microextraction (SPME) fibers were applied in parallel to determine total soil concentrations and bioavailable soil concentrations. The four model hydrocarbons examined represent a range of volatilities and biodegradability; factors that contribute to the ability to maintain concentration in chronic soil toxicity studies. Testing was performed in two phases: Phase 1 determined the necessary equilibration time for the SPME fibers with spiked artificial soils while addressing the previously noted variables of organic carbon content, mixing time and equilibration time. Phase 2 evaluated the test compounds at two concentrations, nominally 15 and 300 mg/kg, under the previously noted variables during a simulated 28 day exposure situation. The potential bioavailability of the four hydrocarbons in spiked laboratory soil was estimated using in situ PDMS coated SPME fibers as surrogates for the soil organisms. Besides, ex situ sampling with SPME fibers was paired with conventional exhaustive extraction techniques to assess discrete time points and to further elucidate changes in bioavailability. SPME measurements indicated steady-state fiber concentrations (except for o-terphenyl) were generally reached within 6 days of soil equilibration. Results revealed a significant decrease in the bioavailability of the volatile compounds due to volatilization and degradation whereas the non-volatile compounds maintained stable exposure. As a result of these experiments, optimal conditions for hydrocarbon soil toxicity test could be determined.

**TU104 Effect of long term application of biosolids to an agricultural soil: potentially toxic elements occurrence and bioavailability**M.

Hidalgo, M. Iglesias, E. Margui, University of Girona / Department of Chemistry; F. Camps, . Wastewater treatment plants produce increasingly large amounts of sewage sludge worldwide. The treatment and disposal of the ultimate product of the sludge (biosolids), includes incineration, landfill and agricultural application as soil conditioners or fertilisers. Although agricultural use seems to be the natural fate of sludge given their high levels of nutrients and organic matter, severe concerns about the presence of contaminants may hinder a more confident and widespread application. The potentially toxic elements (PTEs) include heavy metals and other inorganic elements from domestic, surface runoff and/or commercial and industrial origins which may be found in sewage sludge. When sludge is applied to the land the PTEs will tend to accumulate in the cultivated layer of topsoil and following repeated applications of sludge the PTEs could theoretically accumulate to toxic concentrations which might affect, for example, crop growth and quality, soil fertility and the food chain. In this context, potentially toxic elements concentrations were studied in an agricultural soil repeatedly amended with sludge for sixteen years. Field experiments were carried out at the agricultural experimental station of Mas Badia-IRTA (Girona, NE Spain). Experimental plots were arranged to account for different scenarios: control soil (no fertilizer), chemical fertilization of the soil, biosolids amended soil (dose applied pre-sown) and combined fertilization (biosolids and chemical fertilization). Two different sets of plots were used to crop barley (winter season) and maize (summer season) and PTE concentrations in different parts of the plants were determined. Moreover, several leaching tests and the

Diffusive Gradients in thin Films (DGT) technique were also applied to assess soil metal mobility/bioavailability. An integrated appraisal of the PTE contents in soils and plants and the potential mobility/bioavailability, as predicted by the different applied techniques, related with different soil treatments is presented.

**TU105 Microbiology parameters in Forest soils in The Czech Republic and their use in ecotoxicology** C.C. Cervinka, J. Hofman,

Masaryk University RECETOX / Faculty of Science. Microbial communities are functionally most important component of the soil biota. Soil microbial biomass is an important pool of available nutrients for terrestrial ecosystems and the flow of energy. They are involved in decomposition and mineralization of organic matter and vice versa such as humus formation and also significantly involved in the degradation of some pollutants and maintain soil structure. Therefore, the conditions of soil microbiology are the main indicators of soil quality. Evaluation of the impact on soil biota is important. However, the "reference values" for microbiology parameters in forest soils in the Czech Republic have been missing. Hence, it becomes essential to remove the missing link for comprehensive in forest soil evaluation. The expected benefit further gains the knowledge about relation between forest soils properties and their pollution and ecotoxicity and microbial. The study aims to develop the criteria for evaluating the load on forest soil risk assessment. Also, it will be carried out identification of forest soil in the Czech Republic according to specific levels of environmental risk. At the same time, defined "reference values", which are from microbiology parameter, and critical effects in ecotoxicology tests may indicate inappropriate condition of forest soils and determine the impact of pollution on their quality. However, utilization of soil microbiology parameters has its limit due to homogenized, soil contamination, various environmental factors. So, sufficient number of locations and time is necessary. Sampling sites were considered to represent the variability of soil, coverage, topological and climate conditions of forest. Only top (F) and humus (H) horizons of forest soil from about 120 sites have been measured according to ISO guidelines (ISO 13240-2, 16072, 14240-1, 17155 and 14238). In general, soil microbiological communities are monitored as the whole community or the processes they involve. This study will identify the size of microbial biomass as immobilized carbon ( $C_{bio}$ ), microbial activities associated with carbon exchange as such respiration without/with additional substrate (basal respiration: BR, potential respiration: SIR, respectively) and growth kinetics. Also, ammonification without/ with additional substrate (ammonification: AMO, potential ammonification: PAMO, respectively) will be measured. *Keywords: Forest soil, Microbiology parameters*

**TU106 Simulated persistence of hazardous substances in sewage sludge-amended agricultural soils in Finland**L. Aysto, Finnish

environmental institute; K. Siimes, M. Verta, Finnish Environment Institute (SYKE). As the requirements for waste water treatment are tightened, the amount of sewage sludge produced in the processes is expected to increase. The waste water treatment plants (WWTPs) in the European Union already produce more than 10 million dry tons of sewage sludge each year, out of which Finnish WWTPs produce 1,5 %. Incineration of sewage sludge is minimal in Finland, which calls for environmentally sound disposal. The agricultural application of sludge may be seen as a sustainable way to accomplish this. This idea is supported by the aspirations for increased nutrient cycling as well as the fact that the concentrations of heavy metals Hg and Cd in Finnish sewage sludge have decreased substantially during the last two decades. However, organic contaminants may pose risks and restrict the application of sewage sludge on soils. The occurrence or concentrations of organic contaminants in Finnish sewage sludge have not been monitored. The aim of this study was to simulate the potential concentration levels of contaminants in sludge-amended agricultural soils in Finland. Altogether 150 hazardous chemicals potentially present in sewage sludge were selected from literature. Their persistence in Finnish agricultural soil was simulated using a PECsoil-calculator, originally developed for pesticide registration purposes. The calculator includes first order degradation kinetics, which is affected by soil

temperature according to Arrhenius equation and soil temperature observed in Southern Finland during 20 years. Because of the high uncertainties in the initial concentrations and the degradation rates collected from literature and databases, the simulation results are presented as potential concentration ranges in soil. The predicted concentrations in soil (PEC<sub>soil</sub>) are compared to the lowest no-effect concentrations (NOEC<sub>soil</sub>) found in literature or regulatory threshold values when such have been given. Finally, the contaminants are ranked according to the PEC/NOEC ratio to see which ones present the greatest risks for the application of sewage sludge on Finnish agricultural soils. Results and discussion of potential risks will be presented.

**TU107 Phytotoxicity of heavy metals in different standard soils** J. Yoon, Konkuk University; Y. An, Konkuk University / Department of Environmental Sciences. We evaluated phytotoxicity of heavy metals in OECD and LUFA 2.2 standard soils to investigate the influence of soil properties on the germination, growth, and bioaccumulation capacity of *Phaseolus radiates*. Two types of soils were treated with copper or nickel for five day duration. The presence of heavy metal decreased the plant germination and growth. Bioaccumulations of heavy metals were observed in the roots and shoots of *Phaseolus radiates*, and they are concentration-dependent. Bioaccumulation of roots showed a higher uptake than shoots. We observed that toxicity of heavy metals to *Phaseolus radiates* was enhanced in LUFA 2.2 standard soil compared to OECD standard soil. *Acknowledgement. This subject is supported by Korea Ministry of Environment as the GAIA Project (2012000540011*

**TU108 Amendment of soils with compost and pig manure. Effects on soil functioning and human risk assesment** N. Roig, Universitat Rovira i Virgili; M. Mari, Rovira i Virgili University / Chemical Engineering; E. Marti, Universitat de Barcelona / Productes Naturals Biologia Vegetal i Edafologia; J. Sierra, Universitat Rovira i Virgili / Laboratori d'edafologia; C. Ortiz, Departament d'Agricultura Ramaderia Pesca Alimentació i Medi Natural; M. Nadal, University Rovira i Virgili; M. Schuhmacher, Rovira i Virgili University; J. Domingo, Universitat Rovira i Virgili. Wastewater treatment processes and pig farming generate highly biodegradable solid wastes, for which the final destination is an environmental issue with considerable repercussion for public administrations, that aim the sustainable management of the urban wastes. In this work, the analysis of the systematic use of compost and pig manure as agricultural fertilizers was performed in Agramunt (Catalonia). It has been evaluated the effects on some soil physical-chemical properties and functions and also on vegetal growing, after a large period of application. Moreover, metal accumulation in soil and plants (wheat's straw and seed) and human risk assessment analyses were performed. The objective of this study is to study the effects on environment due to the long-term application of these sub-products in order to enhance soil quality and, simultaneously, manage this type of waste in a sustainable way. In general, it has been observed that the long-term input of pig manure and compost enhance soil properties proportionally to the doses and/or to the frequency of application. The organic amendments have increased the organic matter content, the soil nitrogen and the microbial activity, improving the mineralization processes of carbon and nitrogen and some enzymatic functions. No risk for human health was observed. However, there is a maximum dose, from which the soil properties do not improve and even begin to decline. Furthermore, it was observed that the risk of aquifer contamination by nitrates increase substantially, mainly in the case of pig manure. Therefore, it has been estimated an appropriate application dose of compost and pig manure in order to minimize environmental risk for Mediterranean calcareous soils with fine texture.

**TU109 Analysis of different Lines of Evidence in ecological risk assessment – agricultural area subjected to PAHs emission** Klimkowicz-Pawlas, Institute of Soil Science and Plant Cultivation State Research Institute / Department of Soil Science Erosion and Land Protection; B. Maliszewska-Kordybach, B. Smreczak, Institute of Soil Science and Plant Cultivation – State Research Institute. Agricultural soils are under the pressure of different chemical pollutants, including

PAHs. High carcinogenicity, toxicity and mutagenicity of those compounds may have negative impacts not only on humans, but also on all biotic elements of the soil ecosystems. PAHs can generate direct and indirect risk for most soil functions (habitat, retention and production). For evaluation of the likelihood that adverse ecological effects may occur as a result of exposure to stressors (e.g. PAHs) procedures of the ecological risk assessment (ERA) are applied. The aim of the study was evaluation of the risk in the agricultural area exposed to different PAHs contamination/emission sources (coke and asphalt production and coal mining). The procedure, based on the analysis of three different Lines of Evidence - LoE (chemical, ecotoxicological and ecological), was applied. This methodology is recently recommended for the site-specific risk assessment of terrestrial ecosystems. The study area (about 100 km<sup>2</sup>) covered the territory of arable land in the South-West part of Upper Silesia region in Poland. The distribution of sampling points was aimed to reflect various soil conditions and diverse exposure to local and trans boundary PAHs emission sources. The measured concentrations of PAHs were evaluated according to the Polish guidelines. The battery of biotests was applied to describe ecotoxicological and ecological characteristics of the research area. The effects of PAHs were related to the total and bioavailable content of polycyclic aromatic hydrocarbons in soils. Analysis and integration the results in different lines of evidence allowed to calculate of integrated risk indexes and to delineate the limited area of possible high ecological risk.

**TU110 Derivation of a site-specific remedial objective for Bromacil, a soil sterilant.** G.L. Stephenson, Stantec Consulting / Environmental Remediation Services; R.A. Angell, Environmental Services; K.M. Olavesons, Stantec Consulting Ltd. / Environmental Services; E.J. Shrive, Stantec / Environmental Services; K. Bessie, EBA, A Tetra Tech Company / Soil Science; A. Burk, Cenovus Energy. Bromacil, a synthetic herbicide, was used in Alberta as a soil sterilant by the oil and gas industry to control vegetation on pumping stations and along linear facilities (e.g., pipelines, roads, transmission lines). The rates and frequency of applications, the nature of the chemical, and the increasingly arid conditions in some regions have resulted in concentrations that persist in soil despite the fact that the chemical can be degraded by microbial organisms. These persistent concentrations of Bromacil in soil have the potential to migrate off site and either exert toxicity to crop plants growing in soils on adjacent lands or contaminate ground and surface waters. Therefore, the semi-arid lands with persistent Bromacil concentrations in soil require remediation. In Alberta (and many other jurisdictions), there is no soil quality standard for Bromacil to protect ecological receptors exposed as a result of direct contact with contaminated soil. During the decommissioning of the site, concentrations of Bromacil were measured in soils collected across the site and the risk associated with these exposure concentrations was considered unacceptable for ecological receptors. An ecotoxicity assessment with a battery of test methods and species was conducted with both a fine-textured and coarse-textured soil using chemically-spiked soils in order to generate an exposure series. Subsequently, species sensitivity distributions of the toxicity data (IC25s) were used to derive a site-specific remedial objective (SSRO) for Bromacil in soil for the direct contact exposure pathway. The SSRO based on the 50<sup>th</sup> and 25<sup>th</sup> percentiles of the distribution of inhibiting concentrations (IC25s) for the combined plant and soil invertebrate species data were derived for the two soil types and the agricultural/residential and industrial/commercial land uses.

**TU111 Effect of a waste incinerator in soils from an industrial area of Northwestern Spain: Chemical and ecotoxicological indicator** M. Gomez Mora, Unitat de Toxicologia Experimental i Ecotoxicologia (UTOX-PCB); M. Borrás Suarez, Unitat de Toxicologia Experimental i Ecotoxicologia (UTOX-PCB), Barcelona, Spain; C. van Gestel, Department of Ecological Science, VU University, Amsterdam, The Netherlands / Department of Ecological Science; J. De Lapuente, PARC CIENTIFIC BARCELONA / Unit of Experimental Toxicology and Ecotoxicology; J. Serret Salse, Unitat de Toxicologia Experimental i Ecotoxicologia (UTOX-PCB), Barcelona, Spain; R. Verweij,

Department of Ecological Science, VU University, Amsterdam, The Netherlands. Within an ecological monitoring study, soil samples from an industrial area (Constantí, Cataluña, Spain) were assessed by integrated physico-chemical and ecotoxicological approaches. Soil samples were collected in different areas representing a gradient near a hazardous waste incinerator (zones Z0, Z1, Z2 and Z3) and a non-polluted control (C). The soils were assessed by means of (i) general physico-chemical characterization (ii) quantification of trace element concentrations (Cd, Pb, Cu, Zn, Mn), and (iii) ecotoxicological tests with terrestrial species at different trophic levels. The tests included acute assays with the earthworm *Eisenia fetida* and the oribatid mite *Platynothrus peltifer*, chronic assays with the enchytraeid *Enchytraeus crypticus*, the micro-arthropods *Folsomia candida* and *Opia nitens* and the terrestrial plant *Solanum lycopersicum*, genotoxicity tests (Comet Test) with *Eisenia fetida* and behavioural tests with *Opia nitens*. The endpoints were survival, reproduction, germination, growth, avoidance and, for the Comet Test, % DNA in tail. Zn concentration was highest in soil from the contaminated zone Z3 followed by zones Z1 and Z0, and much higher than in the control soil. Significant differences in *Platynothrus peltifer* adult survival were observed in soils Z1, Z2 and Z3 compared with the control. *Enchytraeus crypticus* and *Opia nitens* adult survival was significantly lower in soils from zones Z3 and Z0 than in the control. Survival of *Folsomia candida* was significantly lower in soils from all study zones than in the control. *Enchytraeus crypticus* and *Folsomia candida* reproduction was significantly lower in all soils compared with the control and in *Opia nitens* it was significantly lower in zones Z0 and Z1. The comet assay applied to *Eisenia fetida*, previously exposed for 14 days, showed a slight but not significant DNA damage in all soils compared with the control. Mite avoidance was slightly but not significantly higher in soils from zones Z0 and compared with the control. In conclusion, toxic effects were found in soils from different sites in the gradient near the pollution source, but not all tests responded in the same way. Our results therefore suggest the importance of using a battery of tests complemented with a physico-chemical characterization of soils to evaluate the ecotoxicological effects of soil pollution. Keyword: Waster industrial incinerator, soil samples, biotest, metal

**TU112 Enhanced Biodegradation Tests; Application to Persistency Evaluations** C. Mead, Harlan Laboratories Ltd / Ecotoxicology dept; N. Best, C. Bayliss, Harlan Laboratories Ltd. With the onset of REACH renewed focus was placed on the biodegradability of substances and the application of the Persistent criteria in PBT assessment. Standard ready biodegradation tests have been designed to be stringent screening tests with limited potential for biodegradation to occur such that a positive result obtained in such a test is unequivocal. However failure to pass the stringent conditions imposed in a ready biodegradation test does not preclude biodegradation of the substance under relevant environmental conditions and hence a fail in a ready biodegradation test should not automatically be taken as evidence of persistency. To help in persistency evaluations, a number of enhancements to the ready biodegradation test methods have been identified. These enhancements aim to improve the environmental relevance of the biodegradation test and to allow the results of such a test to be used in persistency evaluations without the requirement for expensive and time-consuming simulation testing. Here we describe some of the potential enhancements that can be employed in biodegradation tests and present results of various enhanced biodegradation tests that have been conducted.

**TU113 Inhibition Effects on Ectomycorrhizal Mycelium Growth Treated with Commonly Used Pesticides** I. Zunker, Institute of Environmental Chemistry / Institute of Sustainable and Environmental Chemistry; W. Palm, W.K. Ruck, Leuphana University Lüneburg / Institute of Sustainable and Environmental Chemistry. Ectomycorrhizal fungi (ECM) count among the most important microorganisms in the rhizosphere of trees. Mycorrhized plants have an increased and more efficient water and nutrient uptake and are better adapted to the specific challenges of climate change, such as drought stress [1]. This symbiosis can possibly be disturbed by the application of pesticides in agriculture

and forestry. However, quantitative studies are scarce and only a few studies concerning an inhibitory effect on mycorrhization are available [2,3]. Hence, the impact of fungicides on mycorrhizal symbiosis is poorly understood, which is especially true for new and modern active compounds. The impact of authorized fungicides used in agriculture and forestry on the ECM fungi *Pisolithus arhizus*, *Hebeloma crustuliniforme*, *Lyophyllum* sp. and *Cenococcum geophilum* was investigated. Active ingredients quinoxifen, boscalid, tebuconazole and azoxystrobin were examined as well as commercial fungicide formulation; in addition one herbicide formulation was tested. For this purpose an in-vitro test system was established and serial dilutions were prepared from 200% down to 0.01 % of the application rate used in agriculture and forestry together with control measurements at each dilution step. Test tubes were incubated for 29 days at 25°C in the dark and analyzed every 7 days. The evaluation and quantification of the impact of pesticides on ECM was performed determining the radial growth of mycelium corrected by results from the control group. Ecotoxicological parameters like NOED and ED<sub>50</sub> were determined. The ED<sub>50</sub> is thereby defined as threshold value for 50% growth inhibition of the mycelium. For *Lyophyllum* sp. and *Cenococcum geophilum* (both treated with tebuconazole) ED<sub>50</sub> = 7.4% AR and ED<sub>50</sub> = 16.6% AR were found, respectively. In addition, for *Pisolithus arhizus* treated with a commercial herbicide formulation a strong ecotoxicological potential of ED<sub>50</sub> = 0.3 % AR was detected. Besides the discussion of the impact of pesticides on ECM in test tubes, greenhouse experiments were performed. Concentrations of pesticides in soil, rhizosphere and in the root and comparison with experimental ED<sub>50</sub> data will be discussed on the poster. [1] Chakraborty et al. (2000) Environ. Poll. 108, 317–326 [2] Diedhiou et al. (2004) J. Plant Diseases Prot. 545–556 [3] Laatikainen, et al. (2002) Microbiol. Res. 157, 127–137

**TU114 Mobility, toxicity and biodegradation of shale oils in the soils** L. Kanarabik, I. Blinova, M. Sihtmaa, K. Kunis-Beres, A. Kahru, National Institute of Chemical Physics and Biophysics. Shale oil is a synthetic crude oil made by retorting of oil shale. In Estonia, currently different fractions of shale oil are produced and the volume of production is expanding rapidly. This entails risks of environmental contamination, e. g. during transportation of shale oil. However, the information on harmful properties of shale oils and their behaviour in the soil and water ecosystems is very limited. The aim of the current study was to evaluate the mobility and degradation of Estonian shale oils in soils using a combined chemical and ecotoxicological approach. Two fractions of shale oil (light and heavy) were used for spiking of two types of soils (natural soil and sand). The experiments were performed during 6 months (May–November 2012) outside to mimic natural conditions. The toxicity of the contaminated soils to barley (*Hordeum vulgare*) and soil leachates to aquatic organisms (bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*) was investigated after 1, 3 and 6 months of exposure. In parallel, the effect of shale oil contamination on soil microbial community was evaluated. The concentrations of hydrocarbons and PAHs were measured in the tested soil samples and eluates. The chemical analysis and the toxicity tests showed that shale oils were remarkably more mobile in the sand than in natural peat soil, whereas light fraction with lower viscosity was more mobile than heavy fraction. The higher mobility of the light fraction resulted in higher toxicity of the respective aqueous eluates (1:10) of the spiked soil samples to crustaceans and bacteria. Interestingly, after 6 months of ‘aging’ under natural conditions the toxicity of all contaminated natural soil samples to barley was higher than after 1 or 3 months. Thus, the shale oil (bio)degradation products are more phytotoxic than initial compounds. The number of soil heterotrophic bacteria in the spiked soils considerably exceeded the number of bacteria in clean control soil, showing the biodegradation potential of the oil-polluted soils. We conclude that light fraction of shale oil poses a higher hazard to the environment than the heavy fraction but the natural attenuation seems to be a working solution for the remediation of these soils in case of moderate scale accidents. This research is supported by Central Baltic INTERREG IV A Finnish-Estonian project: Risk

**TU115 Evaluation of a Method for Conducting OECD Testing for Transformation of a Material in an Aquatic Sediment System and Toxicity to a Benthic Organism** C. Picard, J. Letourneau, S. McLaughlin, K. Malekani, M. Bradley, H. Aubin, Smithers Viscient. The current European Medicines Agency risk assessment guidance calls for investigation of toxicity to sediment dwelling midge larvae (OECD 218 guideline) when greater than 10% of the test material partitions to the sediment at day 14 or later in sediment/water systems under aerobic conditions (OECD 308 guideline). The typical maximum exposure duration in the OECD 218 testing is 28 days. While this guidance may provide information on the toxicity of a parent compound to a sediment dwelling organism, it does not address the effects of a test material to sediment dwelling organisms over longer durations that may be encountered in the environment as the material undergoes various transformations or partitions from the aqueous to sediment compartment. The following objectives are outlined in this presentation: (1) investigate the biological performance of midge larvae (*Chironomus riparius*) in two natural sediments historically used in OECD 308 testing and compare to performance in OECD formulated sediment. (2) characterize the microbial biomass in these natural sediments as well as the formulated sediment in order to compare the biological activity of the sediments. (3) develop a test method that investigates the transformation of a common pharmaceutical applied to the water in a designated sediment/water test system for approximately 84 days while synchronously exposing multiple populations of midge to the changing test system throughout the duration of the test. Results are presented in this poster, indicating a relationship between exposure and midge emergence in three different populations. With future work, this test method could be used as a possible supplement to the standard OECD 308 and 218 guidelines.

**TU116 New experimental methods for assessing soil toxicity based on the avoidance response of collembolans** J. Bori, Universitat Politècnica de Catalunya UPC; J. Ribo, Technical University of Catalonia UPC; C. Riva, Technical University of Catalonia (UPC). Soil contamination is of increasing concern due to the continued use of chemicals. Moreover, current regulations require communicating the potential for soil contamination when registering chemical substances. Classical soil toxicity tests require exposure of soil-dwelling organisms to the suspected soil for a predetermined period of time to assess the biological impact of soil contaminants. Many standardized assays study reproduction or mortality of organisms indicators of soil health such as earthworms or springtails. However, such tests require exposure times of several days (from 48 hours to 21 days) whereas regulators are in need of quick assessments on soils allegedly contaminated to help them in decision-making processes. In the avoidance test, the behavior of soil organisms moving away from contaminated soil is assessed as the endpoint and recently, the avoidance test with collembolan has been standardized (ISO 17512-2:2011). Although the test is quite efficient in the assessment of the avoidance response, the experimental protocol can be shortened to simplify its execution. In this communication we present a new experimental model derived from the collembolan avoidance test and compare the results obtained with those from the ISO standard avoidance assay with collembolan. Alternative methods developed in our laboratory provide a reliable screening test based on the avoidance endpoint using a paper filter soaked with the soil leachate as the substrate for the performance of the assay and the use of test containers allowing observation of the avoidance response of the test organisms at any exposure time rather than exclusively at the end of the assay. We compared results of avoidance EC50, percentage of avoidance and the exposure time needed for a proper assessment of the avoidance response following the ISO standard and the new and simpler experimental procedures developed in our laboratory. Test soils included natural uncontaminated soils spiked with reference substances together with site-contaminated soils.

**TU117 Separation of Collembolan size classes for the assessment of population-level impacts of organic residues using multi-Gaussian non-linear regressions** E.A. Marks, CREAM; X. Domene, R. Molowny, CREAM / Universitat Autònoma de Barcelona. Biochar is a pyrolyzed biomass product very similar to charcoal whose use is destined as a soil amendment as opposed to storing energy. Some biochars cause toxic effects in invertebrate bioassays, with measurable changes in parameters such as survival, growth and reproduction, allowing the calculation of NOEC and EC. The use of measurable population-level responses of test organisms, such as changes in age structure, has been proposed as a more sensitive endpoint for the assessment of soil quality, especially when no specific harmful contaminant is suspected. Here, we aim to assess the usefulness of a multi-Gaussian fitting approach to detect changes in sub-lethal parameters following exposure to different biochars at increasing concentrations. Our approach is based in the differentiation of somatic length classes of *Folsomia candida* juveniles following the 1-month survival/reproduction assay as additional information that can be gained from the standardized test. The methodology was capable of detecting of small changes in somatic length and size class recruitment due to treatments. It also permitted estimation of biomass for each size class, which provides information at a higher level of ecological significance beyond the standard juvenile count. Finally, it was possible to detect changes in the underlying population structure, which changed with each biochar material tested, thereby providing an additional tool for material characterization.

**TU118 Relative bioavailability of sediment-bound polychlorinated biphenyls in common carp** J. Gaillard, UR AFFPA / URAFFPA, INRA; D. Banas, M. Thomas, A. Fournier, C. Feidt, Université de Lorraine / URAFFPA, INRA. Polychlorinated biphenyls (PCBs) were first identified in environmental media in the mid-1960s and remain a concern in aquatic systems decades after being banned. In Europe, maximum levels of PCBs in fish meant for animal or human consumption are set while PCBs are still under revision for identification as priority substances in the Water Framework Directive. The common carp (*Cyprinus carpio*) is a benthic species commonly cultured in fishponds. When feeding, carps not only re-suspend but may ingest considerable amounts of sediment. The direct ingestion of contaminated sediment may have a significant impact on the accumulation of PCBs for benthic organisms and in the trophic web. A common biotic assessment tool for measuring the bioavailability of sediment-bound PCBs is the study of bioaccumulation in the whole organism. When studying fish, this experimental design does not allow the discrimination of exposure media and uptake route. The availability of sediment-bound PCBs to fish through oral intake is assessed by evaluating the relative bioavailability (RBA). The deposition of selected indicator PCBs (PCB 138, 153 and 180) in fish muscle in response to ingestion of contaminated sediment and contaminated oil are compared. Contaminated sediment is collected in the vicinity of a former oil recycling factory. Seven batches of experimental feed are manufactured, namely control feed, three sediment feed in which contaminated sediment is incorporated at levels of 7, 14 and 21% and three oil feed in which spiked oil is introduced to obtain similar levels and profile. Juvenile common carps are distributed in 18 groups and fed control food during the adaptation period (15 days) and one of the experimental feed during the exposure period (15 days). At the end of the experiment, muscle are collected. Selected PCBs are analyzed in experimental feed and fish muscle from each tank. For both contamination sources (sediment and oil), the amount of individual PCB congeners in muscle linearly increased with the amount of PCBs ingested. Slopes of the two fitted lines could not be differentiated indicating that relative bioavailability is not different from 1.

**TU119 PCB decontamination in accidentally exposed heifers** G. Rychen, A. Fournier, Université de Lorraine / URAFFPA, INRA; P. Marchand, ONIRIS / LABERCA; A. Grunwald, DDCSPP de la Mayenne / Cité Administrative; H. Toussaint, Université de Lorraine / URAFFPA, INRA; D. Matthieu, URAFFPA-INRA / URAFFPA, INRA; B.

Le Bizec, ONIRIS / LABERCA; C. Feidt, Université de Lorraine / URAFPA, INRA. European Union regulation has defined maximum levels for polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (PCBs) in food from animal origin (Regulation N°1259/2011/EU) and in products intended for animal feed (Regulation N°277/2012/EU). Livestock exposed to contaminated forage or soil results in meat or milk unfit for human consumption. Until recently management of these crises involved euthanasia of animals. The question arising is to know if bovines contaminated by dioxin-like compounds may be decontaminated and further used for their initial purpose. The aim of this experiment was to study the decontamination process of eight young accidentally PCB contaminated heifers ("Limousine" breed) removed from their area and fed a non contaminated diet based on hay and grass silage, supplemented by corn and minerals, to allow a daily weight gain of about 1000 g/d. Every two months the animals were weighed and submitted to pericaudal biopsies. Pericaudal fat samples and experimental feed were analyzed for DL-PCBs and NDL-PCBs (ASE extraction and GC-HRMS equipped with a DB-5MS column using the SIM acquisition mode). The eight heifers (weighing  $337 \pm 21$  kg) showed a rather high initial PCBs concentration in fat tissue (from 25-28 pg WHO<sup>2005</sup>-TEQ/g fat). During the experimental period the kinetics of each DL-PCB and NDL-PCB congener followed an exponential decrease mainly related to the weight gain of the animals. After 120 d of depuration, the NDL-PCBs levels were found to be below the threshold value (40 ng/g fat) and after 180 d, the DL-PCBs+PCDD/Fs level was also below the threshold value (4 pg WHO<sup>2005</sup>-TEQ/g fat) for all animals. During this 6 months experiment the mean weight gain of the animals was 180 kg and at the end of this period all heifers were then considered as decontaminated. The estimated half-life is about 40 to 50 days for PCBs. This study indicates that the decontamination process mainly via dilution process during the growing is feasible. However, the length of the process also depends on the initial level of contamination and the changes in adipose tissue of animal.

**TU120 With PAH metabolites all around Europe** U. Kammann, Thünen Institute / Institute of Fisheries Ecology. Polycyclic aromatic hydrocarbons (PAH) are important environmental contaminants which may lead to reproductive effects or formation of liver tumours and associated lesions in fish. Therefore PAH and their biological effects are part of international monitoring programmes and recommendations (e.g. COMBINE, JAMP/CEMP and CORESET). In the light of the European Marine Strategy Framework Directive (EU MSRL) monitoring data and assessment criteria (AC) are brought together for evaluating the environmental status in marine and coastal regions. Usually this is done on a regional scale. ACs for biota are species specific and may differ between the regions. PAHs are rapidly metabolised and excreted via bile in vertebrates such as fish. To assess the PAH exposure of fish, concentrations of the main metabolite 1-hydroxypyrene, was determined in bile of different marine fish species by HPLC with fluorescence detection. The absence of time trends suggested that results of different sampling campaigns and years could be combined. The map of the PAH metabolite 1-hydroxypyrene in fish from different European regions gives a comprehensive picture, even with ACs applied. The results show that PAH metabolite can be used to create a spatial overview on PAH contamination in European marine fish. Concentrations of PAH metabolites differed primarily according to sampling region and secondarily to species. The results are compared to recently developed assessment criteria.

**TU121 Suitability of the Medaka (*Oryzias latipes*) as test organism for endocrine testing.** E. Bruns, Bayer CropScience AG / BCS D ETX - Ecotoxicology; D. Faber; H. Ratte, Institute for Environmental Research. A Fish-Sexual-Development-Test (FSDT) using short term peak-exposure design and a 2 ½ multigenerational Fish-Full-Life-Cycle test (FFLC) under chronic exposure were performed to investigate the suitability of the Medaka for detecting endocrine active substances. As test substances 4-tert-Pentylphenole (4tPP), a weak oestrogen, and Trenbolon (TR), a strong androgen, were used. In the FSDT nominal

test concentrations were 400 µg 4tPP/L and 100 ng TR/L. The exposure was carried out over 3 days (peak-exposures) at 3 different larval developmental stages. During the FFLC nominal test concentrations of 4tPP were 25, 50, 100, 200 and 400 µg/L. In case of TR nominal test concentrations of 50 and 100 ng/L were used. The FFLC started with fresh fertilized eggs (? 12 h) in the F<sub>0</sub> - and was finished in the F<sub>2</sub> - Generation (F<sub>2</sub>) with juvenile fish (28 d post-hatch). For the FSDT and the FFLC the standard endpoints (hatching-success, mortality and development) and the concentration of Vitellogenin (Vtg) were determined. Concerning the sex ratio related endpoints the phenotypic, the histological and the genetic sex were determined. Within the FFLC the reproduction of F<sub>0</sub> and F<sub>1</sub> were investigated. After a short term peak-exposure tested in the FSDT no adverse effects in all treatment-groups were observed. In the FFLC a significant increase in mortality of F<sub>1</sub> was observed at ? 100 µg 4tPP/L. Female Medakas at 50 ng TR/L were larger than females in the control. There was also an increase in the weight of females exposed to 400 µg 4tPP/L and for males exposed to ? 200 µg 4tPP/L. The Vtg-concentrations of phenotypic male fish were significantly elevated in treatment-groups ? 200 µg 4tPP/L. Feminization was observed in the phenotypic sex-ratio at 400 µg 4tPP/L and masculinization was observed in the phenotypic sex-ratio at 50 ng TR/L. The number of eggs/female\*d<sup>-1</sup> decreased after exposure to ? 200 µg 4tPP/L and 50 ng TR/L. The number of eggs/female\*d<sup>-1</sup> increased at 25 and 50 µg 4tPP/L. In F<sub>1</sub> the Vtg-concentration was significantly elevated in case of phenotypic male and female fish after exposure to 100 µg 4tPP/L. In addition there was a significant shift towards male fish in the sex-ratio exposed to 25 ng TR/L. The number of eggs/female\*d<sup>-1</sup> increased at 25 ng TR/L. The number of eggs/female\*d<sup>-1</sup> decreased at 25 µg 4tPP/L. The remaining concentrations tested in F<sub>2</sub> revealed no adverse effects.

**TU122 Effect modelling of intermittent discharge of herbicides accounting for mixture and competition between species** P. Copin, University of Lausanne / Faculty of Geosciences and Environment; N. Chevre, Img / Faculty of Geosciences and Environment. Toxicity of herbicides towards freshwater aquatic species is usually tested for a continuous exposure in laboratory. But, in real environmental conditions, especially after agricultural applications and rain events, aquatic species are typically exposed to non-continuous concentrations, i.e. pulses, of herbicides in streams. These scenarios are characterized by periods of high or low exposure followed by periods of recovery. The effect on aquatic species will therefore depend on the duration of these two periods and on the herbicide concentrations during the pulse exposure. In general, even if the exposure or the concentration is short, the growth of algae can be affected by such events. In a previous study, pulse exposure scenarios of Isoproturon were tested on the alga *Scenedesmus vacuolatus*, in laboratory. A model was developed and validated to predict the algae growth inhibition after exposure. However, in the field, algae are not only exposed to one chemical, but to multiple stressors such as mixture toxicity, physico-chemical changes, nutrient changes, etc. Among others, the competition between species may play a role in the response to a chemical stress. Therefore, for this study, we chose to make more complex the system and we tested i) pulse exposure on one alga with a mixture of herbicides and ii) pulse exposure of one herbicide on two algae in competition. Concretely, for the first kind of exposure, pulses of a mixture of isoproturon and terbuthylazine were applied on the alga *Scenedesmus vacuolatus*. For the second kind of exposure, a methodology had to be implemented to allow the development of two algae in the same growth medium in laboratory. To allow an easy counting, two algae with a different shape were chosen: a circular one (*Scenedesmus vacuolatus*) and a rangy one (*Pseudokirchneriella subcapitata*). This study highlighted the accuracy of the model used.

**TU123 Integrating spatially explicit exposure models with ecological effect models for use in chemical risk assessment** O. Price, Unilever / Colworth Science Park; A. Franco, Unilever; C.M. Holmes, Waterborne Environmental Inc; P. van den Brink, Alterra Wageningen UR / (a) Aquatic Ecology and Water Quality Management Group; (b)

Alterra; O. Jolliet, University of Michigan / School of Public Health. The development of spatially resolved exposure models and their integration with ecotoxicological effects models will enable mechanistic and more ecologically relevant risk assessments of chemicals. The development of a suite of such representative model scenarios that account for spatial and temporal variability for use in prospective risk assessments is a research priority for us. Over the past few years we have initiated work to develop an approach to assess spatial variation in chemical emissions, removal in different media (e.g. wastewater treatment plant models, in-river models) and the environmental fate at various scales (multimedia multiscale modelling). The ambition is to integrate these models to enable the identification of representative exposure scenarios across Asia. In parallel, we are developing approaches to assess the impact of chemical ingredients used in home and personal care products in rivers receiving untreated wastewater (i.e. multi-stressed environments). The development and application of trait-based approaches to define protection goals in diverse ecological regions are being considered. Ecological models that are able to predict direct and indirect effects of stress factors on different levels of ecological organization (individual, population and trophic web models) are a research priority to help contextualize the impact of our ingredients on ecosystem structure and function. We present our conceptual framework for advancing environmental risk assessment of general chemicals and embracing 21<sup>st</sup> century exposure science and ecological assessment. We present an overview of our current research activities and outline the potential benefits to (1) assess spatially resolved concentrations of individual chemicals used in home and personal care products across Asia, (2) develop archetype scenarios for use in prospective risk assessment, (3) conduct source to receptor analysis for use in mechanistic risk assessment and (4) advance ecologically relevant assessment of multi-stressed environments (including mixtures).

**TU124 Oxygen consumption in *Daphnia magna* as a rapid bioindicator of changes in water quality** M.S. Sepulveda, Purdue University / Department of Forestry & Natural Resources and School of Civil Engineering; M.C. Stensberg, Department of Agricultural and Biological Engineering; M. Poterfield, Purdue University. Currently there are a variety of analytical chemistry based water quality monitoring systems which are capable of measuring low level pollutants. However, most are not readily field deployable and in those that are, sample collection strategies are not optimized. We plan to develop a biological based detector of changes in water quality that could be used solely as a detector or used as a trigger system to be coupled with traditional analytical systems. The purpose of the presented research is to determine experimentally whether oxygen consumption in *Daphnia magna* embryos can serve as a suitable indicator of changes in water quality. For this experiment we examined an array of chemicals used in a similar experiment with fathead minnows, all of which have differing mechanism of toxicity: potassium cyanide, cadmium chloride, atrazine, malathion, and pentachlorophenol. Doses used were based on the EPA acute and chronic ambient water quality guidelines for the protection of aquatic life. The effects were first monitored by measuring oxygen flux at the surface of the embryos during exposure, then the experiment was scaled up to optically measuring oxygen concentration change in the microenvironment (modified 96-well plate) around the embryo to determine if the technique could be scaled to a high throughput system. All chemicals elicited a significant change in oxygen consumption in less than two hours during flux experiments, and preliminary data indicates that similar changes will be observed in the microenvironment. The results indicate that *D. magna* embryos serve as a suitable bioindicator species for rapid detection of low-levels of contaminants in water. Because of this response to this broad array of chemicals, our results imply that oxygen consumption may serve as a detector for chemicals, such as emerging contaminants, that may not have had their criteria defined yet.

**TU125 Occurrence of personal care products in surface waters of the Henares-Jarama-Tajo river basin (Madrid, Spain)** B. Eulalia M., INIA / Environmental; J. Pro, M. Torrijos, G. Carbonell, C.

Fernandez, INIA. As with other anthropogenic substances, aquatic systems become the final recipient of personal care products (PCPs), which are found in wastewater treatment plant (WWTP) effluents and surface waters. These compounds continuously enter the aquatic environment largely through effluents from WWTPs through incomplete elimination or sporadic direct wastewater discharge. Even low concentrations of these substances may lead to undesired effects in aquatic systems. Environmental concerns on these compounds are linked to their capacity to be routinely present in aquatic environments. This situation has resulted in their characterization as “pseudo” persistent compounds. This work presents the analytical determination of 26 PCPs in surface waters in the Henares-Jarama-Tajo river basin (Madrid, Spain), including preservatives, fragrances, sunscreen and endocrine-disrupting compounds. A total of 12 sampling sites were chosen along the river basin upstream and downstream of the most populated cities and industrial areas. River-water samples were taken during the four seasons of a year, beginning in October 2011 and ending in July 2012. This study was funded by Spanish projects RTA2010-00004 and CTM2010-19779-C02-01/02

**TU126 Improving chemical risk assessments: Modelling organic chemical concentrations in UK river catchments** S. Comber, Environmental Science; M. Gardner, Atkins Limited; A. Franco, O. Price, Unilever. EU regulations including REACH and the Water Framework Directive are driving a requirement for more realistic impact assessments regarding the use and fate of organic chemicals during wastewater treatment and resulting concentrations in river catchments. The cost of monitoring for low concentrations of organic chemicals is high, which is driving an interest to model concentrations for use in screening and prioritisation exercises. As more data on chemical concentrations in discharges, particularly sewage treatment works, becomes available and models for predicting fate during treatment are developed, then refined risk assessments are possible to better predict levels of chemicals in receiving waters. This project focusses on 12 organic chemicals that are ‘substances of concern’ to the regulators (EDTA, nonylphenol, bisphenol A, oestradiol, ethinyl oestradiol, triclosan, benzo-a-pyrene, diclofenac, TBT, DEHP, benzo-ghi-perylene and pentabrominated diphenylether (BDE-99)). Extensive UK monitoring data for sewage effluents including removal rates and effluent quality are combined with a revised version of the SimpleTreat sewage treatment model and the river catchment water quality model SIMCAT. We use a case-study to predict the persistence and bioavailability of test chemicals in a catchment heavily influenced by sewage effluent (River Tame catchment, West Midlands, UK). The research concluded that in-stream degradation is not likely to be an important factor for non-readily biodegradable chemicals such as these. Chemicals with river half lives longer than eight days are unlikely to decrease significantly in concentration in the time it takes to traverse most UK river catchments. Exceptions, where biodegradation could be important, include triclosan and oestradiol. Suspended solids concentrations in UK rivers tend to be low (

**TU128 UKWIR Chemicals Investigation Programme (CIP) – Removal of Trace Contaminants by Current Wastewater Treatment Processes** M. Gardner; V. Jones, Atkins Ltd; M. Scrimshaw, T. Coello Garcia, Brunel University; E. Cartmell, Cranfield University; B. Ellor, UKWIR. **Summary:** This paper describes the key outputs of the CIP in relation to the performance of wastewater treatment processes and shows how the results of the programme have been used in order to develop future measures to be implemented as part of the WFD. The relative performance of different wastewater treatment processes is discussed in relation to the achievement of good chemical status for surface waters. **Abstract:** The UKWIR Chemicals Investigation Programme (CIP) has provided an extensive set of monitoring data for a wide range of trace substances in the water cycle. The CIP was a three year programme of research that is helping to establish priorities with respect to action to be taken to ensure surface waters meet new environmental quality standards. The CIP was a collaborative project coordinated by UKWIR, serving the needs of Water Companies and



Regulators. One of the three principal parts of the programme was a study of 28 wastewater treatment works (WwTWs) in order to assess both the overall removal trace contaminants and the part played by different elements of each process – primary sedimentation, secondary treatment of various types and, in some cases, different forms of tertiary treatment. The works selected represented a cross-section of works types currently in operation in the UK and include activated sludge, trickling filters, an membrane bioreactor and oxidation ditches. These works were a subset of the larger set of 160 works that had been selected for the previous effluent quality study as representative of UK WwTWs. The total number of samples taken at any given WwTWs was approximately 150. Therefore, approximately 4,000 samples were taken for the determination of up to 64 substances. Over 250,000 determinations were involved. Spot samples were taken throughout the works to include crude, settled sewage, secondary effluent, final effluent and sludge. Data demonstrate that current treatment processes achieve high standards of contaminant removal – often 80 to 90% for many substances. Despite this impressive performance, the stringency of some water quality standards means that wastewater discharges could contribute to risks that water quality in rivers and streams might fail to comply with such standards

**TU129 How does effluent TOC differ between industries? A proposal to understand the nature of monitored organic sum-parameters in terms of their ecotoxicity** C.E. Raptis, Institute of Environmental Engineering; R. Juraske, ETH Zurich; S. Hellweg, ETH Zurich / Institute of Environmental Engineering. Effluent discharges are complex chemical mixtures and are commonly monitored in terms of organic sum-parameters, such as chemical oxygen demand (COD) or total organic carbon (TOC). While relatively easy to measure and useful during the treatment stage for process control, these sum-parameters convey little about the impacts caused by the effluent in the aquatic environment. Whole effluent toxicity (WET) studies provide a holistic view of effluent ecotoxicity, taking into account all emitted substances and their interactions. Here, we propose a methodology to better understand how TOC varies between industries in terms of the ecotoxicity it encompasses. Our method is based on the assumption of additivity of toxicities caused by either fraction of an effluent broadly split into an organic and an inorganic part. Accordingly, we predict the combined toxicity due to the metals measured in the effluent by employing biotic ligand models (BLM), and then subtract this from WET results. The remaining toxicity is attributed to the organic fraction of the effluent, or, in other words, TOC, the most comprehensive measure of organic content. The methodology is particularly data demanding and relies on a number of assumptions, most notably the concentration addition principle for the two broadly defined effluent fractions. However, if successfully applied, it permits the estimation of ecotoxicity endpoints for TOC, which can be employed in analyses for risk assessment or environmental decision support tools, such as life cycle assessment. Here we present the results of a test-of-concept analysis of WET and effluent chemistry data from the pulp and paper industry, and we discuss the assumptions and limitations of the methodology.

**TU130 Lab-scale Approach to Determine Effects of Wastewater Irrigation on Soil Habitat Quality** E. Richter, ECT Oekotoxikologie GmbH / Aquatic Ecotoxicology, Goethe University Frankfurt/Main; A. Wick, T. Ternes, Federal Institute of Hydrology; A. Coors, ECT Oekotoxikologie GmbH. Facing increasing water stress in agricultural land but also natural wetlands, regions such as North-eastern Germany could benefit from reuse of treated wastewater on land. In current German legislation, however, this option is not foreseen anymore due to concerns about groundwater contamination. Moreover, soil quality could be affected by accumulation of (micro-) pollutants present in treated wastewater. Such impacts can be evaluated by monitoring soil quality over the time of wastewater irrigation, but methods for a prospective assessment of long-term risks are lacking. Thus, the aim of our work is to determine the effects of the application of treated wastewater to different soils with respect to their habitat function for organisms and to assess the water quality after soil passage. To this end, we constructed a

lab-scale apparatus of saturated packed soil columns. A pre-test was run with standard soil and treated wastewater spiked with climbazole. Climbazole is applied in personal care products, predominantly as anti-dandruff agent in shampoos. Artificial surface water was used as control solution. The soil columns continuously received 10 L of solution per day for four weeks. At test end, the soil of each column was homogenised and dried to ambient humidity. The percolates were tested as dilution series with the macrophyte *Lemna minor*. The soil was used as substrate in plant growth tests with the monocot *Avena sativa* and the dicot *Brassica napus*. Primary producers were selected as test organisms because they had been identified previously as most sensitive group for climbazole. Chemical analysis indicated that the breakthrough of climbazole did not occur before the second week and that the percolate concentration plateaued at about 60 % recovery after four weeks. Growth of *L. minor* in the respective percolates was inhibited significantly. Likewise, growth of *A. sativa* and *B. napus* was reduced significantly in the soils treated with the test solution. Overall, the toxicity observed in the biotests gave a reliable indication of the climbazole concentrations measured in the percolates and the soil. The variation between the technical column replicates in terms of analytical and biological results was very small. It was therefore concluded that the experimental set-up with the soil column apparatus can be used as a tool to investigate the effects of wastewater irrigation on soil and water quality.

**TU131 Fathead minnow (*Pimephales promelas*) embryo to adult exposure to decamethylcyclotrasiloxane (D5)** J.L. Parrott, Environment Canada / Water Science and Technology Directorate; M. Alaei; D. Wang, Environment Canada; E. Sverko, Environment Canada / Science and Technology. The cyclic siloxane decamethylcyclotrasiloxane (D5) is a high production volume chemical which has recently been assessed under the Canadian Chemicals Management Program (CMP). Cyclic volatile methyl siloxanes (cVMS) are challenge substances in the CMP Batches. To provide toxicity and growth information on a species of relevance to the Canadian environment, we assessed D5 in a fathead minnow (*Pimephales promelas*) embryo to young adult assay. The test was 65 days in length, and exposed fathead minnow eggs to juveniles until near maturity (60 days post-hatch). The D5 concentrations in flow-through fish exposure aquaria were about one-third of nominal D5 concentrations. Fathead minnows were exposed to 0.25, 0.82, 1.7, 3.6, and 8.7 ug/L D5 (mean measured concentrations). The middle to highest D5 concentrations tested were similar to those detected in Canadian wastewater effluents. During the exposure of fathead minnows to D5 there were few effects seen. Egg hatching and larval fish survival and growth were normal. Juvenile fish survival and growth were good in all environmentally-relevant concentrations of D5, and were similar to control fish. The two highest D5 concentrations (8.7 ug/L and 3.6 ug/L, mean measured D5) increased the condition factors of fathead minnows compared to water control and DMSO control fish. Although there were few effects of D5 in our fathead minnow study, the compound was taken up and stored in fish bodies over the 65-day exposure. The bioconcentration factor for D5 in fathead minnows was 4,450 for the lowest environmentally-relevant D5 exposure water concentrations (e.g. river D5 concentrations downstream of wastewater inputs), and 4,920 for all D5 exposure concentrations tested.

**TU132 Effects of Acute Stress on Tilapia (*Oreochromis niloticus*) After Exposure to Bleached Kraft Mill Effluent** C.H. Soares, Universidade Federal de Santa Catarina / Bioquímica; A. Weiler, I. Baptista, Universidade Federal de Santa Catarina. Exposure of fish to pulp and paper mill effluent has been shown to disrupt the function of the hypothalamo-pituitary-interrenal (HPI) axis. In the present study, juvenile tilapias (25), *Oreochromis niloticus*, were exposed for 8 days to diluted biologically treated bleached Kraft mill effluent (BKME) and after that their response to acute mechanical agitation was investigated at 10 - 20 and 60 minutes. 5 fishes were sampled at time. In the same way, 25 control tilapias were monitored. Fishes were maintained in plastic tanks of 310 L, contains 250 L of diluted effluent (1/50) the pH

= 6.8-7.0; and to the temperature of  $25.0 \pm 1.0$  C. A light:dark cycle of 12:12 h was used. Liver glycogen, in addition to blood glucose, cortisol, ALT, AST and hematocrit levels were measured in both fish groups. Fish not exposed to treated BKME exhibited a quite increased hematocrit, plasma glucose and cortisol whereas those exposed to treated BKME exhibited a lower rate of increasing of the respective parameters. Liver glycogen concentration decreased in control fish. ALT and AST plasma activities were higher in fish exposed to BKME. In conclusion, both fish groups exhibited acute physiological stress responses but these responses were altered in fish exposed to treated BKME. Key words: pulp and paper mill effluent tilapia stress

**TU133 Biochemical Changes In Tilapia (*Oreochromis niloticus*) Exposed To Pulp And Paper Mill Effluent, Stress Response** C.H. Soares, Universidade Federal de Santa Catarina / Bioquímica; I. Baptista, A. Weiler, Universidade Federal de Santa Catarina. It is a very well known fact that the handle or adverse environmental conditions provoke physiological and biochemical alterations in fish, which are characterized as stress response. The objective of this study was evaluated the alterations of protein, metabolites and some electrolytes in plasma of tilapia, under laboratory conditions and sublethal concentration of pulp and paper mill effluent. Fifteen fishes were maintained for four weeks in plastic tanks of 310 L, with 250 L of diluted effluent (1/50), the pH = 6.8-7.0 and temperature at  $25.0 \pm 1.0$  C. A light:dark cycle of 13:11 h was used. The triacylglycerol concentration decreased during the first two weeks and stabilized in the subsequent period. Unlike triglyceride, glucose concentration increased during the first week, but decreased in the subsequent two weeks. The plasma chloride level decreased during all the period of experiment. This finding was coherent with the hematocrit variation observed in the same period, which indicated alterations in the blood cell water balance and of electrolytes, probably due to modifications of the gills and liver. The cortisol level was quite high in first and second weeks, decreasing in third and fourth weeks, but did not return to control value. The concentrations of the total plasma proteins were significantly elevated mainly in the second and third weeks. These findings are typical of stress conditions. key words: fish; pulp mill effluent; stress; tilapia

**TU134 Evaluation of methods for the effect-based detection of estrogenic substances in wastewater treatment plant effluent and adjacent rivers** C. Kienle; P. Kunz, Swiss Centre for Applied Ecotoxicology Eawag/EPFL; E. Vermeirssen, Eawag / Dept. of Environmental Toxicology; N. Homazava, Swiss Centre for Applied Ecotoxicology Eawag/EPFL; S. Maletz, T. Floehr, RWTH Aachen University, Institute for Environmental Research / Institute for Environmental Research; H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V); I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy, Physiology and Cell Biology. Estrogenic substances and other micropollutants enter our rivers and lakes via wastewater treatment plants and diffuse sources. In surface waters, they can be found at concentrations where effects on sensitive organisms such as fish have been observed. Their low effect concentrations (in the lower ng/L range) are often below detection limits of chemical analytics. Standardized methods for the investigation and assessment of the condition of streams and rivers in Switzerland are developed within the scope of the project "Coordinated Surface Water Monitoring" in order to integrate structural, hydrological, biological, chemical, and ecotoxicological aspects of water quality. However, to date, no concept for an ecotoxicological assessment of river water quality exists. Therefore the aim of this project was to evaluate three *in vitro* bioassays for the assessment of estrogenicity in waste and surface waters. We evaluated the practical applicability of the assays as well as their sensitivity and suitability for an application by regulatory authorities or private laboratories. Methods should be sensitive, effect-based, simple, cost-effective and easily interpretable thus allowing the identification of locations in need of actions to reduce such substances. Three *in vitro* bioassays were compared; the Yeast Estrogen Screen (YES) and the ER-CALUX® for the detection of the receptor binding and activation potential, and the H295R steroidogenesis assay for the

measurement of interference potential in the steroid hormone production. Samples from 14 rivers and water bodies in Switzerland were screened for their estrogenic potential and chemically analyzed.

**TU135 A case study on chemical mixtures in sewage sludge: Chemical composition and toxicity in the environment** K.B. Norgaard, Department of Environmental, Social and Spatial Change; H. Selck, Roskilde University; K. Syberg, Roskilde University / Department of Environmental, Social and Spatial Change. Sewage sludge is a residual product from the cleaning of wastewater. It contains high amounts of nutrients and is thus used as fertilizer in agriculture. In addition, sludge contains a mixture of chemicals and heavy metals. The composition of sewage sludge is entirely dependent on the sources contributing to wastewater. REACH is likely to have a major influence on the quality and composition of sewage sludge, because REACH regulates the industrial chemicals used in households and industry. The use of sewage sludge is regulated based on Council Directive 86/278/EEC and in Denmark the Statutory Order on sludge (BEK No 1650 of 13/12/2006). These regulations set limit values of chemicals and heavy metals to protect humans and the environment. However, only a limited number of chemicals are regulated. According to establish regulation on mixture toxicity, an important knowledge gap regards the composition of real life mixtures in the environment, and their potential effects on different organisms. This study aims at addressing this knowledge gap in a case based study. The considerations behind selection of a case and the chemicals for mixture risk assessment are of great importance as these will serve as input to make generalizations regarding other cases and to outline procedures for selection of chemicals for mixture risk assessment. This presentation will outline and discuss the selection approach including considerations in regard to those paths that are pursued and those that are not. The parameters discussed include: type of catchment area of the waste water treatment plant, availability of data on chemical composition in sludge and in catchment, and environmental fate of chemicals in the sludge after application to the field. Chemical properties (e.g., hydrophobicity and solubility) will be a determining factor for the fate of the chemicals, and will, as such, provide important information on target compartments (air, water, sediment, soil). This information is crucial for determining which organism may be exposed to a specific chemical mixture and from which exposure pathway. From the case it will be possible to select a relevant mixture and investigate the possible toxicity of the mixture on a chosen organism. We will show how the case can serve as an exemplary case, a case that can be used to extrapolate to other cases in a broader context. This gives a strong foundation for the discussion of implementation of mixture toxicity in European regulation.

**TU136 Developed of a tool for field in situ toxicity determination using luminescence bacteria** P. Masner, Masaryk University / RECETOX Faculty of Science; L. Blaha, Masaryk University, Faculty of Science, RECETOX / Research Centre for Toxic Compounds in the Environment (RECETOX). Luminescence bacteria as *Vibrio fischeri*, has been widely used for toxicity testing of waters. Most of the analyses are designed for laboratory setup. However, sample collection, transport from the field and processing in lab have impact on the final toxicity results. In the present study, we demonstrate modification and use of a "portable luminometer" for field assessment of toxicity with luminescent bacteria. Our experiments aimed to transfer standardized methods from laboratory to the field testing, and included several modifications and validations of respective step. Comparison experiments (lab vs. field results) included different types of luminometers (desktop vs. portable), series of standard substances and natural samples (waters, sediments). The development also includes application of bioluminescent bacteria for testing of solid/suspended material including Flash protocol, which employs direct injections of bacteria and rapid screening of the toxicity response. Differences in sensitivity and limits of detection were estimated by EC 50 values from all data, and lead to the development of well characterized procedure for portable luminometer application in situ. Examples of the biotest application for screening of contaminated samples such as waste water effluents will be presented.

**TU137 Photocatalytic treatment of olive oil mill wastewater using TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> nanomaterials** V. Nogueira, Universidade de Aveiro / Biology; I. Lopes, University of Aveiro / CESAM; T. Rocha-Santos, ISEIT, Instituto Piaget Viseu & Cesam; F. Goncalves, University of Aveiro & CESAM / Department of Biology; A.d. Duarte, University of Aveiro & CESAM / Chemistry; R. Pereira, University of Aveiro / CESAM, Center of Environmental and Marine Studies, University of Aveiro. Olive oil industry produces a highly polluted wastewater which represents an important environmental problem in the Mediterranean region. Olive mill wastewater (OMW) is rich in recalcitrant and phytotoxic organic compounds. Several physicochemical, biological and combined processes have been used to minimize the impact of this effluent in the environment but none of these treatments were totally effective in mitigating their toxicity and subsequently impacts in the receiving systems. In this context nanotechnology arises offering new possibilities for the treatment of wastewaters mainly based on the enhanced physical and chemical properties of nanomaterials, which can remarkably increase their adsorption and oxidation potential. The aim of this work was to investigate the treatability of olive mill wastewater through several systems involving advanced oxidation processes (AOPs) and using as catalysts two nanomaterials (TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub>). Photodegradation was carried out using different combinations of treatments: UV/H<sub>2</sub>O<sub>2</sub>, UV/TiO<sub>2</sub>, UV/Fe<sub>2</sub>O<sub>3</sub>, UV/TiO<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> and UV/Fe<sub>2</sub>O<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> systems. The effects of catalyst dose (2–0.25 g L<sup>-1</sup> of TiO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub>) and oxidant concentration (1M–0.03M of H<sub>2</sub>O<sub>2</sub>) were also investigated. The efficiency of the treatment was assessed through aromatics (270nm) and color reduction (465nm), chemical oxygen demand (COD) and phenolic content measurements. Our results suggest that photodegradation systems associating TiO<sub>2</sub> or Fe<sub>2</sub>O<sub>3</sub> with H<sub>2</sub>O<sub>2</sub> were the most efficient treatment and responsible for reductions of about 39%, 79%, 40% and 78% for aromatic compounds, color, COD and phenolic content, respectively, after 2h of UV, 1M of oxidant and 1.0 g L<sup>-1</sup> of catalyst. Nonetheless, the TiO<sub>2</sub>-assisted photocatalysis (UV/TiO<sub>2</sub>) and the UV/H<sub>2</sub>O<sub>2</sub> treatment also recorded some reduction in terms of phenolic compounds, reaching higher percentages when used 0.75 g L<sup>-1</sup> for TiO<sub>2</sub> (57%) and 1M of H<sub>2</sub>O<sub>2</sub> (67%). Furthermore, the results showed that photodegradation can be enhanced by the use of the catalyst and oxidant together. In this work the use of nanomaterials combined with H<sub>2</sub>O<sub>2</sub> presents a great potential in removing phenols, one of the major toxicity causes in this wastewater. Further research is being carried out to assess the effectiveness of the most promising systems described above in terms of toxicity reduction to *Vibrio fischeri*.

**TU138 Ecotoxicological assessment of ozone treatment in municipal wastewater treatment plants using in vivo bioassays** M. Hammers-Wirtz, Research Institute gaiac; A. Magdeburg, Goethe-University Frankfurt am Main / Aquatic Ecotoxicology. A multitude of micropollutants has been detected in many running waters in Europe. These anthropogenic chemicals are only insufficiently removed in conventional wastewater treatment plants and discharged into the aquatic environment. Therefore advanced treatment processes have been developed and implemented on the large scale to increase the removal of micropollutants. A relatively new promising approach is the use of ozone as an oxidant for the elimination of micropollutants in wastewater treatment plant effluents. However, the use of ozone under economically feasible operation conditions does not result in a complete mineralization of organic substances but rather leads to partially oxidized transformation products. In the joint research project “Study of metabolite formation during the use of ozone in municipal waste water treatment plants” (Project management: IWW, Muelheim, Germany, funded by the Ministry for Climate Protection, Environment, Agriculture, Nature Conservation and Consumer Protection of the German State of North Rhine-Westphalia (MKULNV)) the question should be addressed, whether transformation products which elicit ecotoxicological or human toxicological effects are formed during ozonation. In this project potential effects of transformation products are assessed in real wastewater on three large-scale sewage plants in

Germany differing in their catchment areas. The toxicological examinations cover a broad spectrum of bioassays including *in vitro* and *in vivo* tests. The applied *in vivo* test battery includes two on-site (flow-through) and four off-site acute and chronic tests with representatives of different trophic levels. Here the results of the *in vivo* bioassays will be presented and discussed.

**TU139 Occurrence, alteration and reduction of ecotoxicological effects of hospital wastewater from different advanced treatment steps** M. Hammers-Wirtz, Research Institute gaiac; A. Magdeburg, Goethe-University Frankfurt am Main / Aquatic Ecotoxicology; S. Lyko, I. Nafo, Emschergerossenschaft und Lippeverband. A multitude of micropollutants has become ubiquitously detectable in the aquatic environment, but the ecotoxicological effects of these complex mixtures on the populations and the communities in the aquatic environment are mostly unknown. In the PILLS project investigating the Pharmaceutical Input and Elimination from Local Sources (funded by the European Union through INTERREG IVB, for details see [www.pills-project.eu](http://www.pills-project.eu)) the effluent quality of different treatment steps in a full-scale hospital wastewater treatment plant (HWWTP) was assessed with respect to the ecotoxicological effects and the occurrence of 72 micropollutants (mainly pharmaceuticals). The HWWTP consisted of sequential and parallel combinations of the advanced treatment steps membrane bioreactor (MBR), ozonation, powdered activated carbon adsorption and sand-filtration. The applied ecotoxicological test battery included two on-site (flow-through) and three off-site acute and chronic *in vivo* tests and, additionally, one *in vitro* test. The test battery is characterized by different trophic levels, by acute and chronic toxicity tests as well as different toxicological endpoints. Here the results of the bioassays investigating different treatment steps will be presented and discussed.

**TU140 The occurrence and behaviour of the anticancer drugs cyclophosphamide and ifosfamide in UK wastewater treatment plants and receiving waters** V. Booker, C. Halsall, Lancaster University; L. Neville, A. Johnson, M. Jurgens, Centre of Ecology and Hydrology. Cytotoxic drugs are routinely used in chemotherapy to fight cancer; they work by disrupting critical cellular processes, often through a toxic mode of action. However, there is growing concern regarding the presence of these pharmaceuticals in WWTPs and receiving waters, as they are discharged into wastewaters, largely through the excretion of the unmetabolized drug. This study looks at two commonly used cytotoxic drugs (cyclophosphamide and ifosfamide) in WWTP influent, effluent and receiving waters across England. Wastewater and river water samples were collected through a combination of ‘grab’ and composite water samples, with a detailed survey conducted on the Rivers Calder and Ribble in NW England. Samples were filtered and extracted using a combination of Strata-X and Florisil SPE cartridges in an LC-MS/MS analytical method with a low limit of detection ranging between 0.03–0.12 ng/L. Cyclophosphamide (CP) concentrations ranged from: wastewater influent (0.43–18.02 ng/L); final effluent (0.09–22.69 ng/L) and river water (BDL–3.78 ng/L). Some samples showed interesting results for CP in the influent and effluent wastewaters, whereby the influent CP concentrations were significantly higher than the CP concentrations in the effluent samples. This would suggest that a fraction of the CP arrives in the raw influent water as a conjugated metabolite with subsequent ‘liberation’ of the parent molecule during wastewater treatment processes. Since CP and ifosfamide (IF) are both metabolized into their corresponding oxaphosphorine mustards then this ‘re-activation’ may also occur for IF during sewage treatment. Ongoing work is examining water samples taken at different stages of the wastewater treatment process to examine this effect in detail, including sampling at a WWTP that operates a tertiary UV treatment process. Only limited field studies have reported cytotoxic drugs in the environment and the data presented here allow the assessment of consumption/use figures to concentrations observed in WWTPs and river water.

**TU141 Estimation of biological impact of surface water samples in Kasukawa River using algae, daphnids and fish** H. Watanabe,

National Institute for Environmental Studies / Center for Environmental Risk Research; W. Yin, R. Abe, The University of Tokyo / Graduate School of Frontier Sciences; H. Takanobu, NIES / Center for Environmental Risk Research; A. Nakamura, Graduate School of Frontier Sciences; M. Yamamuro, The University of Tokyo / Graduate School of Frontier Sciences; N. Tatarazako, National Institute for Environmental Studies / Environmental Risk. To prevent human health hazards and conserve the living environment, total 42 substances have been designed as environmental quality standards for water pollution in Japan, and they have been monitored at about 9,000 sites in environmental waters (rivers, lakes, sea, and groundwater) by the Ministry of Environment. However, there is only two substances (Zn and nonylphenol) regulated to protect aquatic life, and the number of field studies which evaluate the total adverse effect of environmental waters to aquatic life is still limited. In this study, to evaluate the total biological impact of river water samples, we used three short-term chronic toxicity tests: algal growth inhibition test (*Pseudokirchneriella subcapitata*), a daphnid reproduction test (*Ceriodaphnia dubia*), and a fish short-term toxicity test on embryo and sac-fry stages (*Danio rerio*). Five river water samples (St.1-5) were collected at the monitoring points of Kasukawa River, in Gumma, Japan. In the results, river water samples from St.3-5 inhibited the reproduction of the daphnid (NOEC values were 10%, 0.0313%, and 5% sample concentration, respectively) and the sample from St.4 also caused algal growth inhibition (NOEC was 10%), while all of the samples did not cause adverse effect on the fish. To investigate the source of high biological impact of St.4 sample to the daphnid, we also conducted the toxicity test on a small effluent which was discharged just before St.4. The effluent caused the adverse impact on all of three organisms; NOEC values of effluent sample to the alga, the daphnid and the fish were 0.625%, 0.01% and 40%, respectively. In addition, ICP/MS analysis found that the concentration of Ni in St.3-4 and the effluent samples were high enough to cause the toxicity to the daphnid. Because Ni concentration was extremely high in effluent sample, this effluent was considered to be one of the sources of the biological impact of river water in St.4.

**TU142 Assessing petrochemical effluents using mesocosms - Designing large scale experiments** K. Cailleaud, Total Petrochemicals France / PERL; A. Basseres, TotalFinaElf; P. Baldoni-andrey, TOTAL RC; K. Den Haan, CONCAWE; G. Whale, SHELL / SHELL Technology Centre. There is increasing recognition that there are limitations to the substance-specific approach for assessing and controlling the environmental fate and effects of effluents. Consequently, many regulators are seeking more holistic techniques such as whole effluent assessment (WEA) to supplement existing approaches. However, to ensure that these approaches are capable of indicating potential environmental effects, it is important to test scientifically robust WEA protocols. In general, WEA methodology assesses toxicity to aquatic organisms using whole effluent toxicity bioassays (WET). WET has relevance for the protection of ecosystems although the relevance and interpretation of results ultimately depends on the tests used. The main objective of the project, financed by TOTAL and the CONCAWE, is to show the distance between WET methodology which is conservative and in situ impact measurement for risk assessment. The difference between WET and in situ impact measurement has been assessed using dynamic outdoor mesocosms. This project has been designed and undertaken in three successive stages which were 1) experimental design and feasibility assessment; 2) understanding the biological responses in effluents and mesocosms and 3) comparing predicted, laboratory and mesocosm effects. This presentation covers the first stage. Initial laboratory experiments assessed the best solution to store and maintain integrity of one effluent (how to minimize volatilization, oxidation and crystallization how to resist to outdoor climatic variation). The next phase included feasibility experiments in mesocosms with one effluent covering how to select, sample and transport the effluent from an industrial site to the mesocosms; and the final phase assessed how the effluents were dosed and how the ecological impact should be measured. The steps leading up to and including the final experiment will be presented and discussed.

The interpretation of the results will presented in 2 other posters.

**TU143 Assessing petrochemical effluents using mesocosms - understanding the biological responses** K. Cailleaud, Total Petrochemicals France / PERL; A. BASSERES, Total / PERL; P. Baldoni-andrey, TOTAL RC; K. Den Haan, CONCAWE; G. Whale, SHELL / SHELL Technology Centre. In the context of the Water Framework Directive (WFD), European surface water should reach good ecological status in 2015. The ecological status is generally assessed with ecological indicators. In some particular cases (sampling difficulties because of access, type of substrate, several industrial wastes in the area), this approach is difficult to be applied. The current project is attempting to link WET methodology determined in the laboratory as an alternative to ecological indicators for refinery effluents. The data described are derived from a project jointly organised by TOTAL and the CONCAWE, which has been developed to assess whether WET data obtained from laboratory assessments can be used to predict effects in dynamic outdoor artificial streams (mesocosms). This project has been designed and undertaken in three successive stages which were 1) experimental design and feasibility assessment; 2) understanding the biological responses in effluents and mesocosms and 3) comparing predicted, laboratory and mesocosm effects. In this second of 3 presentations, the biological data obtained from the mesocosms (benthic invertebrate abundance and biodiversity, diatom abundance and biodiversity, chlorophyll concentration) and impacts observed during the refinery effluent dosing (*Daphnia magna*, *Pseudokirchneriella subcapitata* bioassays and Microtox) and recovery period are described. The results of bioassays show that pure effluent has no effect on daphnia and microalgae and low effect on Microtox whereas effluents fortified with hydrocarbons have effects on Microtox, microalgae and daphnia. The results measured in mesocosms show that chronic exposure (21 days) to pure effluent has no impact on the ecosystem whereas chronic exposure to fortified effluents have significant impact on the ecosystem (mainly on benthic invertebrates and chlorophyll concentrations). However, a rapid restoration is observed in the mesocosms after the stop of the injection. The rationale for selecting which indices should be used and how and when these are considered to be significantly impacted during the course of the mesocosm studies will be presented and discussed.

**TU144 Integrated Biomarker Response as a useful tool to assess landfill leachate toxicity in transplanted freshwater clams** L.F. Oliveira, C.B. Martinez, Universidade Estadual de Londrina / Ciencias Fisiologicas. Landfill leachates are complex mixtures and its toxicity assessment could be difficult and the Integrated Biomarker Response (IBR) should be a useful tool for this purpose. To test IBR applicability, transplanted freshwater clams *Corbicula fluminea* were confined for 1, 5 or 15 d at three sites of a stream (S1, S2 and S3) located consecutively after the discharge of a landfill leachate effluent. The data obtained for these sites were compared with clams recently collected from the sampling site (SS) and clams confined at a reference site (Ref). Several biomarkers, such as ethoxyresorufin O-deethylase (EROD) and glutathione S-transferase (GST) activities, multixenobiotic resistance mechanism, total antioxidant capacity against peroxy radicals, reactive oxygen species content, thiobarbituric acid reactive substances (TBARS) and metallothionein content, were measured in gills and the IBR was calculated. Also, the concentrations of several metals were determined in sediment samples. The results demonstrated that all the sites after the landfill leachate were characterized by higher concentrations ( $\mu\text{g.g}^{-1}$ ) of chromium in sediment samples (SS: 2.45; Ref: 1.56; S1: 3.95; S2: 4.45; S3: 3.82) and there was a visual correlation between chromium concentration and the IBR variation after the confinement for 1 d (SS: 6.12; Ref: 9.04; S1: 10.97; S2: 23.94; S3: 23.47), 5 d (SS: 6.12; Ref: 15.56; S1: 16.29; S2: 16.29; S3: 17.13) and 15 d (SS: 6.12; Ref: 11.98; S2: 33.61; S3: 20.39). In 5 days-test this correlation became easier to visualize (SS: 5.66; Ref: 5.91; S1: 14.25; S2: 7.15; S3: 9.49) when a smaller number of biomarkers were used, especially considering the parameters of the phases I and II of biotransformation (EROD and GST activities) and TBARS. Considering

both the concentrations of chromium in the sediment and the IBR calculated using certain biomarkers, the three points after the landfill leachate could be easily separated from SS and Ref. In 1 and 5 days-tests it was not possible to separate S1, S2 and S3 from each other but after 15 days of confinement, clams of S1 died and S2 had a higher IBR value than S3 evidencing a gradient correlated with the distance from the effluent discharge.

**TU145 Assessing petrochemical effluents using mesocosms - Comparison of predicted, laboratory and mesocosm measured toxicity of petrochemical effluents** M. Comber; K. Cailleaud, Total Petrochemicals France / PERL; A. Redman, ExxonMobil / ExxonMobil Biomedical Sciences; a. BASSERES, Total; P. Baldoni-andrey, TOTAL RC; K. Den Haan, CONCAWE; G. Whale, SHELL / SHELL Technology Centre. The data described are derived from a project jointly organised by TOTAL and the CONCAWE, which has been developed to assess whether whole effluent toxicity (WET) data obtained from laboratory assessments can be used to predict effects in dynamic outdoor artificial streams (mesocosms). Toxicity studies of petroleum refinery effluents were conducted and compared to in-situ impacts, as determined using ecological indices, in the mesocosms. Details of the mesocosm study have been described in other posters. This presentation describes additional studies undertaken to assess whether the toxicity of the effluents and impacts observed in the mesocosms could be predicted on the basis of their analytical composition. Effluent and mesocosm water samples were analysed by high resolution GCxGC, enabling the hydrocarbons present in the samples to be assigned to appropriately described hydrocarbon blocks. The toxicity of these samples were then predicted using the PETROTOX model In this presentation the results of predicted and measured toxicity in both the effluents and the streams will be presented and discussed.

**TU146 Variability of four different in vitro assays for the assessment of estrogenic activity in reconstituted water samples** P. Kunz; C. Schoelau, RWTH Aachen University, Institute for Environmental Research; C. Kienle, N. Homazava, A. Schifferli, Swiss Centre for Applied Ecotoxicology Eawag/EPFL; S. Maletz, H. Hollert, RWTH Aachen University, Institute for Environmental Research; L. Werner, Swiss Centre for Applied Ecotoxicology Eawag/EPFL. In recent years estrogen receptor stably transfected transactivation assays (ER-STTA) are increasingly used to assess estrogenic activity of environmental water samples and have been suggested as suitable tools for monitoring estrogenic activities in surface waters. Such assays are of particular use as they are able to measure the overall estrogenic activity of a sample, including the potent steroids 17-beta-estradiol (E2) and 17-alpha-ethinyl estradiol (EE2), with adopted Environmental Quality Standard proposals (EQS) of 400 and 35 pg/L for E2 and EE2, respectively, both concentrations being below the analytical limits of quantification of most routine chemical methods. The use of simple, sensitive *in vitro* ER-STTA would circumvent the current detection problems and allow to test for EQS compliance, i.e. within the Water Framework Directive, by measuring the total receptor binding and activation potential of estrogens (E1, E2 and EE2) and other substances in an environmental sample by expressing their combined potency in E2-equivalents (EEQs). However, several studies have shown that different ER-STTA lead to different EEQs when analyzing the same environmental sample. Reasons for these differences are known and most likely due to the differences of the assays themselves, for example regarding their sensitivity towards certain substances. But up to now it remains unclear how to use and interpret these results for environmental monitoring. The aim of this study was to compare and interpret EEQs of reconstituted water samples assessed by four commonly used ER-STTA. Furthermore, in order to use ER-STTA for the routine assessment of water quality and EQS compliance checking, the variability and reproducibility of the assays themselves as well as the sample preparation methods (solid phase extraction) have to be known. Therefore, two yeast-based assays, the Yeast Estrogen Screen (YES, Routledge & Sumpter 1996), and its adaptation the lyticase-based

L-YES (Schultis & Metzger 2004), as well as two human cell lines, the commercial ER-CALUX® (ER-mediated Chemically Activated Luciferase gene expression, Van der Linden et al. 2008) and the non-commercial T47D-Kbluc assay (Wilson et al. 2004) were compared. Variability and reproducibility of the bioassays and the sample preparation method were assessed with reconstituted water samples containing estradiol as well as environmentally relevant mixtures (E1, E2, EE2, and BPA) with two of the four in vitro assays, the widely used YES and ER-CALUX®.

**TU147 Quality of meat processing industry wastewater in Vojvodina region, Serbia** J. Radonic, Faculty of Technical Sciences; M. Turk Sekulic, M. Vojinovic Miloradov, Faculty of Technical Sciences, University of Novi Sad; I. Spanik, Slovak University of Technology in Bratislava; M. Sremacki, Faculty of Technical Sciences, University of Novi Sad; O. Vyviurska, Slovak University of Technology in Bratislava; D. Adamovic, Faculty of Technical Sciences, University of Novi Sad; Z. Njezic, Food Institute Novi Sad, University of Novi Sad; Z. Cepic, M. Stupavski, Faculty of Technical Sciences, University of Novi Sad. Meat processing industry is one of the most important branches in Serbia. Slaughterhouses and meat processing plants are characterized by high water consumption and generated wastewater is considerable. Discharged wastewater usually contains large amount of suspended solids, nitrogen in several chemical forms, fats and oils, phosphorus, chlorides and organic matter. The appropriate wastewater treatment technologies cannot be proposed without previous detailed research of water quality. The aim of this work was to identify organic contaminants in the water discharged from the meat industry in Vojvodina region, Serbia. Sampling of three 1.5 L water samples was conducted within the second survey of the research. Samples of wastewater, after the mechanical step of treatment, were collected from the meat processing industries in Novi Sad and its surrounding. Water samples (800 ml volume) were placed in a 1000 ml glass separatory funnel and extracted with two 50 ml portions of dichloromethane for 20 minutes, using automatic shaker device. After extraction, both extracts were combined, dried and placed into heart-shape flask for evaporation to final volume of 1 ml. Large volume injection was used for introduction of 30 µl extract to GC system. The GC analysis was performed using Agilent 7890 gas chromatograph coupled to Agilent 5975 mass spectrometric detector. The system was equipped with PTV injector system. Capillary GC analysis was performed on a 30 m x 250 mm I.D., 0.25 mm df DB-FFAP column. Helium was used as carrier gas. The MSD was used in the SCAN mode for all samples. Identification of compounds was performed using Wiley7n and NIST08 mass spectrum libraries. Many chemical structures of analytes could be proposed regarding obtained mass spectra and retention data. The largest groups of compounds detected in conducted survey are benzenes, phenols and fatty acids. Also, there have been found compounds of interest which will be further elaborated in the paper.

**Acknowledgment:** This research has been supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia within the Project III46009 and Bilateral Project Serbia-Slovakia No. 680-00-140/2012-09/13 and by SRDA project No. SK-SRB-0022-11. M.S. would like to thank Visegrad fellowship program for contract number 51200592 allowing her 6 months study stay at Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, STU, Bratislava, Slovakia.

**TU148 Effect of copper on the development of antibiotic resistance genes within a sediment microbial community** C. Bartel, University of Winnipeg; M.L. Hanson, University of Manitoba / Department of Environment and Geography; C.W. Knapp, University of Strathclyde; C.S. Wong, University of Winnipeg / Richardson College for the Environment. Antibiotics enter and leave wastewater treatment in detectable levels. Their presence in waters may lead to development and proliferation of antibiotic resistance in microbial populations, an emerging environmental problem. This development may be attenuated by other contaminants, for example the presence of heavy metals from both natural and anthropogenic sources. Accordingly, we hypothesized

that when both antibiotics and heavy metals are present, the combined contaminants will induce an increase in antibiotic resistance genes (ARGs) within microbial populations in a manner greater than antibiotics alone. To test this hypothesis, we conducted bench-scale microcosm experiments, in which sediments from an antibiotic- and copper-free source were dosed with the sulfonamide antibiotic sulfamethoxazole (100 ug/L), copper (0.5 mg/g dry weight), or both within synthetic wastewater, to simulate release of effluent to surface receiving waters. Chemicals were measured in sediment and water by liquid chromatography-tandem mass spectrometry or flame atomic absorption spectroscopy, as appropriate. Antibiotic resistance was assessed by plating, as well as by quantification of ARGs by quantitative PCR. A single pulse input of "wastewater" did not result in sufficient microbial growth to assess ARG resistance. Hence, continual inputs twice per week were done. Sulfamethoxazole had a half-life in our mesocosms of approximately 4 days, consistent with photolysis as the major degradation process on this chemical. Copper addition did not appear to shift the makeup of microbial communities, in terms of carbon source preferences via BIOLOG analysis. Preliminary results suggests that copper and antibiotics together may immediately select for resistant bacteria, by decreasing survival of non-resistant microbes. Thus, the continual presence of both contaminants may act as environmental stressors, that may result in gene transfer within the population and a higher proportion of ARGs.

**TU149 Degradation and transfer of polyacrylamide in sludges, industrial and natural waters - Potential impact on aquatic ecosystem: The AQUAPOL project** [a. togola](#), BRGM / Laboratory Division; A. Guezennec, BRGM; N. Marmier, University Nice Sophia Antipolis / LRSAE; N. Desroche, NEXIDIA; C. Hurel, I. Mnif, University Nice Sophia Antipolis / LRSAE; C. Gourlay, Irstea / UR Hydrosystèmes & Bioprocédés; M. Motelica, Université d'Orléans / ISTO; Y. Adam, UNPG; S. Touze, BRGM. Flocculants are widely used in several industrial fields (mineral extracting, food processing industry, treatment of drinking water) to enhance solid/liquid separation in water containing suspended matter. In France, mineral extracting industry is the major user of flocculants. The use of flocculants enables to increase the recycling rate of process water and to decrease sludge volumes, and thus the surface of settling impoundments. Those impoundments are open system with possible exchanges into the near environment (water percolation towards the aquifers, water seepages in natural stream or rivers...), which can lead to the dissemination of flocculants into surface and groundwaters. The question of flocculants innocuousness is now arising as a new environmental issue. The targeted flocculants are composed of polyacrylamide (PAM), a polymer synthesised from acrylamide (AA) and acrylic acid. The final product contains residual acrylamide which is classified as a CMR compound. The environmental issue does not concern the PAM, which is not known as a toxic compound, but the AA and the products of PAM degradation. Although industrialists and administrative authorities in charge of environment protection regard the use of flocculants as an important environmental matter, there is no scientific and multidisciplinary study which can help them to take appropriate measures to prevent potential impact on the ecosystems. In this context, the global objective of "AquaPol" project is to study the behavior of AA, PAM and products of PAM degradation in process and natural waters and their impacts on the balance of aquatic ecosystems. The effort will be put on the particular case study of the mineral extracting industry. The heart of this project will be based upon in situ characterization of the dissemination of PAM, AA and products of PAM degradation in connexion with the physical, chemical, microbiological and hydrogeological properties of the studied environments. This study will be surrounded by analytical developments, an eco-toxicological study, a laboratory characterization of transfer and degradation mechanisms and the development of bio-hydrogeochemical model. One of the main purposes is to bring to flocculants users and administrative authorities the scientific basis which can enable them to implement the appropriate measures to prevent potential environmental damages linked to the use of flocculants.

**TU150 Source identification of pesticide and biocide contamination in a Karst aquifer** U. Bollmann, Aarhus University; T. Wagner, S. Birk, University Graz; [K. Bester](#), Aarhus University / Environmental Science. Karst aquifers are widely used as drinking water resources. Recent studies showed that karst springs can be contaminated with organic micro pollutants. Within this study the water from the different springs from one karst aquifer as well as the main sinking stream were analysed before, during and after a storm water event in order to examine the occurrence of different organic pesticides and biocides. Contaminants from both urban (tebuconazole, carbendazim, diuron, isoproturon, terbutryn) as well as agricultural origin (dichlorobenzamide, atrazine, carbendazim, isoproturon, tebuconazole) could be detected in the water with concentrations in the low ng/L

**TU151 Three-spined stickleback: Gene expression in a Sentinel Species after exposure to effluent from a sewage treatment works.** P.J. Baker, Glasgow Caledonian University / School Health Life Sciences; [C. Kneller](#), C. Engelke, C. Engelke, J. Redshaw, S. Morrison, B. Miller, Scottish Environmental Protection Agency; J. Craft, Glasgow Caledonian University / School Health Life Sciences. Previous studies have shown that gene expression can be monitored in the three-spined stickleback (*Gasterosteus aculeatus*) following exposure to environmental pollutants<sup>1</sup>. In this study male fish were collected from the River Lugar, Ayrshire, Scotland, upstream and downstream of a sewage outfall. A selection of the fish collected downstream of the outfall were then maintained for 5 weeks in clean water (DSC). The expression of 5 genes (choriogennin H, Choriogennin L, Estrogen Receptor Alpha, Estrogen Receptor Beta and Prostaglandin D2) previously shown to be sensitive to exposure to sewage effluent were then measured in the livers of the three groups of fish using qPCR. Results: Statistically significant changes in levels of expression were observed in all genes except ERBeta. The Downstream Group (DSG) had significantly higher levels of PGD2 than in the upstream (USG). The USG had significantly higher expression of Choriogennin L and PGD2 than in the DSG. When the DSG and DSC were compared the levels of Choriogennin H, Choriogennin L and ERalpha were significantly reduced showing effects of removal from the polluted water. When the expression levels of choriogennin H were compared previous data sets the DSC were similar to those collected upstream of the sewage outfall two years before and to a control set of fish collected from a clean site. DS fish had similar Choriogennin H as a set two years earlier. When compared to fish exposed to controlled levels of Ethinyl Estradiol (EE) the DS male fish had levels of choriogennin H as fish exposed to 10ng/l of EE for 4 days. Conclusion: The three spined-stickleback is again shown to be sensitive to environmental pollution, but more importantly not only are these changes in gene expression measurable but are shown to be so in a real world situation. **Hepatic transcriptomic and metabolomic responses in the Stickleback (*Gasterosteus aculeatus*) exposed to ethinyl-estradiol** Katsiadaki, I and Williams, TD and Ball, J and Bean, TP and Sanders, MB and Wu, H and Santos, EM and Brown, MD and Baker, P and Ortega, F (2009) Hepatic transcriptomic and metabolomic responses in the Stickleback (*Gasterosteus aculeatus*) exposed to ethinyl-estradiol. Aquatic Toxicology

**TU152 Responses of three fish species to remedial actions at select sewage treatment plants** G. Tetreault, University of Waterloo / Aquatic Ecosystem Protection Research Division; P. Marjan, University of Waterloo; M. Fuzzen, University of Waterloo / Biology; J. Bennett, M.E. McMaster, Environment Canada / National Water Research Institute; G.J. Van Der Kraak, University of Guelph / Department of Integrative Biology; S. Kleywegt, Ontario Ministry of the Environment / Standards Development Branch; [M.R. Servos](#), University of Waterloo / Department of Biology. Recent studies have identified significant impacts on fish populations and communities in Canada associated with wastewater effluents across an urban gradient. Alterations in endocrine function including changes in gene expression, changes in the regulation of sex steroids, and stress responses have been documented. In addition the occurrence of intersex (oocytes in testes), including visible eggs in

testes has been recently observed near wastewater outfalls. The purpose of this study was to enhance our understanding of the linkages between environmental contaminants in effluents of differing quality and biological responses in fish and fish populations. In particular this research focuses on the wastewater effluents in an urbanized reach of the watershed using chemical, biochemical and whole organism responses using caging experiments and wild fish collections. Wastewater effluent and river water samples were collected near the caged fish during the exposures (at 0, 7 and 14d) to characterize: nutrients (ammonia, nitrate/nitrite), metals, and selected pharmaceuticals (atrazine, carbamazepine, diclofenac, fluoxetine, ibuprofen, venlafaxine). This study also investigates the relative species sensitivities among Rainbow Trout, Fathead Minnow and native Rainbow Darters exposed to wastewater effluent using caging experiments. A subset of the caged fish was injected with 17 $\alpha$ -ethynylestradiol (10 mg/kg wet-weight) in corn oil or with a placebo as a positive control. Blood was collected from trout and minnows for analysis of plasma vitellogenin level. Gonad and liver sections from each of the caged fish were evaluated for gene expression (mRNA) for key receptors and enzymes in the Hypothalamus-Pituitary-Gonadal axis such as aromatase, estrogen receptor, and vitellogenin. As ammonia from the effluent is an environmental concern, gill tissue was collected for histopathology. Similar endpoints were collected from wild reference and wastewater effluent exposed Rainbow Darters. In addition, the regional municipality is currently implementing a massive infrastructure upgrades at several of the municipal wastewater plants in the watershed creating a unique opportunity to study the impacts of remedial actions. The outcome of this study will provide watershed managers with a better understanding of the relationships among contaminants and responses in fish to support decisions related to remediation and infrastructure investments.

**TU153 Tracking the effectiveness of TBT regulation in Korea: Temporal trends of butyltin compounds in finless porpoises (*Neophocaena asiaeorientalis*)** M. Choi, National Fisheries Development and Research Institute / Research Scientist; Y. An, K. Park, Cetacean Research Institute (CRI), National Fisheries Research and Development Institute; I. Lee, D. Hwang, J. Kim, National Fisheries Research and Development Institute; H. Moon, Hanyang University. The concentrations of butyltin compounds (BTs) were measured in the livers of finless porpoises (*Neophocaena asiaeorientalis*) caught off the Korean coast in 2003 and 2010, to assess the effectiveness of legislative action against BTs. The concentrations of BTs ranged from 65 to 1400 (average: 370) ng/g wet weight, within the ranges reported by previous studies. The levels of BTs in almost all the samples exceeded the suggested threshold value, implying potential adverse health effects from the BT exposures. Concentrations of BTs were significantly correlated with body length, weight, and age of finless porpoises, but were not correlated with sex. Spatial differences in the concentrations of BTs were not observed between Yellow and South Seas, while there was a significant decrease in BTs between the sampling years of 2003 and 2010. Our result indicates that the effectiveness on TBT ban has reached to marine mammals in the coastal waters of Korea.

**TU154 Butyltin concentration in Water, Sediment and Periwinkle Tissues from Miller Creek of Warri, Nigeria.** E.T. Ogbomida, National Centre for Energy and Environment / Ecotoxicology and Environmental Forensics; L.I. Ezemonye, University of Benin / Animal and Environmental Biology. Aquatic pollution by tributyltin (TBT) and its derivatives monobutyltin (MBT) and dibutyltin (DBT) has become a matter of great concern due to their distribution in water, sediment and bioconcentration in benthic organisms. Tributyltin is an organotin pesticide mainly used as antifouling agents in paints, disinfection of circulating industrial cooling waters and slime control in paper mills. Monobutyltin (MBT) and dibutyltin (DBT) are byproducts and/or metabolites of tributyltin (TBT) that are used in several household products including polyvinylchloride (PVC) plastics. To assess impact of TBT and its derivatives from Miller Creek, surface water, sediment and Periwinkles *Tympanotonus fuscatus* var *radula* samples were collected and analyzed using gas chromatography coupled with flame

ionization detection (GC-FID) with detection limit of 0.001 $\mu$ g/l. The results of the survey showed that tributyltin (TBT) and its derivatives were detected in water, sediment and periwinkle samples. In water samples TBT, DBT and MBT levels ranged from 0.01 $\mu$ g/l to 0.04 $\mu$ g/l, in sediment samples TBT, DBT and MBT levels also ranged from 0.01 $\mu$ g/l to 0.05 $\mu$ g/l while in periwinkle snails *Tympanotonus fuscatus* var *radula* TBT concentrations ranged from 0.01 $\mu$ g/g to 0.02 $\mu$ g/g while its derivatives were not detected. In all the samples, TBT was predominant. The measured mean concentrations in water, sediment and Periwinkle samples exceeded the ecotoxicological benchmark of 0.01 $\mu$ g/l recommended by US Environmental Protection Agency (USEPA) indicative of potential environmental risk. This survey provides baseline data of tributyltin compound contamination from Miller Creek and suggests further environmental monitoring of other Nigeria harbours.

**TU155 Tracking the effectiveness of TBT regulation in Korea: Temporal trends of imposex in rock shells (*Thais clavigera*)** M. Choi, National Fisheries Development and Research Institute / Research Scientist; H. Moon, Hanyang University; J. Yu, Marine Environment Impact Assessment Center, NFRDI; H. Cho, Chonnam University; H. Choi, National Fisheries Research and Development Institute. We investigated the temporal trend in contamination of butyltin compounds (BTs) along the Korean coast using imposex and tributyltin (TBT) burden in gastropods (*Thais clavigera*) as a biomonitor. *T. clavigera* were collected from 26 locations with different shipping activities between 2004 and 2009 after restrictions on TBT-based antifouling paints were imposed in Korea. In the present study, imposex indices and TBT tissue concentrations significantly decreased over time from 2004 to 2009, confirming effectiveness of TBT regulation. However, the imposex in 2009 samples was still found. Significant high imposex indices and TBT tissue residues were found in large ports containing commercial and ferry services, compared with small ports and background areas. Imposex indices in background areas had decreased to zero, suggesting recovery from imposex caused by BT contamination. These results suggest that recovery from TBT contamination occurs in areas with very low maritime activities faster than areas with high maritime activities.

**TU156 Persistent Organic Pollutants in Three Whales Species from Brazilian Coast** J. Leonel, Universidade Federal do Rio Grande; S. Taniguchi, University of São Paulo / Physical Oceanography department; J. da Silva, Cidade universitária; S. Siciliano, J. Moura, Escola Nacional de Saúde Pública, da Fundação Oswaldo Cruz; R.C. Montone, Instituto Oceanográfico Universidade de São Paulo / Departamento e Oceanografia Física Química e Geológica. Cetaceans which have a long life-span and a low capacity to metabolize the organic pollutants are subjected to long-term accumulation and consequently probable chronic toxic effects from the persistent organic pollutants (POPs). Therefore, POPs have been widely studied in different marine mammals species around the world. However, these data are still scarce from Brazilian coast species, especially for whales. This study aims to evaluate the occurrence of PBDEs, PCBs and chlorinated pesticides in blubber samples from three whale species: Bryde's whale (*Balaenoptera brydei*, n=3), dwarf minke whale (*Balaenoptera acutorostrata*, n=1) and southern right whale (*Eubalaena australis*, n=2). Dwarf minke whales occur in polar, temperate, and tropical waters, whereas Bryde's whales prefer highly productive tropical, subtropical and warm temperate waters worldwide. Southern Right whales occur from temperate to polar latitudes. Neither PBDEs nor PCBs were detected above the detection limit and the few chlorinated pesticides detected presented levels lower than those found in other cetaceans from Southern Hemisphere. Concentrations of p,p'-DDE in dwarf minke whales and Bryde's whales (81,46 and 46,99 ng g<sup>-1</sup> ww, respectively) were one order of magnitude higher than in Southern Right whales (8.5 ng g<sup>-1</sup> ww). This difference can be related to the diet preferences because the primary food source for Southern Right whales is copepods and krill, while dwarf minke whale and Bryde's whales diets, besides zooplankton, include also small schooling fish, such as anchovies, which belong to a higher trophic level. The lack of other DDTs, especially the p,p'- DDT,



indicates no recent input of DDT. Mirex was detected at same level in the three species (1.54 – 6.15 ng g<sup>-1</sup> ww); whereas heptachlor and HCB were detected only in Southern Right whale, 4.57 and 34.70 ng g<sup>-1</sup> ww, respectively. Although POPs levels are low, they showed that these species are exposed to these pollutants and suggest that their accumulation are related to their feeding.

**TU157 Toxic effects of fipronil and its photodegradation products on zebrafish embryo development** B. de Campos Ventura-Camargo, Institute of Biosciences, São Paulo State University (UNESP) / Department of Biology; M. Casado, Environmental Chemistry; E. Oliveira, IDAEA CSIC / Environmental Chemistry; E. Prats, CSIC; S. Pelayo, Institute of Environmental Assessment and Water Research (IDAEA-CSIC); S. Diez, IDAEA-CSIC / Environmental Chemistry; M. Marin-Morales, Department of Biology Institute of Biosciences, São Paulo State University (UNESP); B. Pina, IDAEACSIC / Environmental Chemistry. Agriculture has been considered one of the sectors that cause more environmental contamination by releasing different toxic chemicals to the environment. Fipronil is a phenylpyrazole insecticide extensively used in tropical and subtropical crop applications. Both the parental compound and its photodegradation products have long been considered a potential toxic risk to many non-target species. We present here an analysis of the acute toxicity of fipronil and of their photodegradation products using zebrafish embryos (days 1-7 post fertilization). Newly spawned embryos (4 hpf) were exposed to four concentrations of fipronil (either non-irradiated or irradiated with a solar xenon lamp emulating tropical irradiation levels), from an estimated relevant environmental concentration (5 ppb) to a high sublethal concentration (500 ppb, nominal concentrations in fish water), using 3,4-dichloroaniline 3,7 mg/l as positive control. The data show a dose-dependent induction of different toxic effects, including morphological and behavioural abnormalities and death. These effects occurred at 5 to 10 times lower nominal concentration when the fipronil stock was previously exposed to solar irradiation, confirming the higher toxicity of its photodegradation products, which were produced even during the shortest irradiation treatment (1h 30 min). This would be especially critical in tropical regions, with high solar irradiation and where fipronil is widely used. We concluded that residual dosages of the fipronil insecticide resulting from the leaching of culture fields near to water resources can negatively interfere with aquatic ecosystems, mainly due to the toxic potential of such chemical for promoting abnormalities and death of developing fish and other additional non target organisms. These data illustrate the case in which a given pesticide may originate highly toxic photoproducts when exposed to high irradiation levels, as it occurs in the tropics.

**TU158 Environmental evaluation through analysis of linear alkylbenzene in sediments as an indicator of exposure to sewage in coastal area, Santos, Brazil** S.t. Sasaki, Instituto Oceanográfico da Universidade de São Paulo / Oceanografia Química; S. Taniguchi, M.C. Bicego, Instituto Oceanográfico da Universidade de São Paulo. The contamination of coastal areas by human activities has been intensified in recent decades. The discharge of sewage is a major source of pollution in coastal regions. In Brazil it is estimated that only 20% of the sewage receive treatment before to discharge to the sea. The introduction of sewage into aquatic ecosystems can be monitored by analysis of sediment. An organic chemical markers are the linear alkylbenzenes (LABs) are used in many studies. The main use of LABs is the formation of linear alkylbenzene sulfonated (LAS), used in the formulation of detergents, but 5% of LABs remain in the formulation as residue and once in marine environment can be preserved in sediments. The aim of this study was to evaluate the introduction of sewage in the marine environment by the analysis of linear alkylbenzenes in sediments in a highly urbanized coastal region (Santos, Brazil). Santos is in the Brazilian southeastern coast, where the most important industrial complex of the country and the biggest port of Latin America are located with important contribution of waste disposal. The 15 samples were extracted with a mixture of hexane / dichloromethane 50% and purified on a column of silica and alumina. Identifications and

quantitations were made by gas chromatography with mass spectrometry detector (GC/MS). The LABs concentrations found ranged from 6.7 to 1234 ng g<sup>-1</sup> (dry weight), which are comparable to other studies that evaluated the densely urbanized around the world the contribution of sewage. In relation to individual LABs, samples were enriched with high input of C<sub>18</sub>-LAB isomers followed by C<sub>17</sub>, C<sub>16</sub> and C<sub>15</sub>-LAB. Since the C<sub>18</sub>-LAB isomers are more hydrophobic than their homologues<sup>13</sup>, they can be associated to the particulate material, and thus be available for the sediment. The results of LABs in the sediments analysed showed they are good indicator of sewage input in coastal areas.

**TU159 Analysis of volatile organics in the industrialized region of Tropical Singapore using Thermal Desorption Gas Chromatography Mass Spectrometry** G. Wong; S. Ng, R.D. Webster, Nanyang Technological University. Singapore is a developed nation in tropical Southeast Asia that has one of the world's largest petrochemical industries on its mainland and its western offshore islands. Volatile organic compounds (VOCs) are pollutants that are potentially emitted from chemical industries during chemical production and waste disposal. Although the detrimental effects of VOCs to the global environment and human health have been well-established and widely known, the monitoring of atmospheric VOCs by regulatory boards in Singapore are not complete. The National Environment Agency, which is the leading public organization responsible for monitoring air quality in Singapore, only reports the pollutants standards Index (PSI) which measures 5 major atmospheric contaminants (particulates > 10µm, SO<sub>2</sub>, CO, O<sub>3</sub> and NO<sub>x</sub>). A wider range of VOCs for analysis are normally limited to indoor emissions. In addition to that, Singapore suffers serious air quality problems annually from the burning of forests by neighbouring countries to create new land for palm oil plantations. Unless extensive atmospheric investigations are conducted continuously, there is no other way in knowing accurately what kind of VOCs are present. 48 common VOCs were detected in the air near the industrialized region of the nation by sorbent tubes active sampling and Thermal Desorption Gas Chromatography Mass Spectrometry (TD-GCMS). An analytical method for these compounds was established and validated for these 48 compounds. Method validation characteristics such as linear regression coefficients of at least 0.99, breakthrough values of 5% or lower, high repeatability with relative standard deviation below 10% were reported. Criteria established by the United States Environmental Protection Agency (USEPA) for sorbent tube sampling (EPA TO-17) compendium method were achieved for most target VOCs.

**TU160 PCBs, organochlorine and currently used pesticides in precipitation and high volume air samples from the watershed of Lake Victoria, Uganda** K. Arinaitwe, Makerere University / Chemistry Department; D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division; B.T. Kiremire, Makerere University / Department of Chemistry; T. Harner, Environment Canada / Atmospheric Science & Technology Directorate; P. Fellin, Airzone One Inc; R. Hecky, University of Minnesota, Duluth campus; C. Teixeira, Environment Canada; D. Mubiru, Kawanda Agricultural Research Institute. The large surface area of Lake Victoriaprovides for elevated potential for atmospheric loading of pollutants. Persistent organic pollutants (POPs) have been detected in the lake's sediments and fish samples but source elucidation has not been done. Towards this end, we measured PCBs, organochlorine pesticides (OCPs) and currently used (CUPs) pesticides in air and rain samples, from 2008-2010, at Entebbein Uganda. Air samples were taken weekly (on a 6 day-rotation basis) using a TE-1000 PUF high volume air sampler (Tisch Environmental Inc., OH, USA) and polyurethane foams. Precipitation samples were collected using an automatic MIC-type sampler, equipped with a rain sensor, and Teflon XAD-2 wet-packed columns. The samples were analysed for PCBs, and OCPs using GC-ECD and for CUPs using GC-negative ion MS. Preliminary partial results for air data (for 21 samples taken weekly from 2008-2009) show the tri-, penta- and octa-PCBs to be the predominant congeners. SPCBs (sum of 107 individual and co-

eluting congeners) had a mean concentration of 265.5 pg/m<sup>3</sup> (range 56 – 1729 pg/m<sup>3</sup>). P,p'-DDT and p,p'-DDE were the major OCPs with SDDT levels varying between 7-80 pg/m<sup>3</sup>. The frequently detected CUPs were chlorpyrifos, lindane and  $\gamma$ -endosulfan. The levels in these air samples will be compared with previous measurements in air samples from the same watershed. The complete data from all the 56 air samples and 10 rain samples will be presented. Temporal trends of levels of these compounds in high volume air samples and precipitation fluxes will be discussed. Air mass back trajectories will be used to estimate directional sources of the sampled air while precipitation profiles will be used to estimate wet loading of these chemicals into Lake Victoria.

**TU161 Assessment of the natural variation in HOC sorption to dissolved organic carbon.** S. Heijden, M.T. Jonker, Utrecht University. Being an important determinant of bioavailability of hydrophobic organic chemicals (HOCs) in environmental systems, dissolved organic carbon (DOC) has endured as an object of interest for environmental chemists for several decades. As a result, a sizeable amount of data is currently available on DOC-water distribution coefficients (K<sub>doc</sub>). To bridge the gap between observation and application, attempts have been made to capture the sorption behavior of these chemicals to DOC within the neatness of a model. This however proved problematic, as a large degree of variation exists among K<sub>doc</sub> values in the literature. This variation amounts up to nearly 2 log units, which presumably is related to the non-conformity of methods, labs, and DOC-samples. Though for very hydrophobic HOCs sorption to DOC is of greater relevance, their reported K<sub>doc</sub> values are generally also affected by more uncertainty due to a more pronounced disagreement between methods. In an attempt to study the separate variation in K<sub>doc</sub> values solely introduced by the type of DOC, a single, robust method (i.e. passive sampling) was applied in a single lab to measure K<sub>doc</sub> values for more than 80 HOCs (including PAHs, PCBs, organochlorine pesticides, chlorobenzenes, and petrochemicals) spanning a broad log K<sub>ow</sub> range from about 4 to 10. DOC samples included filtrates of 9 fresh European surface waters of distinctly different origin and 2 reference materials. Results indicated that differences in K<sub>doc</sub> values varied with the compound group and were typically as large as 0.5 log units, although differences of between 1 and 2 log units were not uncommon for PAHs and petrochemicals. The variation was not easily explained by DOC characteristics (UV spectra, size and type fractions), although for some chemicals a significant relationship was observed with the pH of the DOC solution. These data emphasize the oversimplification of the generic regressions with log K<sub>ow</sub> currently used for K<sub>doc</sub> estimation.

**TU162 Biocides emissions from renders: Comparison of desorption equilibria and leaching from walls** U. Bollmann, Aarhus University; K. Styszko, University of Science and Technology Krakow; K. Bester, Aarhus University / Environmental Science. Houses are increasingly equipped with thermal insulation to increase the energy efficiency of heating. The renders of these systems are often equipped with biocides, such as terbutryn and carbendazim, to protect the façade surfaces of the buildings. It is known that these biocides can be mobilized from the materials if rainwater gets into contact with them and are then leached out. Successively they may reach surface waters. However, the exact leaching processes are under discussion. In the recent study the leaching of biocides from façade renders in a semi-field experiment was compared to desorption equilibria experiments performed on pulverised material in the laboratory. The desorption constants from the laboratory experiments were documented under diverse conditions (pH value, organic modifier of the plaster etc.). Additionally the initial emissions of the semi-field experiments were assessed. During the first months the 1 m<sup>2</sup> artificial walls in the field emitted up to 150 mg single biocide each per rain event. Desorption coefficients ranged between 1 and 1000. For the comparison also other physico-chemical properties, such as water solubility and octanol-water coefficients, were taken into account.

**TU163 Sorption of xylene isomers by selected soils and soil colloids** M. Balseiro, Universidad de Santiago de Compostela; C. Monterroso,

Soil Science. Xylene isomers (ortho-, meta- and para-xylene) are volatile and soluble compounds included in the BTEX group. These compounds are carcinogenic and affect the central nervous system in humans, being petrochemical activities the main source of environmental emissions. The retention of xylenes by soils and soil components affects its mobility and final environmental fate and strongly determines the efficiency of decontamination techniques. A comparative study of the retention of xylene by a selection of soils and soil colloids was made to determine the influence of the physicochemical properties of the sample, the concentration of xylene, ageing, pH and temperature on retention. For this purpose, spiked samples were used and analyzed by headspace-gas chromatography-mass spectrometry. The presence of organic matter had a significant effect on the retention of xylenes by soils or colloids and the retention was even perceptible at the moment of contamination. Retention was so significant that very high temperatures were required to achieve a significant release of xylenes from soil. The retention by inorganic soils and colloids was much weaker and the migration of xylenes was considerable, even at room temperature. The pH had little effect on xylenes retention and there were only slight differences in the retention by inorganic samples. This study contributes to understand the environmental behavior of xylene isomers and will be useful for optimizing remediation of contaminated soils and risk assessment.

**Acknowledgements.** This research was supported by the Consellería de Innovación e Industria of Xunta de Galicia (INCITE08PXIB200136PR) and by the Ministerio de Ciencia e Innovación and FEDER (CTM2009-14576-C02-01).

**TU164 Development and results of the SPME-method to determine sorption of cationic surfactants to clay and organic matter.** Chen, Institute for Risk Assessment Sciences; N. Timmer, Utrecht University / IRAS; J.L. Hermens, Utrecht University; S.T. Droge, Utrecht University / Analytical Environmental Chemistry. Cationic surfactants are a challenging but poorly studied group of environmental contaminants. Only little data is available on the environmental fate and ecological toxicity of these hydrophobic, positively charged compounds. A SPME-method to sample the free concentration of cationic surfactants was developed, using 7  $\mu$ m polyacrylate coated fibers. After successful validation and calibration, this fiber method was used to determine sorption of different cationic surfactants (i.e. various linear alkyltrimethylammonium and dialkyldimethylammonium structures, and individual benzalkonium structures) to clay and organic matter, the two most important sorption sites in natural soils and sediments. Concentrations in samples were measured using LC-MS/MS, which was sufficiently sensitive and selective for adequate identification and quantification of all samples. In addition to sorption to soil components, adsorption to glassware and various septa was also evaluated. The methods and data originating from these experiments will be used to assess and predict the bioavailability of cationic surfactants in fate studies with natural sediments.

**TU165 Adsorption/Desorption testing of complex mixtures or UVCB chemicals** M. Gassen, Harlan Laboratories / Metabolism EFate; A. Wehrhan, Harlan Laboratories. The determination of K<sub>d</sub> and/or K<sub>oc</sub> values is of importance to understand the mobility of a chemical in the environment. Several test methods are available, and are well established for chemically defined single compounds. However through REACH requirements, testing strategies are needed to obtain adsorption/desorption data for more or less well defined chemical mixtures, such as polymeric mixtures. ECHA guidance acknowledges that for complex mixtures, a single K<sub>d</sub> or K<sub>oc</sub> value may not be adequate, and recommends a representative value or a range of values. If the batch equilibrium method (OECD 106) is used, the analytical method for quantification of the UVCB is a crucial element of the testing strategy, and we discuss the advantage of generic versus specific methods discussed. We provide arguments in favour of specific methods like LC-MS/MS and show examples how to select representative components of the mixture based on chain length, molecular weight, or clogP.

**TU166 Sorptive Bioaccessibility Extraction and Sorptive Physiologically Based Extraction of soils: combining a mobilization medium with an absorption sink** V. Gouliarmou, Aarhus University Science and Technology Faculty / Environmental Chemistry and Microbiology; E. Christiansen, Aarhus University / Department of Environmental Science; C.D. Collins, Reading University / Department of Geography and Environmental Science; P. Mayer, Technical University of Denmark / Department of Environmental Engineering. In principle, soil bioaccessibility extraction methods are simple dissolution experiments, where the fraction of compounds that is transferred to the extraction medium is measured and considered to be bioaccessible. For hydrophobic organic chemicals (HOCs) such techniques can lead to underestimation of bioaccessibility when the capacity of the extraction medium is insufficient to provide infinite sink conditions for the target compounds. A Sorptive Bioaccessibility Extraction (SBE) method was thus developed and validated, which integrates the key processes of desorption from the matrix and subsequent consumption or depletion.[1] Cyclodextrin was used as a diffusive carrier to enhance desorption from the matrix, while a silicone rod was used as a dominating sink that continuously absorbed the HOC molecules from the cyclodextrin solution. The silicone rod was then solvent extracted and the HOCs measured by GC-MS. For wood soot, the SBE method yielded PAH bioaccessibility estimates that were 3 - 24 times higher compared to a cyclodextrin extraction without a sink. This new bioaccessibility extraction concept was extended to the area of human bioaccessibility research, by exchanging the cyclodextrin with simulated digestive fluids. This resulted in the development of a Sorptive Physiologically Based Extraction Test (sorptive PBET).[2] The study demonstrated that the inclusion of an absorption sink into an established bioaccessibility extraction method (1) is rather simple, (2) can have a major impact on the obtained results, especially for the more hydrophobic compounds and high  $K_{oc}$  samples, (3) increased the extraction capacity of the system by orders of magnitude and (4) can simplify and increase the robustness of the analytical procedures. [1] Gouliarmou, V.; Mayer, P. 2012 Sorptive Bioaccessibility Extraction (SBE) of Soils: Combining a Mobilization Medium with an Absorption Sink Environ. Sci. & Technol. 46: 10682-10689 [2] Gouliarmou, V.; Christiansen, E.; Collins, C. D.; Mayer, P. 2012 Sorptive physiologically based extraction of contaminated solid matrices: incorporating silicone rod as absorption sink for hydrophobic organic contaminants. Environ. Sci. & Technol. In revision.

**TU167 Sequential extraction procedures to characterize non-extractable residues** A. Eschenbach, University of Hamburg / Institute of Soil Science; K. Oing, University of Hamburg; E. Thumm, German Federal Environment Agency (UBA). Non-extractable residues (NER) are defined as chemical species that cannot be extracted by methods which do not significantly change the chemical nature of these residues or the structure of the matrix. For market authorization of active substances and agents (e.g. pesticides) NER formation will be quantified in transformation studies. At present NER formation is considered mainly as substance dissipation and sink for substances. Depending on different processes of NER formation some parts of NER could be remobilized. The potential environmental hazard of these NER should be assessed. So there is a need to understand the interrelation of the specific formation processes of NER and their availability or remobilization potential for environmental risk assessment. Experimental data indicate that NER could result from a - fixation of chemicals by covalent bonding to organic matter - physical entrapment of environmental chemicals in the organic matter - transformation of chemicals into biogenic substances. A literature survey was carried out to prove whether the application of different extraction approaches could differentiate binding types of NER originating from various active substances from pesticides, biocides and pharmaceuticals. Also the aim of this study is to develop a sequential extraction procedure to characterize the NER-fractions with different hazardous potential.

**TU168 Modelling kinetics of POPs uptake in different soils** K. Smidova, Faculty of Science; C. van Gestel, Vrije Universiteit

Amsterdam / Ecological Science; J. Hofman, Masaryk University RECETOX / Faculty of Science. Chemical properties such as hydrophobicity, bioavailability in soil, and biodegradation of chemicals are among the important factors which may significantly affect the bioaccumulation process in soil organisms. Good understanding of these factors may help in risk assessment and data extrapolation between different soils. When searching the literature, the information about kinetics of POPs uptake is incomplete. The main reason is probably the time required for such experiments. This study attempts to model uptake kinetics of five POPs (phenanthrene, pyrene, DDT, lindane, and PCB 153) in earthworms *Eisenia fetida*. Six soils with the wide range of soil properties: TOC (0.47 – 20.2%), CEC (187 – 450 meq/100 g), C:N (2.9 – 16.8), and different land use (arable soils, grasslands, forest soils) were studied. These soils might represent the land sources of the Czech Republic. The earthworms were exposed in laboratory contaminated soils from 1 to 21 days (odd numbers only). Chemical degradation in soils was also accounted. The results showed: Using a one-compartment model it was possible to model uptake kinetics in *Eisenia fetida*, biodegradation strongly influenced uptake of PAHs, the number of days required to reach equilibrium concentration differed between soils and chemical compounds, and uptake kinetics were significantly dependent on TOC content.

**TU169 Novel methodological procedure to evaluate the polycyclic aromatic hydrocarbons uptake by *Medicago sativa* in phytoremediation** C. García-Delgado Autonomous University of Madrid / Agricultural Chemistry; F. Yunta, Universidad Autónoma de Madrid / Geology and Geochemistry; A. Garate, E. Eymar, Universidad Autónoma de Madrid. An original methodological procedure was validated in order to quantify the potential of *Medicago sativa* culture to be used for polycyclic aromatic hydrocarbons (PAH) phytoremediation. A new device based in isolation of two-compartment root-shoot was developed. Washed quartz sand was spiked with an acetone solution of fluorene (Flu), phenanthrene (Phe), anthracene (Ant) and pyrene (Py) to achieve 100 mg kg<sup>-1</sup> of each PAH. Polluted sand was introduced in methacrylate cylinders without and with spent mushroom compost (SMC) to enhance PAH biodegradation. Onto each cylinder, other similar cylinder was placed but containing either clean sand or clean sand plus a plant of *M. sativa*. Both, upper and lower cylinders were separated with a metallic mesh allowing the root crossing. Biodegradation process was keeping for 14 days at 25°C. Content of each PAH in sand, root samples (lower and upper cylinders), and shoots from *M. sativa* were determined by HPLC-PDA. None PAH were detected in upper sand. The PAH content in sand+SMC treatment were lower than sand with *M. sativa*. However, *M. sativa* treatment decreased the 4PAH percentage (22%). No biodegradation rate was enhanced when PAH contents were compared between SMC+*M. sativa* and SMC treatments. Significant concentrations of all tested PAH were found in lower roots (737 – 2682 mg·kg<sup>-1</sup>), upper root (150 – 1124 mg·kg<sup>-1</sup>) and shoots (0.70 – 8.91 mg·kg<sup>-1</sup>) suggesting that PAH translocation in *M. sativa* was taking place. The lower roots growing in sand+SMC did not present differences of Flu and Phe concentration and showed higher concentration of Ant and Py than roots growing in sand. However, the final PAH concentration in sand+SMC was lower than sand, therefore SMC enhanced the PAH availability to plant. The PAH translocation factor (TF) in *M. sativa* between lower and upper root and shoot were determined. TF values in lower and upper root were between 11 and 42%. *M. sativa* grown on sand+SMC presented lower TF as general pattern than *M. sativa* grown on sand. The TF between upper root and shoot were very low (0.31 – 2.14%), so the PAH translocation to shoot appear to be impaired. Therefore, phytostabilization appears more likely than phytoextraction to PAH phytoremediation. Consequently, *M. sativa* was able to enhance the PAH degradation in sand, accumulate high concentrations of PAH in root and translocate a small part of PAH to shoots. The device suggested allow us to check PAH mobilization in plant tissues.

**TU170 SOLID PHASE MICROEXTRACTION OF PERSISTENT ORGANIC POLLUTANTS FROM NATURAL AND ARTIFICIAL**

## SOILS AND COMPARISON WITH BIOACCUMULATION IN EARTHWORMS

**L. Bielská**, Research Centre for Toxic Compounds in the Environment Faculty of Science Masaryk University / Research centre for toxic compounds in the environment; K. Smidova, J. Hofman, Masaryk University, Faculty of Science / Research centre for toxic compounds in the environment. Total organic carbon content (TOC) has been suggested as an extrapolation basis between different soils and identified as an important parameter in the sequestration of soil pollutants. The aim of the study was to verify the role of TOC in soil for pollutant fate by measuring the extractability of pollutants by means of solid-phase microextraction (SPME) from three natural and three artificial soils having comparable TOC and aged for 56 days. Further, the study aimed to assess the suitability of SPME to predict bioaccumulation of tested compounds by *E. fetida* in these soils. The results revealed decreased extractability of tested compounds with increasing TOC and pollutant-soil contact time. The extractability was higher from natural soils compared to artificial analogues having comparable TOC. The results also showed the SPME was able to precisely ( $r = 0.66-0.96$ ) estimate the bioaccumulation (*E. fetida*) of tested compounds. Based on these results, we assume that extrapolation from natural to artificial soils cannot be based solely on TOC. We also suggest that SPME with its cost and time saving features can be used as a surrogate for bioavailability assays.

## TU171 Bioaccumulation of Nonylphenols (NPs) and Polycyclic Aromatic Hydrocarbons (PAHs) in Tapes Philippinarum in the Venice Lagoon (Italy)

**n. ademollo**, IRSACNR; L. Patrolecco, Water Research Institute - National Research Council; S. Valsecchi, Water Research Institute Italian National Research Council IRSACNR / Water Research Institute; S. Polesello, Water Research Institute CNR / Water Research Institute; V. Matozzo, M. Marin, University of Padova / Department of Biology. Biomonitoring is a scientific method for assessing the health status of the environment to natural and synthetic chemicals, based on sampling and analysis of organism's tissues and fluids. This technique relies on the knowledge that chemicals induce markers reflecting this exposure: the marker may be the chemical itself. Different models have been used to study the bioavailability and the bioaccumulation of contaminants: the Biota-Sediment Accumulation Factor (BSAF) predicts that in the organism's tissue the chemical residue can be estimated from the partitioning of the compound between the lipid fraction and the organic carbon in the sediment. In this work the BSAFs of NP and PAH in *Tapes philippinarum* from the Venice lagoon were calculated, with the aim to verify if the routine biomonitoring studies are reliable in contaminated sites. A field campaign from October 2003 to June 2004 in three sites of the Venice lagoon was realised: Marghera characterised by high contamination levels of industrial origin, Campalto located close to a sewage treatment plant and Poveglia benchmark site, located near one of the lagoon mouth. Results showed that Marghera and Campalto sediments were more contaminated by NPs and PAHs than Poveglia. In each sampling date, the highest NP concentrations (64-247 ng/g d.w.) were measured in Marghera sediments while the lowest one (28-99 ng/g d.w.) in Poveglia. The higher mean concentrations of PAHs were found in Marghera sediments in April and June (516 and 364 ng/g d.w. respectively), while in October and January the higher values were detected in Campalto, with a mean PAH concentration of 821 and 668 ng/g d.w., respectively. Different trends were observed in the contamination of clams: the Poveglia site, in April, showed the highest NP and PAH contamination with values, respectively, of 181 and 1160 ng/g f.w.. From these values, BSAFs were calculated, which resulted always highest at Poveglia for both classes of contaminants. The BSAF trend appears to be inversely related to the contamination level of the sites. Environmental stressors can have an impact on the health status of the clams, altering their normal feeding behavior and, as a probable consequence, reducing the bioaccumulation rates. This could mean that the concentration measured in biota are not necessarily representative of the biota exposure with the risk of losing the direct correlation between sediment and biota concentrations in contaminated sites.

## TU172 Influence of sorption on bioavailability and biodegradation of secondary alkane sulfonates (SAS) in marine sediments

**R. Baena-Nogueras**, University of Cadiz / Physical Chemistry; P. Lara Martin, Universidad de Cadiz / Physical Chemistry; E. Gonzalez-Mazo, University of Cadiz / Physical Chemistry. Coastal marine ecosystems are often influenced by wastewater discharges from surrounding populations. Surfactants, with a worldwide production over 10 million tons per year, are among the organic contaminants showing highest concentrations in wastewater. Most available studies on this topic deal with the distribution and fate of alkylphenol polyethoxylates (APEO) in aquatic systems, as some of their degradation intermediates are endocrine disruptor compounds (EDC). Linear alkylbenzene sulfonates (LAS) have also been extensively studied as they show the highest worldwide production volumes. Environmental data on many other surfactants, however, are still scarce. This is the case of secondary alkane sulfonates (SAS), one of the major anionic surfactants used in the market of dishwashing, laundry and cleaning products. Although this compound is easily removed during wastewater treatment, previous studies have shown that SAS and other anionic surfactants such as LAS accumulate in sludge as they are hardly biodegraded during anaerobic digestion. Recently, anaerobic degradation of LAS was confirmed in marine sediments due to the presence of sulfate reducing bacteria. This research has focused on determining whether SAS are biodegradable or not in absence of oxygen in the marine environment, and, if that happens, on the role of sorption on the speed of the biodegradation. First, sorption experiments were performed using several amounts of sediments (0.5-5 g) and SAS concentrations (1-10 mg/kg). Distribution coefficients ranged from 54 to 1505 L/Kg depending on the SAS homologue considered. Thus, we observed that the sorption capacity was much higher for those homologues having longer alkyl chains (e.g., C17-SAS) rather than for more polar homologues such as C14-SAS. Later, biodegradation experiments using anoxic marine sediments and seawater were conducted. SAS anaerobic biodegradation was observed for the first time, reaching overall values up to 98% in 166 days. Half-life values ranged from 20 days (C14-SAS) to 37 days (C17-SAS), showing that the speed of this process significantly depends on the sorption capacity and, therefore, bioavailability, of each SAS homologue.

## TU174 Successful Isolation of Effective Oil-biodegraders Growing at Low Temperature

**V. Pham, Kyonggi University**; **S. Jeong**, Kunsan National University / Dept of Environmental Engineering; J. Kim, Kyonggi University. We isolated 17 bacterial strains from oil-contaminated soils collected in March, identified them through 16S rRNA gene analysis, and found very effective petroleum-biodegraders at low temperature (10°C). During 14 days incubation, 5 strains degraded around 100% TPH in culture medium (150 mg-oil/L). Those are *Pseudomonas simiae* G1-10, *P. korensis* Gwa2-10, *P. migulae* Gwa5-10, *P. taiwanensis* Y1-4-10, and *Rhodococcus qingshengii* Gwa1-10. Other 12 strains showed 30-90% biodegradation efficiencies. Therefore, we expect that the isolates in this study will be useful for bioremediation in oil-contaminated soil and groundwater, especially during winter season. A further study will be done with oil-spiked soils mixed with/without foam. (This study was supported by the GAIA project (RE201202062))

## TU175 Comparison of different techniques to improve the biodegradability of poorly water-soluble organic chemicals in an OECD 301 test

**C. SWEETLOVE**, Research & Innovation; J. CHENELE, L'Oréal; Y. BARTHEL, C. AUCHET, M. BOUALAM, EUROFINS / Expertises Environnementales; J. Lharidon, LOREAL / Life Sciences Direction. The OECD Guidelines for the testing of the ready biodegradability of chemicals in the aquatic environment (standardized methods 301 A to 301 F and 310) indicate the method's applicability according to the chemical solubility. However, they do not provide specific recommendations upon the assessment of poorly water-soluble chemicals. Yet, it is recognized that low water solubility may lead to an underestimation of the biodegradability of tested chemical by limiting its bioavailability towards microorganisms. In the literature, only few guidance documents and publications have proposed technical

adaptations to improve the bioavailability of poorly soluble products. They show that the implementation of such adaptations can improve the results of biodegradation. This poster compares 9 operating conditions for dispersing organic chemicals with low water solubility. Test chemicals were: a solid (anthraquinone) and a liquid (isodecyl neopentanoate). The 9 operating conditions were assessed in parallel in the same ready biodegradation test using the same inoculum. The conclusion focuses on the most interesting technical adaptations and brings out their interest in a standardized test.

**TU176 Effect of *Cytisus striatus* and bacterial inoculants on HCH bioavailability and its impact on the efficiency of phytoremediation of contaminated soils** P. Santos-Ucha, Departamento de Edafología e Química Agrícola, Universidade de Santiago de Compostela; C. Becerra-Castro, Instituto de Investigacións Agrobiolóxicas de Galicia (IAG), Consejo Superior de Investigaciones Científicas (CSIC); B. Rodríguez-Garrido, A. Prieto-Fernandez, Instituto de Investigacións Agrobiolóxicas de Galicia (IAG), Consejo Superior de Investigaciones Científicas (CSIC); P. Kidd, Instituto de Investigacións Agrobiolóxicas de Galicia (IAG), Consejo Superior de Investigaciones Científicas (CSIC); C. Monterroso, Soil Science. The phytoremediation of soils contaminated by organic compounds can greatly benefit from plant-bacterial associations, however the remediation success depend on the bioavailability of the contaminant in the soil. Low bioavailability hinders biodegradation, while high bioavailability affects the survival and activity of plants and microorganisms. In previous studies, *Cytisus striatus* (Hill) Rothm with microbial inoculants have been shown to enhance the dissipation of the hexachlorocyclohexane isomers (?-, ?-, ?-, and ?-,HCH), although their efficiency depended on soil properties and plant/inoculant combination. The aim of present study was to test the effects of soil properties and plant/inoculant combination on the HCH bioavailability in contrasting contaminated soils. Soil samples were collected from the A and B horizon (A and B soil) of an alumi-umbric Cambisol in the surroundings of Santiago de Compostela (Galicia, NW Spain) and contaminated with 0 or 65 mg HCH kg<sup>-1</sup>. *Cytisus* plants were either not inoculated (NI), or inoculated with the endophyte *Rhodococcus erythropolis* ET54b and the HCH-degrader *Sphingomonas* sp. D4 on their own or in combination (ET, D4 and ETD4), and grown for 4 months. Soils were analysed for total and bioavailable HCH after 0, 2 and 4 months. HCH was analysed in plant after harvest. Bioavailable HCH was significantly lower in the A soil than B soil due to the HCH retention by organic matter. High bioavailable HCH led to phytotoxicity and low plant growth was reported in the B soil. Both plant and microbial inoculants significantly modified HCH bioavailability, and these effects were soil-,plant/inoculant- and isomer-dependent. Bioavailability was directly related with HCH dissipation. **Acknowledgements.** This research was supported by the Consellería de Innovación e Industria of Xunta de Galicia (INCITE08PXIB200136PR) and by the Ministerio de Ciencia e Innovación and FEDER (CTM2009-14576-C02-01/02).

**TU177 Enhanced biodegradation of diesel fuel present in a cold-soil-condition by surfactant foam spraying** S. Jeong, Kunsan National University / Dept of Environmental Engineering; J. Jeong, Kunsan National University; J. Kim, Kyonggi University. Bioremediation of fuel contaminated soil has been ineffective in winter season or a cold condition. The number and activity of micro-organisms falls down in low temperature conditions. Continuous biodegradation is needed even in winter if the contaminated site should be reused as planned. This study isolated low-temperature-microorganisms capable of degrading fuel from fuel-contaminated-soil and mixed them with a surfactant solution to prepare the remedial solution. Surfactant foam was generated with the remedial mixture solution and sprayed onto the diesel contaminated soil. Degradation of diesel was monitored as time elapsed. All experiments were conducted in a cold-chamber below 10 °C. The experiment results showed that surfactant foam being placed on the soil insulated soil from cold air temperature and helped micro-organisms to keep their activity and degrade diesel fuel. (This study was supported by the GAIA project (RE201202062))

**TU178 Chemotactic biomobilization of PAH-degrading bacteria with fungal zoospores** R. Sungthong, Department of Agrochemistry and Soil Conservation; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiología / Agroquímica y Conservación del Suelo. Within the framework of bioavailability limitations to functional bacteria toward their carbon sources like PAHs, we present here a novel biomobilizer approach by using fungal zoospores. We studied zoospores from a rhizosphere oomycete, *Pythium aphanidermatum* to assist the bacterial dispersal of the motile, naphthalene degrading strain *Pseudomonas putida* G7. The concept was examined in several aspects. First, we evaluated possible negative influences between zoospores and bacterial cells. The bacterium did not influence the induction and production of zoospores by the oomycete. Cellular morphology and motility of the zoospores tested using a microscope connected to CellTrak 1.5 motility analysis software did not reveal negative influences. Secondly, co-motility analyses by a modified capillary assay with suspensions containing bacterial cells and zoospores evidenced that the zoospores promoted the dispersion of the bacterial cells. This was supported by the significantly higher numbers of bacterial cells and zoospores entering into capillaries filled with an attractant for zoospores (ethanol), as compared to controls for attractant and zoospores alone. Finally, we obtained further evidences by optical microscope observation, confirming that *P. putida* cells exhibited positive chemotaxis to encysted zoospores, what supported a mechanism for this co-mobilization based on chemotaxis. This novel biomobilizer concept might be developed further as to improve inoculants for current bioremediation strategies.

**TU179 Tactic response to nanoparticles induces bacterial deposition on porous materials and NAPLs** C. Jimenez Sanchez, Inst de Recursos Naturales y Agrobiol de Sevilla; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiología / Agroquímica y Conservación del Suelo. Nanoparticles may be toxic to motile bacteria but at sub-lethal concentrations can cause specific tactic responses, characterized by repellence and a motility pattern built on abrupt changes of direction in individual cell trajectories. Our research focuses on how these responses can promote bacterial adhesion to solid surfaces (as sand or silicone) and the accumulation at the interface of nonaqueous-phase liquids (NAPLs) and water. Indeed, our results suggest that the cell motility pattern of a naphthalene degrader bacterium (*Pseudomonas putida*) caused by low concentrations (0.2 mg/L) of silver nanoparticles (AgNPs) can help bacterial cells to intercept with the surface of porous materials and NAPLs. We used a sand porous material packed in a column system to test the effect of AgNPs on bacterial transport and deposition, together with batch adhesion experiments performed to test the bacterial affinity to sand and to silicone. We also characterized the motility behavior by capillary assays and by analyzing the movement of individual cells through computer-assisted motion analysis, determining the rate of changes of direction. An adhesion method, already described for hydrocarbons, was also applied for the study of the effect of AgNPs on the bacterial adhesion to the interface of liquid DEHP, which was used as a model NAPL. We suggest that nanoparticles may be used to promote bacterial adhesion to surfaces of solids, for example in a bioreactor, to promote biodegradation of sorbed pollutants, or to improve the biodegradation of a contaminant in a NAPL, by enhancing the bacterial colonization of the interface between the NAPL and the aqueous phase.

**TU180 Rhizosphere enhances PAH bioavailability through root exudates: DOM-related effects and chemotaxis** M. Cantos, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC); C. Jimenez Sanchez, Inst de Recursos Naturales y Agrobiol de Sevilla; M. Tejeda-Agredano, Instituto de Recursos Naturales y Agrobiología de; M. Grifoll, Departament de Microbiologia, Universitat de Barcelona; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiología / Agroquímica y Conservación del Suelo. Exudates represent between 40 and 90% of organic compounds released into the soil by the roots. These substances provide enough carbon and energy to enhance up to 500 times the microbial biomass in the soil. This effect is relevant because

the success of rhizoremediation of PAHs depends largely on the ability of contaminant degraders to settle in soil and on the plant growth that drive organisms to colonize roots efficiently. In this work we examine possible ways in which root exudates may also promote the bioavailability of the pollutants. Results obtained in our work indicate that the exudates released *in vitro* by sunflower roots present the same superficial tension that the basal medium used, indicating the absence of surfactant capacity. Chemical analysis of exudates showed that the organic compounds, released into the medium up to a TOC content of 130 mg/L, included carbohydrates as glucose and fructose, amino acids as glutamine, aspartic acid and isoleucine and fatty acids as palmitic and stearic acids. We tested the bioavailability-promoting properties of the exudates with two different model soil bacteria, *Mycobacterium gilvum*, able to degrade HMW PAHs such as pyrene, and the chemotactic strain *Pseudomonas putida*, a naphthalene degrader. Exudates promoted the mineralization of crystalline pyrene by *M. gilvum*, likely through a dissolved organic matter (DOM)-related mechanism that caused an enhanced solubilization of the chemical. Besides, root exudates caused a powerful chemotactic attraction in *P. putida*, changed its cell motility behaviour and promoted the transport of this strain through sand columns. We propose that the guided modifications on the conditions where the plants are growing, such as a targeted phosphorous or potassium deficiencies or an increased availability of nitrogen (as nitrate form), may cause important variations in the TOC level and the composition of the exudates released by the roots, with implications for bioavailability of PAHs.

**TU181 Optimize the plant function in the Yangtze Three Gorges Reservoir** Z. Chen, Institute for Environmental Research Biology V / Environmental Biology and Chemodynamics; X. Yuan, Chongqing University / College of Resources and Environmental Sciences; B. Schmidt, RWTH Aachen University / Inst. for Environmental Research (Biology V); A. Schaeffer, RWTH Aachen University / Chair of Environmental Biology and Chemodynamics. Periodic, anti-season changes of the water level develop a unique ecosystem in the Three Gorges Reservoir (TGR). Many vascular plants disappeared and consequently the flora of the littoral zone changed from the species to the community level due to hydrological alteration. However, some species exhibiting resistance to flooding survived. These remnant plants are gradually becoming the dominant species and are vital for sustainable management of TGR. The aim of the study is to determine the fate of organic pollutants in these dominant plants. We investigated the distribution of plant biomass and plant diversity in the drawdown area of TGR, selected several dominant species for lab-experiments. So far, *Lemna gibba*, *Lemna minor* and *Cynodon dactylon* are target species. We will use <sup>14</sup>C labeled propanil as model substance. Propanil is a widely used rice herbicide, which is known to be converted to the main product 3,4-dichloroaniline (DCA). Both, cell suspension cultures and whole plants will be used for the studies. The cell suspension culture is a suitable model system for metabolism studies while the whole plant can be used for fate studies under conditions which are similar to those in the environment. All studies are performed by applying radioactively labeled compounds in order to follow metabolic pathways and establish mass balances.

**TU182 Effects of carbonaceous sorbent amendment on observed plant growth and predicted bioavailability in a multi-pollutant contaminated soil** A. Brennan, University of Strathclyde; E. Moreno-Jimenez, Universidad Autónoma de Madrid; C. Switzer, University of Strathclyde. Contaminated land management strategies are increasingly focused upon reducing bioavailable fractions of the contaminants of greatest concern. Immobilisation of contaminants by carbonaceous sorbent amendment is gaining ground as a remediation strategy but work to date in soil only experiments suggests it may have variable effects in a multi-contaminant environment. With the aim of investigating the effects of carbonaceous sorbent amendment on plant growth and end point contaminant bioavailability in a contaminated soil system, a field soil contaminated with polycyclic aromatic hydrocarbons (PAHs) and metals was used to grow maize in a controlled growth chamber. Three

treatments in a plant pot experiment using maize biochar; pine biochar and commercial activated carbon were compared to an un-amended control. An additional series of controls without plants was set up. Plant growth characteristics such as chlorophyll content, shoot to root biomass and contaminant uptake to roots and shoots were assessed. Results suggest that maize biochar and activated carbon amendments show the most potential in terms of improving plant growth and reducing contaminant uptake compared to soil without amendment. Passive sampling techniques were employed to assess soil bioavailability, using rhizon samplers and non-exhaustive extractions for metals and metalloids and polyoxymethylene passive samplers for PAHs. By further defining the conditions in which sorbent amended soils successfully reduce contaminant bioavailability and improve plant growth, this work aims to inform field scale applications.

**TU183 Reduction of PCBs bioavailability by condensation of organic matter: assessment of linearity and concentrations in tissues of orally exposed piglets.** D. Matthieu, URAFPAINRA / URAFFA, INRA; A. Fournier, Université de Lorraine / URAFFA, INRA; T. Duval, Université de Lorraine; G. Rychen, C. Feidt, Université de Lorraine / URAFFA, INRA. Risk assessment of Polychlorinated biphenyls (PCBs) contaminated soils is a critical point in sanitary management all the more that little reliable information on transfer of PCBs to humans *via* involuntary soil ingestion is available. Indeed, young children with their hand-to-mouth activity may be concerned by an exposure to contaminated soils. In order to assess the possible impact of soil Organic Matter (OM) condensation onto bioavailability of NDL-PCBs a study completed in two parts was designed. The first study investigated the dose-response relationships in selected tissues. The second study was subsequently completed to evaluate the incidence of organic matter condensation on NDL-PCBs levels in target tissues. In the first study, eight male piglets were orally exposed during 10 days to one of the four levels (190, 560, 940 and 1 310 ng NDL-PCBs.Kg<sup>-1</sup> of Body Weight (BW) per day) of a corn oil spiked by Aroclor 1254. NDL-PCBs concentrations in adipose tissue, in liver and in muscles were determined for each congener by GC-MS after extraction and purification. Except for PCB 28, the analyzed levels were above the LOQs. A regression analysis showed a significant linear relationships between the administrated doses and the levels measured in the various adipose tissue, liver and muscle (R<sup>2</sup>>0.70) in a range of dose of 190 ng to 1310 ng NDL-PCBs. Kg<sup>-1</sup> of BW. In the second study, three Artificial Soils (AS) were prepared according to OECD guideline 207. One Standard Soil (SS), containing no organic matter, and two amended versions of this SS with Fulvic Acid (FA) or Activated Carbon (AC) were prepared to obtain 1% mass of organic. This study involved fourteen young male swine randomly distributed into 4 contaminated groups (3 replicates) and a control one (2 replicates). During 10 days, the piglets were fed AS or a corn oil spiked with 19 200 ng of Aroclor 1254 per g of dry matter (6 000 ng/g of NDL-PCBs) to achieve an exposure dose of 1 200 ng NDL-PCBs.Kg<sup>-1</sup> of BW. Variance analyses were performed onto resulting levels of NDL-PCBs into tissues as repeated measures using MIXED procedure of SAS software. Significant treatment effect and two distinct groups of treatments were found: on one hand oil, SS and FA, on the other hand control and AC. This study highlights that condensed OM (AC) strongly reduces bioavailability whereas less condensed part (FA) doesn't seem to have a significant effect.

**TU184 Exceptionally strong sorption of infochemicals to activated carbon reduces their bioavailability to fish: implications for in situ sorbent amendments?** L. van Mourik, Utrecht University / Institute for Risk Assessment Sciences; M.T. Jonker, Utrecht University. The addition of activated carbon (AC) to sediments is a new approach to remediate contaminated sites. AC strongly sorbs hydrophobic organic chemicals like PAHs and PCBs, thereby reducing their bioavailability and uptake in organisms. Because of its high sorption capacity, AC might however also be expected to sorb other chemicals, which are not contaminants, but have important ecological functions instead. Examples of such chemicals are infochemicals or pheromones, i.e., compounds serving as chemical inter- and intraspecies information

vectors. In the present study, we investigated the sorption of two (of the few) known infochemicals, hypoxanthine-3-N-oxide (H3NO) and pyridine-N-oxide, to five different powdered ACs. Sorption isotherms of these small molecular, polar fish kairomone substances appeared highly nonlinear, with logarithmic Freundlich sorption coefficients of up to 7. At physiologically-relevant concentrations, sorption of the compounds was up to 5 to 7 orders of magnitude stronger than what can be expected on the basis of hydrophobic forces only (i.e., their logKow value, being around 1), indicating exceptionally strong binding to specific sites. This binding effectively reduced the bioavailability of H3NO to fish, as was demonstrated in a behavioral assay in which goldfish were exposed to the kairomone in the absence and presence of AC. The predator-avoiding (freezing) behavior of the fish was significantly reduced down to the negative control level by the addition of AC. In addition to the presently investigated chemicals, many more infochemicals might be expected to be captured by AC particles, including hormones like estradiol and testosterone, which serve as sex pheromones for fish and are known to sorb relatively strongly to AC as well. The results of this study indicate that AC additions to sediments may have previously unrecognized, subtle, unwanted side effects through the potential binding of ecologically-important compounds.

#### **TU185 Impact of carbonaceous amendments on the freely dissolved concentrations, chemical activities and diffusive mass fluxes of PAHs in soil**

G. Marchal, NERI University of Aarhus; K.E. Smith, Aarhus University / Department of Environmental Science; Z. Frkova, Aarhus University / Environmental Science; L. Wollensen de Jonge, Aarhus University / Department of Agroecology; U.G. Karlson, Aarhus University / Department of Environmental Science. Carbonaceous soil amendments are applied to contaminated soils to strongly sorb polycyclic aromatic hydrocarbons (PAHs), reducing their freely dissolved concentrations and limiting bio-uptake and toxicity. An agricultural soil was spiked with five PAHs (phenanthridine, phenanthrene, anthracene, fluoranthene, and pyrene) and then mixed with different carbonaceous soil amendments: activated carbon (AC), biochar, or compost. Over 2 months, the PAH freely dissolved concentrations ( $C_{free}$ ), chemical activities and diffusive mass fluxes into a silicone sink were measured. To investigate the role of the root rhizosphere in promoting biodegradation, a parallel set of pots was planted with radish (*Raphanus sativus* L.). For the unplanted AC amended soil, after 1 week the  $C_{free}$  of the 5 PAHs decreased by up to 94% compared to the unamended soil and thereafter showed only minor additional decreases over the 2 months. This was reflected in the diffusive fluxes of the PAHs into the silicone sink, which dramatically decreased by 96% compared to the unamended soil. In contrast, the addition of charcoal led to only rather modest reductions in the initial values of  $C_{free}$ , between 37 and 61% after 1 week, compared to the unamended soil, leading to smaller reductions in the diffusive fluxes into the silicone sink of between 24 and 70%. Compost had no significant effect on initial or final  $C_{free}$  values compared to the unamended soil, but still reduced the diffusive uptake fluxes by 38 to 74%. Compared to the non-sterile and unplanted soils with the different amendments,  $C_{free}$  was lower in the corresponding planted soils, and the diffusive fluxes into a silicone sink were also reduced by more than 55%. For the different treatments, only in soil amended with AC the sum chemical activities of the five PAHs was measured below the 0.01 to 0.1 threshold for baseline toxicity. Therefore this study suggests that adding AC may reduce acute toxicity of PAHs in agricultural soil, and that plant-promoted biodegradation may also be an important mechanism for the natural attenuation of PAHs. Keywords: carbonaceous soil amendments, PAHs, bioavailability, toxicity, *Raphanus sativus* L.

#### **TU186 Total and bioaccessible PAH concentrations in biochar**

I. Hilber, Agroscope Reckenholz Tänikon Research Station / Natural Resources and Agriculture; F. Blum, Agroscope Reckenholz-Tänikon Research Station ART; H. Schmidt, Delinat Institute for Ecology and Climate Farming; V. Gouliarmou, Aarhus University Science and Technology Faculty / Environmental Chemistry and Microbiology; P. Mayer, Technical University of Denmark / Department of

Environmental Engineering; T. Bucheli, Agroscope ART / Analytical Chemistry. Biochar is produced by pyrolysis of biomass to be used as a soil improver. Its many positive effects may be undermined by the presence of considerable amounts of polycyclic aromatic hydrocarbons (PAHs). Whether or not these PAHs are bioaccessible once biochar is amended to agricultural soils has not yet been investigated systematically. To assure product quality and its safe application, we determined total concentrations and bioaccessible fractions of PAHs in a series of commercially produced biochars, using recently developed methods. For total PAH concentrations, samples were extracted with toluene for 36 hours, followed by GC-MS (Hilber et al. 2012). Bioaccessible PAH concentrations were determined by Sorptive Bioaccessibility Extraction (SBE) using cyclodextrin as diffusive carrier and silicone rod as sorptive sink (Gouliarmou & Mayer, 2012). The SBE results will be: 1) related to total concentrations, 2) compared to PAH bioaccessibilities of other black carbon forms and 3) related to freely dissolved PAH concentrations (chemical activities) of the very same biochars quantified earlier with polyoxymethylene (POM) as non-depletive passive samplers (Hale et al. 2012). All together, the regulatory framework for biochar production, quality assurance and application is still under development. This offers unique opportunities to test and implement novel risk assessment tools such as chemical activity (measured by POM) and accessibility (measured by SBE) of the pollutant. Such methods are crucial for an integrative soil (pollution) management accounting nowadays only for total concentrations. References: Gouliarmou, V., Mayer, P. 2012 Sorptive Bioaccessibility Extraction (SBE) of Soils: Combining a Mobilization Medium with an Absorption Sink Environ. Sci. Technol. 46, 10682–10689. Hilber, I., Blum, F., Leifeld, J., Schmidt, H.P., Bucheli, T.D. 2012 Quantitative determination of PAHs in biochar – a prerequisite to assure its quality and safe application. J. Agric. Food Chem. 60, 3042-3050. Hale, S., Lehmann, J., Rutherford, D., Zimmerman, A.R., Bachmann, R.T., Shitumbanuma, V., O'Toole, A., Sundqvist, K.L., Arp, H.P.H., Cornelissen, G. 2012 Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars. Environ. Sci. Technol. 46, 2830-2838.

#### **TU187 Adsorptive bioremediation for spent motor oil contaminated soil**

E.R. Strijakova, Institute of physicochemical and biological problems in soil science RAS. Keywords: soil bioremediation, spent motor oil Oil spills have become a serious problem in environments with the ever-increasing resource exploitation, transportation, storage, and accidental leakage of oil and petroleum products. The aim of our research is to study the influence of two adsorbents on bioremediation rate of spent motor oil (SPM) contaminated soil. Granular activated carbon (GAC, Electrostal, Russia) and peat product Natursorb (Canada) were used for these purposes; they were mixed with the soil at doses 2,5 and 5% (w/w). An association of petroleum utilizing strains *Rhodococcus* sp. X5 or S67, *Pseudomonas putida* BS3701 and *Pseudomonas* sp. 142NF were also inoculated into the contaminated soil, separately or together with the adsorbents. Pilot-scale experiments were carried out with grey forest soil (Pushchino), contaminated with 5% SPM. The experimental soils (except untreated control samples) were treated through regular mixing, watering and fertilizing with mineral nutrition (NPK). Total content of hydrocarbons and oxidized hydrocarbon products were determined in the soil with IR-spectrometry. In addition, phytotoxicity of those soils was determined by express-method based on germination and biomass of clover white (*Trifolium Repens* L.). The results indicated two stage degradation of spent motor oil in the soil. About 30 or 35% of hydrocarbons remained in the soil in the end of the 1<sup>st</sup> season, and about 10 or 20% of petroleum hydrocarbons were detected in the soil in the end of the 2<sup>nd</sup> season of soil treating. However the degradation of SPM was accompanied with accumulation of oxidized petroleum hydrocarbons, which were more mobile compared to petroleum hydrocarbon and highly toxic for clover seeds. The soil amendment with GAC substantially reduced bioavailability and mobility of those toxic metabolites and thus the adsorbent prevented their penetration to underground water and reduced their toxic effect on degrading microorganisms and plants. Soil



amendment with Natursorb was less effective because of its degradation at presence of high doses of mineral fertilizers, that accompanied by soil acidification up to pH 5,0 and less. The influence of inoculated petroleum degraders was also low effective. Thus, granular activated carbon substantially accelerated biodegradation of SPM in grey forest soil and reduced leaching of toxic components to underground water and accumulation in plants.

**TU188 Bio2Remediation as effective and self sustainable tool to reduce persistent contaminants and to contrast accumulation of the new ones.** S.-. Manente, Ca Foscari University of Venice / Department of Molecular Sciences and Nanosystems; S.-. De Pieri, Ca' Foscari University of Venice / Environmental Sciences, Informatics and Statistic Dept.; C.-. Stocco, Ca' Foscari University of Venice / Molecular Sciences and Nanosystems Dept.; G. Ravagnan, Ca' Foscari University of Venice. Bio Remediation is a patented technology developed by Ca' Foscari University of Venice. This *in situ* remediation's technology consists in a forced oxygenation of the water layer upon the sediment, in order to re-activate the microbial community capable to biodegradation and so to stimulate a renewed biocenosis. Then, a biocapping suitable to create a sediment surficial oxic layer and to strongly limit pollutant's release from the deeper sediment layer is forming in a treated area. The Bio Remediation was already applied in two different very polluted sites in the Venice Lagoon. Here, according to ecotoxicological point of view, we want to describe the results obtained applying this technology to Arsenal Vecchio basin, a polluted area of the ancient venetian shipyard, about one year after the end of the treatment. This area was chosen for the experimentation because of its limited size, shallow water and (nowadays) reduced boat traffic. The final aim was to compare the results obtained in the treated area vs. the no-treated area's ones, in order to verify the treatment maintenance property, being the 16 polycyclic aromatic hydrocarbons (PAHs), focused by the U.S.-E.P.A., as priority pollutants to trace organic pollution with and without Bio Remediation treatment. The content, the kind, the relative and absolute distribution of PAHs has been investigated in this sensitive area, strongly affected by various old and recent anthropogenic activities. It is really evident that PAH's concentration decreases in treated area clearly after *in situ* remediation treatment with Bio Remediation, showing both reduction of their absolute amount and different percentage distribution. Finally, in order to understand the PAH's sources, diagnostic ratios (DR) as PAH's contamination indexes have been applied to both treated and no-treated areas, suggesting a from fossil fuel combustion and/or from grass, wood or coal combustion origin, depending on the particular sample site. In conclusion, this work highlights that Bio Remediation is pertaining as a new approach for the treatment of the contaminated sediment *in situ*, in particular showing a strong capacity both to reduce the amount of old persistent contaminant and to contrast the accumulate of new pollutant's input.

**TU189 In vitro testing of biofuels – Ecotoxicity tests for alternative fuels** S. Heger, Institute for Environmental Research RWTH / Department of Ecosystem Analysis; K. Bluhm, Institut für EnvironmResearch RWTH Aachen Univ / Dept. of Ecosystem Analysis; T. Seiler, RWTH Aachen University / Institute for Environmental Research (Biology V); A. Schaeffer, RWTH Aachen University / Chair of Environmental Biology and Chemodynamics; H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V). The increasing global demand for energy, e.g. for the fossil fuel dominated transport sector, requires the development of alternative energy sources. Due to declining production capacities and environmental concerns of fossil fuels, such as emissions of greenhouse gases (GHG), governmental support for the development of renewable energy sources, such as biomass-derived fuels, increased considerably. A rise in biofuel production and consumption increases the risk of a release into the environment. The considerable lack of ecotoxicological data on biofuels impedes a sufficient assessment of their environmental hazard potentials. Applying ecotoxicological biotests as part of a comprehensive ecotoxicological investigation allows an identification of

potential hazards for the environment and, therefore, the identification of the most environmentally friendly biofuel even at a very early stage of the development process. This study focused on the investigation of the three biomass-derived substances ethyl levulinate (EL), 2-methyltetrahydrofuran (2-MTHF) and 2-methylfuran (2-MF) that were identified as potential biofuel components: The ecotoxicological investigation was conducted by means of *in vitro* biotests for assessing the acute cytotoxicity, 7-ethoxyresorufin-O-deethylase (EROD) activity and genotoxicity. In addition, the ecotoxicological hazard potential of two petroleum-derived reference fuels (gasoline fuel and diesel fuel) and an established biodiesel (rapeseedoil methylester (RME)) was investigated and compared to the potential biofuels. Cytotoxicity tests revealed the highest cytotoxic potential for 2-MF, whereas 2-MTHF showed the lowest cytotoxicity. Cytotoxic effects were also found for the petroleum-derived gasoline and diesel fuel. EROD activity was not induced by the investigated fuels. Genotoxicity was investigated only for the potential biofuels where 2-MF showed significant effects. With regard to the environmental hazard potential, 2-MF revealed the highest toxic potential, whereas 2-MTHF revealed the lowest toxic potential. Moreover, this study showed the suitability of adapted *in vitro* biotests for an ecotoxicological investigation during the development of biofuels. Acknowledgement: This work was performed as part of the Cluster of Excellence "Tailor-made fuels from biomass", which is funded by the Excellence Initiative by the German federal and state governments to promote science and research at German universities.

**TU190 A sustainable use of biocides – Examples for disinfectants, rodenticides and products for the protection of façades** S. Wiecek, S. Gartiser, Hydrotox GmbH; M. Burkhardt, HSR, Hochschule für Technik Rapperswil; R. Gross, Öko-Institut e.V.. The Directive 2009/128/EC established a framework action plan for achieving a sustainable use of pesticides and aims at minimizing hazards and risks for human health and the environment. Measures to accomplish these goals are proposed, e.g. the improvement of the application of pesticides or the reduction of the use of hazardous substances. Up to now the directive focuses on plant protection products (PPP). However, the possibility for extending the scope to biocides is kept open. Just like PPPs biocides are designed to have an effect on organisms. Thus, their use should be minimized as far as possible to decrease their effects on human health and the environment. This objective has already been included in article 3 of the Biocidal Product Directive 98/8/EC as well as in article 17 of the European Biocidal Product Regulation (EU) 528/2012 which is to be applied from September 1, 2013. The new regulation calls for a report on measures geared to the sustainable use of biocidal products until 2015 (article 18) where the already existing contributions to a sustainable use and additionally needed measures shall be described. Examples for measures that could be analyzed are: the obligation to minimize the use of biocides, for instance by taking into account preventive measures, the need for education and training or public awareness raising. The Federal Environment Agency of Germany conducts a project on how to achieve a minimization of risks caused by the use of biocides. In this project product type (PT) specific measures for a sustainable use will be compiled. The considered PTs are disinfectants and algicides not intended for direct application to humans or animals (PT 2), products used for veterinary hygiene purposes (PT 3), products for the protection of façades (PT7/10) and rodenticides (PT 14). Questionnaires were sent out to different stakeholders, e.g. user groups or associations, to gain insight in the practical work with the respective biocidal products and to hear their opinions on the efficacy and practicability of possible measures. That way, a package of feasible measures for a sustainable use of biocides will be developed. This poster will present a first set of possible measures for the respective PTs. Comments on the measures will be considered during the future work within this project. In a preceding project measures for PT 8, PT 18 and PT 21 were assembled: <http://www.umweltdaten.de/publikationen/fpdf-l/4261.pdf>.

**TU191 Evaluation of environmental improvements from the substitution of fossil-based ingredients with bio-based ones in**

**cosmetic industry** M. Secchi, V. Castellani, E. Collina, University of Milano Bicocca / GRIS- Department of Environmental Science; S. Sala, Joint Research Centre European Commission / Sustainability Assessment Unit - Institute of Environment and Sustainability. Pharmaceutical and personal care products (PPCPs) are widely used in our daily-life. Their formulation includes a wide range of fine chemicals ingredients, which are mainly derived from fossil resources. Nowadays, there is a growing demand of more "natural" and bio-based cosmetic products, arising from a more conscious approach to consumption and life-styles by citizens.. We present an application of Life Cycle Assessment (LCA) to two cosmetic products, in order to assess the environmental effects of an Eco-innovation process: the substitution of one or more fossil-based ingredients with a patented bio-based one, produced from the by-products of olive oil industry (EP WO2012131624). A cradle-to-gate LCA was run using ReciPE and Eco-Indicator 99 LCIA methods. Despite cosmetics are widespread consumer goods, it is very difficult to find LCA studies in literature which investigate the environmental impacts of this kind of products, also because of proprietary concerns by the chemical industries. In addition, existing LCA databases of life-cycle inventory (LCI) results do not provide specific datasets about fine chemicals currently used as ingredients in many personal care products. Therefore, for the LCI of our case study it was necessary to approximate many ingredients included in the formulations given by the companies with bulk or fine chemicals LCIs, chosen because they had similar molecular structures or similar synthesis, manufacturing or refining processes. Results show that the substitution of traditional fossil-based ingredients with the innovative bio-based one can produce significant environmental benefits to the entire production chain, since the more critical life cycle stages in a traditional cosmetic production chain are those related to the manufacture of the ingredients. Moreover, the innovative ingredient shows good environmental performances also because it is made using by-products of an existing bio-based industry (olive oil industry) instead of using bio-based raw materials from dedicated cultivars. Results are further discussed in terms of uncertainty related to the lack of inventory data for fine chemicals and accuracy of characterization factors for chemical substances in current LCIA methods.

**TU192 Solvents in the Value Chain of the Lithium Ion Batteries** B. Simon, Helmholtz Institute Ulm; M. Weil, KIT / Institute of Technology Assessment and System Analysis. The growing amount of electric devices in our everyday life demands new, effective, light weight and mobile energy source. Therefore rechargeable batteries are substantial building stones of our future. There are indications that the critical requirements of "green chemistry" are not always ensured by recent battery technologies. Often different sorts of organic materials are used for the production of batteries, which can have several effects along the whole life cycle. These materials are not seldom highly volatile and possess toxic properties. Even if a proper handling is aimed total avoiding of human exposure is difficult. This is pertinent to development phase and to other life cycle phases (such as the production or recycling). The method of life cycle assessment (LCA) gives a hand to identify of processes and materials in the value chain of batteries which have high environmental impact. The development and production phase offer opportunity reducing the related environmental impacts due to the significant effect of designing on the environmental characteristic in these periods of a product. Only in close cooperation with technology developers, system analyses can help to get a deeper understanding of development related ecological risks. In this way it is possible to get insights into the the preparation process of samples for electrode materials and electrolytes whereat the reserachers come into contact often with different solvents. Furthermore the production process of electrodes consumes different solvents as well, which evaporate during the manufacturing. The recycling of batteries can be carried out by pyrometallurgical and hydrometallurgical manner. Both of the hydrometallurgy and the downstream processes of smelting methods are using solvents for leaching of metals from battery materials. The study focus on possible ecological hot spots of the development and other life cycle phases of batteries which includes toxicity issues. This

information gives a hand to make proposals for a more green battery technology and early identification of toxicity related innovation risks.

**TU193 Software Supported Approach to Replace Compounds with Toxicological or Ecotoxicological Concerns in Chemical Mixtures** R. Kühne, R. Ebert, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry; T. Müller, A. Sengespeick, E. Jelen, Fraunhofer Institute of Environmental, Safety and Energy Technology (UMSICHT) / Research and Development; G. Schuurmann, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry. Industrial mixtures of chemicals that may serve as, e.g., flame retardant, pigment, emulsifying agent, etc., typically contain compounds with different levels of toxicological or ecotoxicological hazards. In order to avoid unwanted effects, concerning substances need to be replaced by less harmful chemicals. The range of potential substituents is obviously restricted to compounds yielding a similar desired effect with regard to the application of the mixture. A software system is envisaged for automated proposals of suitable substituents. Alerting components of the mixture are identifying by examining their toxicological and ecotoxicological profile through database lookup, QSAR models and automated read-across. The inspection also includes physical-chemical properties. Candidates for substitution are checked in the same manner. A conceptual approach to address mixture effects is included. The software development is based on coupling the existing systems GEVIS and ChemProp. The OSIRIS edition of ChemProp [1] is publicly available free of charge due to a bilateral license agreement already. The study is financially supported by the IGF project FORMULA (17176 BG / 2) of the IUTA Duisburg through the AIF within the framework of the program to support industrial research co-operation (IGF) by order of the German federal ministry of economy and technology. [1] Chemical Properties Estimation Software System (ChemProp) 5.2.8, 2012. UFZ Department of Ecological Chemistry, <http://www.ufz.de/index.php?en=6738>.

**TU194 QSPR prediction of ready biodegradability of fragrance materials** S. Kovarich, SIN Soluzioni Informatiche Srl; L. Ceriani, Department of Theoretical and Applied Sciences; E. Papa, QSAR Res. Unit Environ. Chem/Dep. Structural Functional Biology; P. Gramatica, University of Insubria / QSAR Res. Unit Environ. Chem. Ecotox./Dep. Structural & Functional Biology. Biodegradation is among the basic information required for risk assessment for the evaluation of environmental fate and persistence of chemicals. The "benign by design" concept requires the knowledge of the biodegradability potential to be available in the earliest phases of the chemical production processes, and *in silico* approaches like QSA(P)Rs (quantitative structure-activity(property) relationships) represent useful tools to predict biodegradability of chemicals even before their synthesis. In this study the attention was focused on a specific class of emerging pollutants, studied within the EU-FP7 CADASTER Project, i.e. fragrance materials. Despite the large consumption and exposure to these chemicals, limited information is actually available regarding their health effects and environmental fate. Classification models, based on the *k*-NN (*k*-Nearest Neighbors) method, were developed to predict ready biodegradability, on the basis of measured data for a heterogeneous set of 136 fragrances and structurally similar chemicals. Hundreds of 1D- and 2D- theoretical molecular descriptors were calculated by the commercial software DRAGON and the freely available tool PaDEL-Descriptor. The genetic algorithm was applied to select modeling variables (models were explored up to 5 molecular descriptors). Classification performances were evaluated by calculating percentages of ready biodegradable (RB) and not ready biodegradable (NRB) chemicals correctly classified, and the overall classification accuracy (OA%). Models were externally validated on an additional set of 45 fragrances, whose biodegradation data were measured within the CADASTER Project or provided by RIFM (Research Institute for Fragrance Materials). The applicability domain (AD) of the models was assessed taking into account the structural space defined by the descriptors used in the models. The developed models are able to correctly classify both RB and NRB chemicals, with OA over 80% for

training set chemicals and around 70% for fragrances included in the validation set. In conclusion, the proposed QSPRs are useful tools to support risk assessment of fragrance materials. In line with the “benign by design” concept, they could be used *a priori* for a rational design of new commercially and environmentally compatible alternative fragrances, which are potentially less persistent in the environment.

**TU195 Stressors, ecosystem services and ecosystem functioning: a first approach in the Minho River estuary (NW Iberian Peninsula)**

L. Guilhermino, Instituto de Ciências Biomédicas de Abel Salazar / Laboratory of Ecotoxicology; R. Sousa, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, laboratory of Ecotoxicology and Ecology; & 3CBMA – Centre of Molecular and Environmental Biology, Department of Biology, University of Minho.; C. Antunes, University of Porto, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research & AquaMuseu do Rio Minho. The Minho River estuary has a length of near 40 Km making the natural border between Portugal and Spain and being an important source of resources for local human communities of both countries. It has been considered a low impacted estuary. However, in the last decades both anthropogenic and natural pressures have been increasing with potential negative effects on ecosystem functions and services. The benefits from ecosystem services of the Minho River estuary are not quantified and there are no estimates on the economic impacts that changes resulting from these pressures may have. Therefore, the objective of this study was to perform a first approach to the services that the estuary of Minho River provides, on the main pressures that are interfering with ecosystem functioning, and on the reflexes that this may have considering ecological, socio-economic, and human wellbeing perspectives. In addition to the services that estuaries in general provide (e.g. carbon sequestration), Minho river estuary provides important provisioning (e.g. fresh water, food), regulating (e.g. nutrient cycling, waste processing, regulation of natural hazards) and cultural (e.g. tourism) services which are essential for regional economy. Furthermore, several species have their Southern limit of distribution in this estuary, and it is a natural habitat for emblematic species in the Iberian Peninsula, including several migratory fish (e.g. eel, salmon, shad, sea lamprey), endangered mammals (e.g. otters), and several invertebrates that are in risk of extirpation. Important threats include habitat loss and fragmentation, water regulation by upstream dams, effluents from urban and industrial activities, introduction of invasive species, and alterations resulting from global climate changes (e.g. heat waves). A conceptual framework for the problem including the potential implications for ecosystem functioning and services will be presented and discussed. This study was carried out in the scope of the LTER project “3M\_RECITAL – Minho, Mondego and Mira estuaries observatory: long-term variation of ecological status as a response to natural and human induced changes. Implications for management and restoration”, funded by the Portuguese Foundation for the Science and the Technology and FEDER COMPETE funds (LTER/BIA-BEC/0019/2009).

**TU196 Risk assessment of water scarcity in a low flow**

**Mediterranean river basin due to climate change** M. Marques, R. Bangash, V. Kumar, M. Schuhmacher, Rovira i Virgili University. Mediterranean basin is considered one of the most vulnerable regions of the world to climate change and with a high potential risk to present problems related to water scarcity in the next years. These impacts on water availability, water quality, as well as their impacts on the human society and economy make it a key issue on the EU agenda. In this study, Francolí River basin (NE Spain) was selected to assess the effects of the different climate change future scenarios (A2 and B1 from IPCC, for the years 2011-2040, 2041-2070 and 2071-2100) and further compared to the base scenario (1971-2000) in water provisioning terms. Francolí is a small river of Catalonia with an irregular low flow (~2m<sup>3</sup>/s). As a Mediterranean river, its flow is subjected to high interannual and seasonal variability. The river basin has been under considerable pressure for water availability and water quality due to the increasing water demand related to the population growth and land use

and land cover changes. Furthermore, climate change is an added key factor causing water stress in the Francolí River basin. All these pressures were the main motivation to model and map the impacts on water provisioning ecosystem service using InVEST tool to elucidate general patterns and changes caused by climate change impacts. This tool enables decision-makers to assess the tradeoffs associated with alternative choices. The results show that the highest impacted regions are the region which are at the extreme boundary of water provision (least and most affected region). Consequently, on one hand, the water stressed area located at the upper Francolí is likely to be desertified and, on the other hand, flow reductions in the source area will reduce the flow of the Francolí River causing water scarcity in the basin. InVEST application is validated by comparing the obtained results with Sacramento mass balance. It can be safely concluded that the use of InVEST is promising in a river with low flow characteristics to assess the water scarcity risk. Regarding the results, the stakeholder engagement should take urgent measures and policy choices through the introduction of a River Basin Management Plan to mitigate the already existing water scarcity risk and assure the provision of adequate amount of water in the basin.

**TU197 Classification of freshwater ecosystem functions in**

**Mediterranean river basins** R. Bangash, Departament d'Enginyeria Química, Universitat Rovira i Virgili.; V. Kumar, Environmental Engineering Laboratory / Departament d'Enginyeria Química, Universitat Rovira i Virgili.; M. Sanchez-Canales, F. Javier Elorza, Universidad Politécnica de Madrid, / Escuela Técnica Superior de Ingenieros de Minas; V. Acuna, Institut Català de Recerca de l'Aigua (ICRA) / Parc Científic Tecnològic de la Universitat de Girona M. Schuhmacher, Environmental Engineering Laboratory / Departament d'Enginyeria Química, Universitat Rovira i Virgili,. The Mediterranean basin is considered one of the most vulnerable regions of the world to climate change and with a high potential to present important problems in water availability in the next few years. The predicted future scenarios for this region present an increased frequency of floods and extended droughts, especially at the Iberian Peninsula. Global change impacts on water availability, water quality and ecosystem services in Mediterranean river basins of the Iberian Peninsula, as well as their impacts on the human society and economy makes it an issue high on the EU agenda. Ecosystem function is the capacity of natural processes and components to provide goods and services that satisfy human needs, either directly or indirectly. These benefits are often regulated by terrestrial ecosystems and include provisioning services such as water supply for drinking, power production, industrial use and irrigation, as well as regulating services such as water purification and erosion control. Three different types of rivers (low, medium and high flow) are selected to evaluate the ecosystem services based on the flow characteristics. There are four primary groups of ecosystem functions: (1) regulatory functions, (2) habitat functions, (3) production functions and (4) information functions. All those functions that are affected by fresh water such as water supply for drinking, power production, industrial use and irrigation etc. are analysed in Mediterranean river basin. Georeferenced maps are essential to identify areas of river catchments that provides ecosystem services and vulnerable to climate change. Integrated Valuation of Ecosystem Services and Tradeoffs-InVEST is used to determine the degree of change in water provisioning, and the obtained results will also help to identify the areas of the basin that are most impacted by climate and global changes in Francolí, Llobregat and Ebro river basins (NE Spain). Quantification of the ecosystem services followed by mapping will provide an understanding of the prevailing situation in all three Mediterranean river basins. And it will be highlighted that current risk assessment practice may not protect biodiversity, ecosystem functions and services. Participatory nature of this study with stakeholders can provide better communication of water research that helps to develop more sustainable water management strategies.

**TU198 Evaluation of the ecosystem services of a crop field in**

**Portugal** M. Santos, CESAM DeptBiology / Department of Biology

and CESAM; R. Morgado, E. Esperanca, University of Aveiro / Department of Biology and CESAM; J. Van Wensem, TCB; S. Loureiro, Universidade de Aveiro / Biology. Agricultural fields provide ecosystem services that contribute to human well-being and they maintain their productivity due to exogenous stimulus as pesticide and fertilizers application. Thus it is pertinent to evaluate if the current method for maintaining crop field's productivity would affect the environmental health status of these ecosystems in terms of their biological diversity and function. The aim of this work was to evaluate the ecosystem services of an agricultural field (production of summer flowers) and compare those services with the services provided by a contiguous reference site (pine tree field). More specifically we have assessed the soil function (nutrient cycling, organic matter breakdown) and measured the community structure of the soil (abundance, biomass, diversity and trait dominance). As a result, a comparison between the two fields was made, stressing the effects in the function and structure of the soil. The ultimate goal was to establish a relationship between the ecological sustainability of different fields and the ecosystem services provided.

**TU199 How land use affects freshwater communities and the services they provide** S. Alsolmy, The University of Sheffield / Department of Animal and Plant Sciences; L. Maltby, The University of Sheffield / Dpt of Animal Plant Sciences. Macroinvertebrates play an important role in the functioning of freshwater ecosystems and underpin many important ecosystem services. However, fresh waters are influenced by their landscape context and the structure and functioning of their biological communities are affected by changes in habitat quality resulting from how the adjacent land is used. In rural landscapes, two major land uses are arable and pasture. Both are managed, but how they are managed and the inputs they receive (e.g. nutrients, pesticides) differ. In this study we compared invertebrate communities in streams and ponds located in arable and pasture dominated catchments. Twenty four sites were sampled – 6 arable ponds, 6 arable streams, 6 pasture ponds and 6 pasture streams – and invertebrates were identified to family level and counted. The effects of land use on invertebrate communities and the potential consequences for ecosystem services delivery will be discussed.

**TU200 Evaluation of soil related ecosystem functions and services at the Okavango basin and impact of land use and climate change** J. Luther-Mosebach, L. Landschreiber, A. Groengroeft, University of Hamburg; A. Eschenbach, University of Hamburg / Institute of Soil Science. The Okavango basin extends from the central highlands of Angola over the mid stream regions in northern Namibia to one of the biggest inland deltas in the world in Botswana. Within the international research project "The Future Okavango" (TFO) funded by the German Ministry for Education and Research (BMBF) an interdisciplinary approach has been developed to assess the impact of land use and climatic change on Ecosystem functions and services (ESF&S). In this study the assessment of soil related ESF&S is investigated with special focus on provisioning and regulating services. This assessment is carried out in two steps. In a first phase the natural scientific condition in the basin are analysed and described on the basis of four representative research sites. In the second step land use scenarios coupled with climate change scenarios are used as input for models to show changes in ESF&S relative to the status quo. The integration of pedological data in this overarching approach is realised by a combination of field, laboratory and model applications. In this area main parameters and processes to estimate soil quality are the soil water availability and groundwater recharge, soil carbon pools and fluxes as well as nutrient dynamics

**TU201 Changes in bacterial community structure and functioning in a degraded soil amended with organic waste** A. Barra Caracciolo, National Research Council / Water Research Institute; M. Bustamante, Consejo Superior de Investigaciones Científicas / Soil and Water Conservation and Organic Waste Management; M. Cinicia, National Research Council / Water Research Institute; M. Di Lenola, M.

Luprano, National Research Council / Water Research Institute; I. Nogues, National Research Council / Institute of Agro-environmental and Forest Biology; G. Paola, National Research Council / Water Research Institute. Although the application of organic amendments is considered a suitable tool for improving the quality and fertility of degraded soils, few studies have been conducted in semiarid climates to evaluate the effect of such practice on the structure and function of the soil microbial communities. Organic amendments may contain heavy metals and/or other micro-contaminants and therefore affect the composition and activity of soil microorganisms. In this work, we studied the changes in the structure (abundance and diversity) and functioning (viability and dehydrogenase activity) of the microbial community in a loam soil located near Rome (Italy) after adding a cattle and/or pig compost to restore the soil. Cattle or pig compost was applied at a low (30 t/ha) or a high dose (60 t/ha) on soil samples which were maintained in microcosms for 6 months. Moreover, some soil microcosms were treated with the fertilizer NPK and others (non-treated soil) were used as controls. rosemary plants were planted in half of the entire microcosms set up (48 microcosms). Rosemary was used both for its ability to grow in semi-arid regions and because its root system is able to protect soil from erosion. At different times (0, 30, 120 and 180 days) the bacterial abundance, cell viability and dehydrogenase activity were measured in the 12 different experimental conditions. The soil organic carbon and nitrogen content together with the bacterial community diversity were also assessed at 0 and 180 days. With an increase in carbon and nitrogen soil content, in the presence of cattle and pig compost, an increase of bacterial abundance was also observed. However, the bacterial activity was significantly influenced by the presence of the rosemary, without considering the allochthonous carbon and nitrogen input. The highest values for microbial community biodiversity were found in the co-presence of the plant and a low concentration of cattle or pig compost. The use of organic amendments, which reduces the costs of their disposal as waste, together with the planting of species suited to Mediterranean semi-arid areas seem to be an appropriate strategy for improving the soil quality and restoring the ecosystem services provided by microorganisms.

**TU202 Predictive models for the sorption of pharmaceuticals to sewage sludge** L. Berthod, Astrazeneca University of Portsmouth; G.C. Roberts, Astrazeneca UK Ltd / Brixham Environmental Laboratory; A. Sharpe, AstraZeneca UK; G.A. Mills, D. Whitley, University of Portsmouth / Pharmacy and biomedical sciences. Over the past two decades concerns over the presence of pharmaceutical residues in the environment have grown considerably. Pharmaceuticals mainly enter the aquatic environment through effluent discharges from wastewater treatment plants. Pharmaceuticals that are not biodegraded during treatment processes can also be removed through sorption to the sewage sludge. As part of the environmental risk assessment for these substances, it is beneficial to have robust models capable of predicting their environmental fate. The predictive models currently used for the sorption of chemicals are based on soil, and predominantly use the partition coefficient to organic carbon ( $K_{oc}$ ), a variable that is not measurable directly by experimentation. These soil-based models are, however, inappropriate for use with sewage sludge due to significant differences in complexity and variability between the two matrices. Furthermore, these models are mainly optimised for neutral organic chemicals, and only a few consider ionic substances. As many pharmaceuticals are ionic (particularly acids and Zwitterions) the application of most existing models to these chemicals may not be appropriate. This work is based on the partition coefficient ( $K_d$ ) of pharmaceuticals (both ionic and non-ionic) in sewage sludge, using data collected from the literature, grouped according to ion class. The dataset is composed of a broad spectrum of pharmaceuticals, including antibiotics, anti-inflammatory, cardiovascular, anticancer, central nervous system drugs, hormones and synthetic musks. The performance of existing soil-based models for the prediction of  $K_{oc}$  from the octanol/water partition coefficient ( $K_{ow}$ ), are evaluated for each ion class, using estimates of the fraction of organic carbon ( $f_{oc}$ ) to relate  $K_{oc}$  to  $K_d$ . New models, developed specifically for sorption to sewage

sludge and taking into account the variation in ion class, are presented for the dependence of  $K_d$  on  $K_{ow}$ . In addition, multiple linear regression techniques are applied to derive models for the prediction of  $K_d$  from various sets of commonly-used molecular descriptors. The performance of the new and existing models is compared.

**TU203 Effect of inorganic electrolytes on the sorption of charged organic compounds** P. Bauerlein, T. ter Laak, R. Hofman-Caris, P. de Voogt, S. Droge, KWR. A wide variety of environmental compounds of concern are acids or bases. Therefore, they are often present as charged species in drinking water (sources). Removal of these compounds is often not straightforward with common techniques such as oxidation, activated carbon or membrane filtration. For that reason we were looking into ion-exchange polymers as an (additional) extraction material for this class of compounds. We tested various organic compound that are representative for common contaminants, such as pharmaceuticals, in the environment. The sorption affinity of these compounds was tested in a dynamic HPLC-setup, using pure water as mobile phase and custom packed columns. The method had the advantage that changing the water matrix could easily be done. Various water matrices were used to evaluate the effect of inorganic ions on the sorption affinity of the organic compounds. As sorption materials the OASIS polymers (MAX, MCX, WCX) were chosen. Furthermore, the adsorption to activated carbon was compared to the adsorption to the OASIS polymers. It was found that the sorption affinity of the compounds is nonlinear and depends on the composition of the water phase. This indicates that ion-exchange is the main sorption mechanism. Not only the concentration of the inorganic ions has an impact on the sorption but also the nature of the ion.  $Ca^{2+}$  has a bigger influence on the sorption affinity than  $Na^+$ . Sorption of organic cations is five fold less in  $Ca^{2+}$  solutions compared to similar concentrations in  $Na^+$ . Similar effects can be observed for inorganic anions. Also here the nature and the concentrations do effect the sorption. The apolar part of the molecule plays an important role in the sorption process, too. The more pronounced the apolar part is, the stronger the molecule binds to the ion exchanger polymer. Additionally, it can be said that sorption to the ion exchanger exceeds sorption to activated carbon.

**TU204 Accumulation affinity of organic cations to phospholipid membranes: monolayer columns, bilayer beads, dissolved unilamellar vesicles** N. Timmer, s.T. droge, Utrecht University / IRAS. To assess environmental risk and toxicity of ionizable compounds, measured liposome/water partitioning (Klip-w) coefficients are preferred over the traditional octanol/water partitioning coefficient ( $K_{ow}$ ) if compounds are largely ionized at environmental pH. For bases, the  $K_{ow}$  does not include the electrostatic interaction that strongly attracts the charged species to the anionic phosphate group, and  $K_{ow}$  underestimates the Klip-w by orders of magnitude. Several tools to determine Klip-w on phospholipids are available, each having advantages and drawbacks. The HPLC column method with Immobilized artificial membrane (IAM) coating allow for high throughput, but consist of covalently bonded monolayers and often require extrapolation from several solvent-water mixtures to fully aqueous conditions. TRANSIL beads consist of non-covalently bound phospholipid bilayers to a porous support phase, which can be readily centrifuged out of aqueous solutions. Dispersions of unilamellar liposome bilayer vesicles can be created by extrusion through 0.1  $\mu m$  filters, but require third phase measurements of the freely dissolved fractions. We measured sorption to phospholipids by all three methods for the same diverse group of strongly basic primary, secondary, tertiary and quaternary amines, at a pH where the neutral species are present only in minute fractions. For the bilayer vesicles, a newly developed passive sampler (C18/SCX) was used. The results from the different techniques are comparable within an order of magnitude, several obvious deviations will be discussed. Comparison with physicochemical parameters shows that the Klip-w of organic ions is not readily modeled.

**TU205 Acute toxicity of benzyl dimethyl dodecyl ammonium chloride to different species: linking free concentration to**

**bioavailability** Y. Chen, Institute for Risk Assessment Sciences; s.T. droge, Utrecht University / IRAS; J.L. Hermens, Utrecht University. Acute toxicity tests with a cationic surfactant, benzyl dimethyldodecylammonium chloride (C12-BAC), were performed on sediment organism (*Lumbriculus variegatus*), Rainbow Trout gill-derived cell line (RTgill-W1) and fresh water algae (*Chlorella vulgaris*). Different endpoints including organism survival, cell metabolic activity and algae photosynthesis inhibition were studied. With the presence of artificial sediment and protein for *L. variegatus* and RTgill-W1, respectively, the bioavailability of the chemical were decreased, which was determined by measuring the free concentrations of the surfactant using polyacrylate SPME fiber. The free concentration-based dose response curves were similar with the curves obtained for medium only tests, suggesting free concentrations is a better dose indicator instead of total concentrations. Sorption of the compound to sediment was much stronger than to protein, which predominantly determined the bioavailable fractions and accorded with the toxicity data. A mitigation factor of 8 was obtained in the test of 4 g/L sediment; reduced toxicity of a factor of 4 was calculated due to binding to 10 g/L bovine serum albumin. Additionally, sorption of surfactant on plastic well, cells, algae along the time and the mass balance of the test system were carefully investigated.

**TU207 Respiratory uptake efficiency of perfluorooctane sulfonate (PFOS) in fish** T. Sakurai, National Institute for Environmental Studies / Center for Environmental Risk Research. Understanding the bioaccumulation process is a key component of environmental risk evaluation of chemicals. Uptake efficiency is an important parameter in the models describing the bioaccumulation kinetics. This study examined the uptake efficiency of PFOS in fish at the respiratory surfaces based on a laboratory experiment and analysis of literature data. PFOS is persistent in the environment and is expected to accumulate in surface water, in which it is ionized. Respiratory uptake efficiency of PFOS in a marine fish, the marbled flounder (*Pseudopleuronectes yokohamae*), was investigated in our laboratory. Two-year-old flounders were exposed to dissolved PFOS at an average concentration of 74 ng/L for 28 days, and then depurated for 84 days (water temperature averaged 17.3 °C). The respiratory uptake rate constant for fish whole body was estimated at 22 L/(kg-wet-fish d). This value corresponded to a respiratory uptake efficiency of PFOS 3.2% that of oxygen, based on the ventilation rate of the fish estimated from a respiration measurement of marbled flounders, and assuming that the respiratory uptake efficiency of PFOS was proportional to that of oxygen. Few respiratory uptake efficiencies have been reported for PFOS in fish. We therefore analyzed reported respiratory uptake rate constants of PFOS in a similar way by using allometric estimation of oxygen demand by the fish. The experimental result and the data analysis resulted in the respiratory uptake efficiency of PFOS 0.8%–10% that of oxygen, for marbled flounder, rainbow trout, common carp, and bluegill. By taking into account the uptake efficiency of oxygen, this range corresponded to the respiratory uptake efficiency of PFOS of roughly 0.6%–7%, which was lower than those typically reported for neutral hydrophobic compounds. We note, however, that higher values of respiratory uptake rate constant were reported with increasing hydrophobicity for surfactants and perfluoro acids in the literature, and that these values correspond to uptake efficiencies up to about 100% based on an analysis as described above. Investigation on the mechanisms responsible for the uptake of ionized chemicals at the respiratory surfaces of fish has been limited, and further investigation would contribute to a better prediction of the uptake efficiency of ionizing compounds.

**TU208 Environmental risk assessment: which pH-range is relevant in the environment?** T.W. Schmidt, Harlan Laboratories Ltd / Ecotoxicology & Registration. The term "environmental pH" is widely used in the environmental risk assessment of chemicals, but the ranges used differ even between guidance documents, e.g. pH 4-9 in OPPTS 835.0001 (2008) or pH 5-9 in ECHA Chapter R.7A – Endpoint Specific Guidance (2012). The Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market

considered the same range for the determination of the partition coefficient, obviously considering this range environmentally relevant. The range was extended to pH 4-10 by Commission Directive 94/37/EC of 22 July 1994. These obvious inconsistencies regarding the range of the environmental pH are problematic since the fate of some ionisable chemicals in the environment (e.g. water, sediment, soil) strongly depends on the pH within the respective medium: its solubility and bioavailability may vary at different environmental pH values since the unionised form of the compound tends to be more hydrophobic than the ionised form. In a first step, a synopsis of different regulatory sources is shown which are relevant for regulatory assessment regarding the different finalities (e.g. PPP, MHP, VMP, Biocide, Industrial Chemical) of chemicals. In the second step, pH values of relevant media for the entry of chemicals in the environment are collated in a literature survey. Depending on the results of the comparison between the pH-ranges of the survey and the recently used guidance documents, a justification for the selection of one general environmentally relevant pH range may be given.

**TU209 Thermodynamics Study of Ferrous Ion Binding to Keratin from Sheep Wool** Y. Zhao, . Ferrous ion has been shown to play a critical role as a mordant in dyeing hair, thus prompting intense investigations into the formation of metal-hair protein complexes. Keratin is the key structural component of hair and accounts for more than 90% of hair dry mass. In this study, Isothermal titration calorimetry (ITC) measurement has been used to study the associated thermodynamic properties of ferrous gluconate and keratin solution from sheep wool. ITC has been widely used to quantify metal-protein binding affinity and associated thermodynamics. By fitting the enthalpy data with the identical independent binding sites model, the association constant (K), stoichiometry (n), free energy (G) and enthalpy (H) associated with the interaction were obtained. 1 mole keratin can bind with 9 mole ferrous gluconate. The large positive entropy value obtained shows that  $\text{Fe}^{2+}$  binding to keratin is entropically driven. A systematic investigation of the binding of ferrous gluconate and keratin has been carried out by ITC at temperatures of 298 K, 308K and 318K. It turns out that increasing temperature results in an increase in the binding affinity, further suggesting that the interaction is entropy-driven. Positive entropy observed also indicates the changes in the hydration of the keratin upon  $\text{Fe}^{2+}$  ion binding to the protein.

**TU210 Identifying population-relevant endpoints for ecotoxicological risk assessment and hazard identification for endocrine active chemicals** L. Weltje, BASF SE / Agricultural Centre; J.R. Wheeler, Syngenta Ltd. Most ecotoxicological tests measure individual-based endpoints (survival, growth, development and reproduction) to assess the impact of a substance on an organism or group of organisms. In addition, an increasing number of biomarker endpoints are measured, especially for endocrine active chemicals (e.g. vitellogenin in the case of fish). However, the goal of an ecotoxicological risk assessment for which these data are generated is to protect populations of organisms. In contrast, the protection goal of a human (toxicological) risk assessment is the individual. Hence, the ecological risk assessor needs to select endpoints of population relevance for use in the risk assessment. This ultimately creates the need to reduce complex data sets to values, which are predictive of population level impacts. This is exemplified by the analysis of toxicological studies designed for human health assessments (individual protection) for the derivation of endpoints for wild mammal assessments (population protection), but equally applies to other eco/toxicological studies. This distinction may appear trivial, but in practice relies on a thorough understanding of the biological (i.e. defining what is actually adverse) and statistical properties of the test system. It prompts the question: Which endpoints are relevant for populations and what level of impact [change] on these individual-based endpoints is tolerable for a population? Further, apical (integrative) and mechanistic endpoints to investigate specific modes or mechanisms of action (e.g. endocrine disruption) need to be weighted in the overall evaluation. Identifying the population-relevance of endpoints is relevant for the environmental risk

assessment, but in future it may also be important when assessing chemicals against criteria for determining 'endocrine disrupting properties' of chemicals. This poster explores aspects to the selection of endpoints of population relevance, which will be illustrated with examples.

**TU211 Interpreting fish full lifecycle study data: a case study for population relevance** J.R. Wheeler, Syngenta Ltd; H.O. Krueger, Wildlife International Ltd / Aquatic Toxicology; S. Gallagher, Wildlife International, LTD; K. Benyon, Syngenta Ltd. Fish full lifecycle studies represent the gold standard for fish long term testing. They assess a multitude of endpoints measured over at least two generations. As such they generate complex datasets that need careful interpretation in order to derive a single study endpoint that may be reliably used for environmental risk assessment. Ideally this endpoint should be related to the protection goal of the assessment – namely the protection of fish populations. Study interpretation needs to consider the biological test system, statistical properties, magnitude of effect and the relation of the endpoint to the population relevance question. Here we present a case study of a plant protection product fish full lifecycle study that investigated 45 endpoints over 2-generations in fathead minnow. The data demonstrate the need for a holistic evaluation of all of the evidence, where endpoint outliers are considered opposite the consistency of effects within the study and other fish long term data is utilised.

**TU212 Differing sensitivities of juvenile and adult *Potamopyrgus antipodarum* under chronic exposure to cadmium and TBT** Theis, Goethe University Frankfurt / Aquatic Ecotoxicology; D. Brettschneider, Johann Wolfgang Goethe University / Aquatic Ecotoxicology; K. Ruppert, Goethe Universität / Aquatic Ecotoxicology; C. Geiss, U. Schulte-Oehlmann, Johann Wolfgang Goethe University / Aquatic Ecotoxicology; J. Oehlmann, Johann Wolfgang Goethe Universität Frankfurt am / Aquatic Ecotoxicology. Molluscs are one of the most widely distributed taxa that inhabit both terrestrial and aquatic habitats. Due to their ecological importance and sensitivity to a wide range of toxicants, including endocrine disrupting chemicals, molluscs represent a group of invertebrates which seems to be highly suitable for risk assessment studies. In the framework of the OECD test guidelines program, a project was funded by the environmental authorities of Germany and the United Kingdom to support the preparation of a *Detailed Review Paper (DRP) on Molluscs Life-Cycle Toxicity Testing*. One of the species that was considered as a promising test species is the parthenogenetic and ovoviviparous freshwater snail *Potamopyrgus antipodarum*. To investigate a potential extension of an existing Partial Life Cycle test protocol to a Full Life Cycle design, which incorporates both juvenile and adult life stages, a comparative sensitivity analysis with juvenile and adult *P. antipodarum* was performed. Neonates and adult snails have been exposed to the metal cadmium and the endocrine disruptor tributyltin (TBT). Nominal cadmium concentrations were 1.56 up to 50 µg/L. TBT concentrations ranged from 15 to 480 ng TBT-Sn/L. The experiments were performed over 28 days at 16°C in a semi static test design. Mortality was assessed for both life stages. Juvenile snails' specific growth rate and reproduction of adults were investigated as endpoints. We determined significantly lower embryo numbers and effects on juvenile growth under exposure to both chemicals. For cadmium-exposed groups,  $\text{EC}_{50}$  values were 10.1 µg/L for embryo numbers and 3.88 µg/L for juvenile growth. Calculated  $\text{LC}_{50}$  values were 36.5 µg/L for adults and 7.14 µg/L for juvenile snails.  $\text{EC}_{50}$  values for TBT were 102 ng TBT-Sn/L for adult *P. antipodarum*. TBT also significantly affected juvenile snails' growth ( $\text{EC}_{50}$ : 163 ng/L) and survival rate ( $\text{LC}_{50}$ : 89.3 ng/L).  $\text{LC}_{50}$  in adult snails could not be calculated due to low mortality in the tested concentration range. Based on calculated  $\text{EC}_{50}$  and  $\text{LC}_{50}$  values, higher sensitivity of juvenile snails compared to adults could be confirmed, except for TBT when comparing reproduction and juvenile growth as endpoints. This underpins the advantage of Full Life Cycle tests, which allow evaluating the impact of substances on all life stages and may help identifying the potential impact of a test compound on physiological functions during development.

### **TU213 How protective is Non-Target Arthropod risk assessment?**

**K. Swarowsky**, German Federal Environment Agency UBA; D. Suessenbach, German Federal Environment Agency (UBA); F. Streissl, EFSA / Pesticide Unit. The European Food safety authority (EFSA) recently developed a methodology to define Specific protection goals (SPGs) for the ecotoxicological risk assessment of pesticides (EFSA, 2010). The next step is the definition of specific protection goals for groups of non-target organisms in consultation with risk managers. It needs to be investigated whether the current risk assessment achieves an adequate level of protection to address the SPGs. According to EFSA (2010) “for each key driver (taxonomic group or other ecological entity) a reference tier should be identified, based on the most sophisticated experimental or modelling risk assessment method currently available that addresses the specific protection goal. This reference tier will then be used to calibrate lower tiers using simpler methods that are practical for routine use.” How to calibrate trigger values according to protection goals has been previously illustrated using the example of the HQ trigger for predatory mites (Swarowsky et al. 2012). In the current presentation we apply this concept using data from EFSA Draft assessment reports to evaluate the level of protection of the current risk assessment for Non-target Arthropods. References: European Food Safety Authority (EFSA) (2010). Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). EFSA Journal 2010;8(10):1821 Swarowsky, K., Streissl, F., Bopp, SK.(2012)Calibration of hazard-quotient trigger values for pesticide risk assessment for non-target arthropods. Poster Presentation, 6th SETAC World Congress 2012, Berlin, Germany

### **TU214 Ecological risk assessment of a potentially polluted soil.**

**Three methodological approaches to a practical case.** A. Vicente-Garcia, University of the Basque Country; J. Iglesias, Dinam Ingenieria S.L.; M. Soto, University of Basque Country / Zoology and Animal Cell Dynamic; **A. Rodriguez Ruiz**, University of the Basque Country / Zoology Animal Cell Biology. Soils are a fundamental and hardly-renewable resource for life in Earth. Therefore, soil risk assessment is an important duty. Basque environmental administration, inspired by the Dutch system, has established Intervention Values (IVA) for pollutants in soil. A-values correspond to baseline reference values, whereas C-values are those levels that pose an unacceptable risk for life. Concentrations between B- and C-values indicate that exists a potential risk and therefore further examination of toxicity is required before intervention measures are decided. In the present work, the ecological risk assessment of a potentially polluted real soil was carried out. This soil contained a mixture of metals and organic compounds at concentrations between B- and C-values (26.0 mg Cu, 55.5 mg Ni, 2.5 mg benzo[a]pyrene, 7.3 mg fluoranthene and 5.5 mg pyrene/Kg dry weight soil). Thus, the risk assessment of the potentially polluted soil needed to be performed. For this purpose, three different methodologies were used: a simulation software, an standard assay and a novel assay. The simulation software applied (Arriskugest) calculates the risk to ecosystem functioning by the species sensitivities distribution and was designed to help on the specific ecological risk assessment. Besides, two tests were carried out in the laboratory on *Eisenia fetida* earthworms: The OECD acute toxicity test adapted to real soils was performed. Growth and survival were recorded. The *in vitro* Neutral Red Uptake (NRU) assay was also carried out, indicating lysosomal membrane stability of the coelomocytes of earthworm exposed to the potentially polluted soil. The bioaccumulation of the compounds above B-IVA value was also assessed. The OECD assay did not show negative effects on *E. fetida* exposed to the potentially polluted soil, nor the simulation software found risk on none of the measured compounds, but on Pb and benzo[a]anthracene, where showed uncertain risk. In fact, Pb was bioaccumulated in *E. fetida* after 14 days of exposure. However, NRU assay showed the existence of a general stress on the coelomocytes of *E. fetida* exposed for 3 days to the potentially polluted soil. Thus, the

earthworm population immune system was damaged. Therefore, we conclude that the use of alternative methods like the NRU, which are early warning assays, is a useful and sometimes necessary tool for environmental risk assessment. Funded by the UPV/EHU (UFI 11/37) and Basque Government (GIC07/26-IT-393-07).

### **TU215 Ecological Risk of a contaminated landfill in the Basque Autonomous Community based on the TRIAD approach**

**L. Gutierrez**, Tecnia Research Innovation / Energiey and Environment; E. Ciprian, P. Menger, Tecnia Research & Innovation / Energy and Environment; **A. Rodriguez Ruiz**, University of the Basque Country. / Ekoiz-Berrilur Res. Consortium, CBET Res. Grp., Dept. Zoology and Animal Cell Biology.. Ecological Risk Assessment (ERA) methodology based on the TRIAD has been applied on a potentially contaminated area surrounding a landfill with a double objective i) to validate the developed ERA methodology for assessing Basque soil quality and ii) to assess the ER in the area potentially affected by a landfill. “Larregain” is a non authorized landfill of 32,700 m<sup>3</sup>, located in Hernani. ER has been calculated in two sampling points located downstream the landfill: “cat” and “car”. Reference soil “ref” was collected from non-contaminated soil in the upper area of the landfill. The results of the tests must be normalized to make them comparable and integrable. The normalization process consists on transforming tests into a common “ecosystem impairment” scale from zero (no impairment) to one (maximum impairment) according to the framework expalined in [2]. It is assumed that the risk is zero at the reference site and therefore. The “impairment values” are then integrated in the three LoEs risk indexes (RI): IR\_ch, IR\_e-tox and IR\_ecol. The outcome of RA in each tier depends on the value of the integrated RI and the standard deviation [4]. For the ecological risk interpretation a combination of WoE approaches have been used: quantitative and a qualitative assessment [3]. Both sampling points show a moderate risk in the first tier derived from the maximum value of IR\_ch due to the high presence of metals in soil. However they are not presented in toxic concentration neither bioavailable fractions as the values of IR\_e-tox and IR\_ecol indicate respectively in tier 1. Moreover, IR\_ch in tiers 2 and 3 are derived from the metal content in plants and earthworms collected from the site and in both cases the value is zero. Despite the high metals concentrations in soil, pollutants are not bioavailable for soil organisms. This fact may be due to the lime treatment applied to the landfill, that avoid contaminants mobility. To conclude, it can be said that there is no ecological risk in the surroundings of the landfill at this moment because of the basic pH provided by the lime. However, there will be a potential risk as the pH goes down due to the lime washing with the precipitation and runoff. Moreover, the ERA methodology developed for BAC has been validated and it is suitable for ecological risk assessment as it reflects the current situation in the landfill.

### **TU216 The Risk Assessment for bees as an example of how to translate protection goals into relevant endpoints in ecotoxicological studies.**

**J. Boesten**, Alterra / ERA team; F. Streissl, EFSA / Pesticide Unit; R. Luttik, Working Group on Bee Risk Assessment of the European Food Safety Authority; K. Swarowsky, German Federal Environment Agency UBA; M. Arena, C. Scentes, M. Clook, A. Rortais, F. Sgolastra, G. Arnold, Working Group on Bee Risk Assessment of the European Food Safety Authority. The Guidance Document on bee risk assessment is the first guidance document developed in the pesticides area where the methodology of defining specific protection goals based on ecosystem services has been followed. This approach allowed to make a link between protection goals, measurement endpoints and population relevant effects. Pollination, hive products (for honey bees only) and biodiversity (specifically addressed under genetic resources and cultural services) were identified as relevant ecosystem services to protect. Regulation (EC) No 1107/2009 lists survival and development of colonies, effects on larvae and effects on bee behaviour as attributes to protect. In consultation with risk managers specific protection goals were set. The specific protection goals include thresholds for magnitude of effects and temporal and spatial scales of effects and exposure assessment goals.



Based on the agreed protection goals relevant endpoints were identified to be measured in laboratory, semi-field and field tests. A tiered risk assessment scheme was developed which should make sure that the specific protection goals are met at each level. A Honey bee model was used to translate effects on colony size to daily forager mortality rates. This enabled the mathematical deduction of assessment factors which are to be applied in first tier assessments. Key words Pesticides, Bees, Protection goals, Risk assessment, Guidance

**TU217 The SETAC Environmental Monitoring Action Group (EMAG) – current status of the work of the birds and mammals subgroup** C. Wolf, Tier Solutions GmbH; M. Clook, CRD HSE / Environment Branch; J. Ludwigs, Rifcon GmbH; S. Norman, Ridgeway Eco; P.J. Edwards, Syngenta Ltd; J. Pascual, BASF SE; E. Bonneris, Bayer CropScience SAS / expertise and stewardship; P. Prosser, FERA; A. Barletta-Bergan, GAB Consulting GmbH. Bird and mammal risk assessments for individual pesticides carried out under EU Regulation 1107/2009 routinely use the relevant guidance document issued by EFSA (EFSA, 2009). This guidance outlines procedures of toxicity and exposure assessments for lower tiers. If concern is raised regarding lower tier assessments it may be possible to refine the risk assessment and although a range of options are available, detailed guidance is lacking as regards how to proceed. One possible refinement mentioned in EFSA (2009) is the use of field studies. The Environmental Monitoring Action Group for Pesticides (EMAG-PEST) of SETAC has been investigating the range of both pre and post-authorisation studies that have been conducted as well as the experience gained, with a view to consolidating 'best practice'. A survey of regulators and notifiers was carried out to determine what studies have been carried out, the next step is for the subgroup to develop proposals for the conception, execution and interpretation of wildlife monitoring field studies for both, pre- and post-approval conditions

**TU218 A holistic program of intensive field monitoring for birds & mammals: Overview of 6 years of studies in the EU on the insecticide chlorpyrifos** C. Wolf, Tier Solutions GmbH; B. Giessing; R. Ditttrich, F. Sotti, S. Wilkens, Tier3 Solutions GmbH; S. Norman, P. Manson, Dow Agrosciences; G. Weyman, Makhteshim Agan (UK) Ltd. As a widely-used organophosphate insecticide, chlorpyrifos (CP) is often the focus of regulatory attention. This is the case for birds & mammals. CP has a relatively high acute toxicity to birds (quail) & mammals (rat) in standard lab studies, with acute toxicity also driving the results of long-term lab studies where treated diet is provided constantly with no alternative. When results of these lab studies are compared with worst-case generic estimates of field-residues on diet & worst-case food consumption rates, a high risk is predicted. These 'Tier 1' assessments can be made more realistic with analysed residues of CP on field-collected samples of arthropods & vegetation, to derive a 'Tier 2' assessment. In most cases, even Tier 2 indicates potential high risk. The paradox is that in countries with post-registration surveillance, the UK Wildlife Incident Investigation Scheme being the main example, no bird & mammal poisonings have ever been reported for CP. This is in context of 40 yrs major usage in arable, vegetable & fruit crops. However, 'no evidence of effect' is not the same as 'evidence of no effect' & the rigorous demands of EU regulations require more-intensive field evidence to demonstrate safety. To give a strong basis for regulators to evaluate the real risk, a major program of field studies (2007 – 2012) was funded by the manufacturers. This was conducted by expert teams of RIFCon, tier3 solutions & Ecotox Ltd, under GLP which requires all observations to be meticulously reported. Intensive monitoring studies have been done for this program in: cabbages/Poland, grapes/France, apples/Italy, apples/UK, apples/Czech Republic, citrus/Spain. To study acute effects on birds & mammals, state-of-the-art radio-tracking of birds (e.g. small insectivores) & mammals (e.g. woodmice & voles) was done. In total, ca. 300 birds were radio-tracked during & after spraying of crops, with no effects observed. Long-term responses of populations were also studied for birds in citrus for 3 yrs (2010-2012; 2.4 kg/ha) & for mammals in citrus (2008 & 2011; 2.4 kg/ha) & apples orchards (2008; 1 kg/ha). A 3 yr project is ongoing in UK apple orchards (2012 is 1st yr).

Species-diverse, abundant populations of small insectivorous & omnivorous birds, & small mammals, have been found in these treated crops, with no apparent population impacts from CP use. The poster will summarise the 6 yrs of data.

**TU219 A large-scale monitoring program involving farmers for auto-evaluation of the farming practice and the effects of pesticides on birds** E. Bonneris, Bayer CropScience; A. Ossard, Bayer CropScience France; R. Barfknecht, Bayer CropScience Aktiengesellschaft, BCS AG-D-EnSa-ETX; F. GODET, Bayer CropScience France. Regulatory authorities require comprehensive environmental risk assessments to be conducted before a pesticide can be registered for use on specific crops. As mentioned in the guidance issued by EFSA (EFSA, 2009[1]), an holistic weight-of-evidence approach based on the results of laboratory studies, modeling exercises, field studies, and available incidents reports can be presented as final step of the risk assessment to birds and mammals. However, in some case, in order to confirm the efficacy of the mitigation measures under practical field conditions and get additional information, post-approval monitoring programs can be requested. At present, no guideline exists which gives recommendations how to implement such monitoring programs. Different complementary approaches can be therefore envisaged to get a better comprehensive overview of the risk of use of the pesticide and support the development of adequate stewardship actions at field level. In such context, an 'extensive' monitoring approach with multiple field study sites was put in place during the drilling period of cereal seeds in the autumn season of 2012 in France. At that period, some pesticides in solid formulations like granules (i.e. molluscicides applied in the form of slug pellets) and seed treatments (i.e. imidacloprid) known for their acute toxicity to birds are applied. This specific active and large scale monitoring program was initiated with more than 200 farmers who accepted to record their farming practice, assess the quality of their drilling and the non-intentional effects of imidacloprid based seed treatments and molluscicides on birds. Each farmer was asked to apply easy access protocols prepared in considering the methodological approaches developed in the framework of the national biovigilance network able to address side-effects of agricultural practices[2]. Such type of active monitoring program can be considered as an appropriate way to work with farmers to record wildlife incident data and to make them much more concerned by the respect of appropriated mitigation measures for the protection of birds. <br clear="all" /> [1] Guidance Document on Risk Assessment for Birds and Mammals (Sanco/10997/2009). EFSA Journal 2009 7(12): 1438, 358 p [2] DGAL, Vade-mecum de l'observateur en biovigilance, 2012, 57 p

**TU220 Pesticides indicators for river water quality assessment** C. Halkett, Agence de l'eau Artois-Picardie; j. prygiel, Agence de l'eau Artois-Picardie; D. Lotty, Agence de l'eau Artois-Picardie; E. Prygiel, Université Lille; G. Billon, University Lille1. Pesticides monitoring is achieved from the Water Framework Directive (WFD) and local networks so that data acquisition protocols largely vary according to sites, sampling frequency, substances... Moreover, due to the public works contract regulations, the data collected often came from several laboratories that use different protocols, update methods as well as quantification and/or detection limits. For all these reasons, water administrators have to manage large and heterogeneous pesticide data sets. The main objectives of this study are: (i) to set up a data exploitation protocol and (ii) to propose pesticides indicators to discriminate contaminated sites and substances to be kept under surveillance. Pesticides data have been collected from the monitoring Artois-Picardie water basin networks for the period 2007-2011. 99 monitoring sites and 174 active substances or metabolites have been considered for this study, half of them belonging to herbicides. Two pesticides indicators, one for sites and one for substances have been proposed based on a scoring system. For their construction, different criteria have been considered such as the number of detected substances (diversity of substances), the number of detections for all substances, the importance of standard overtaking (WFD EQS (Environmental Quality Standard) and/or national limits), the role of substances when exceeding

limits, the substances concentrations (when no standard exist). All these criteria are weighted by a confidence level. A last criterion allows taking into account the trend of substances concentrations which include the updating of laboratories practices. The application of these two indicators to the Artois-Picardie 2007-2011 pesticides dataset shows that pesticides are ubiquitous but that the very contaminated sites remain relatively rare and are located in well known urban and agricultural pressures areas. These indicators allow to clearly discriminate the monitoring sites, and the resulting classification is also reliable with the chemical and ecological status assessments. So, some of the pesticides belonging to the Artois-Picardie water basin 'top ten' are listed in the WFD chemical status substances list or in the substances list of the national ecological status. Finally these indicators allow to highlight contamination by non statutory pesticides.

**TU221 Surface water monitoring campaign as a pragmatic tool serving French regulatory needs** A. JAMES-CASAS, F. BOTTA, V. DULIO, S. ANDRES, INERIS. In France, monitoring of chemicals in water is being carried out by Water Agencies in the French River Basins. A first state of the art of concentrations of chemicals in water bodies was done among 2007 to 2009 monitoring data, demonstrating that monitoring was effective for some pesticides/biocides but that most of the active substances were not covered by routine monitoring. Following this step, it was decided to specifically include some pesticides and/or biocides active substances in two exceptional monitoring campaigns (for groundwater in 2011-2012 and surface water in 2012, respectively) in order to collect primary information on these substances. The general process for the substances selection to be included in the campaign was based on a prioritisation lead for all types of chemicals not already covered by the 2007-2009 monitoring in order to highlight chemicals of concern. This prioritisation was conducted according to several criteria, among which use, environmental hazard, human health hazard, PBT-like properties, and suspected endocrine disrupting properties. A total of ca. 2400 substances were screened allowing highlighting of ca. 300 chemicals, including pesticides, biocides, industrial chemicals, pharmaceuticals, etc. Furthermore, an adaptation was made specifically for French overseas departments with an additional weight given to further biocides and pesticides, namely insecticides for vector control recommended by World Health Organization. The results of the analysis of the surface water monitoring campaign are presented with a focus on pesticides and biocides. This work shall allow identification of substances potentially of concern and a better plausibility prediction of pesticides and biocides linked to environmental risks. In turn, risks possibly identified might serve in feedback regulatory needs if deemed necessary, allowing a better protection of the aquatic environment.

**TU222 A new Geo Database to increase the Benefit of Groundwater Monitoring Programmes for the German Registration Process of Plant Protection Products** A. Mueller, Federal Environment Agency / IV; D. Guerniche, RLP AgroScience GmbH; S. Karl, UDATA; W. Koenig, German Federal Environment Agency (UBA); O. Mayer, B. Schultze, UDATA; K. Thomas, M. Trapp, RLP AgroScience GmbH. Over the past years it became evident from regular groundwater monitoring programmes in Germany, that despite the conservative character of the German risk assessment for groundwater a number of active substances and metabolites are repeatedly found to reach the groundwater exceeding the authorisation limit concentrations. If this is the case, the authorisation holders of the respective pesticides are asked to investigate the findings and to clarify the cause of each entry into the groundwater. Another way to make use of groundwater monitoring studies is to evaluate the efficiency of risk mitigation measures to ensure the target groundwater protection level for certain uses of pesticides. For both kinds of investigations general requirements for proceeding and documentation are given in Aden et al. (2002). Up to now more than 70 studies related to around 20 active substances and/or their metabolites are available in the German Federal Environment Agency (UBA). The studies are containing a total of more than 700 monitoring point related data sets which are showing a distinct heterogeneity in quality and

extend of the provided data. Thus, the objective of the new geo database is first to combine all available data sets in a geo referenced database and second to allow a comprehensible evaluation and assessment of the provided information. In addition, the database contains nationwide spatial information on soil properties, climatic and hydrogeological conditions and is compatible to the geo information system ArcGIS. An integrated GIS-Tool allows a) adding spatial information on data sets where they are missing, b) to contribute to the identification of the causes of pesticide entries into groundwater on the basis of spatial and temporal geo statistical analyses and c) to evaluate the worst case character of monitoring sites chosen for special groundwater monitoring studies.

**TU223 In lab and in situ assessment of pesticide effects on aquatic organisms: key role of groundwater monitoring of Ariège alluvial plain (France).** N. BARAN; S. BLANCHET, CNRS; L. GAUTHIER, Ecolab; G. IMFELD, Université de Strasbourg - LHyGeS; C. MAAZOUZI, P. MARMONIER, Université Claude Bernard Lyon 1 - LEHNA; F. MOUCHET, Ecolab; C. PISCART, Université Claude Bernard Lyon 1 - LEHNA; A. RIBERON, Université de Toulouse - EDB; S. VUILLEUMIER, Université de Strasbourg - GMGM. Pesticides emitted into the environment may contribute to complex mixtures including the parent molecules, degradates in association with other pollutants. It is of paramount importance to implement suitable monitoring procedures to assess the fate of pesticides, their transfer to groundwater and the effects of water quality on aquatic and terrestrial organisms. The alluvial domain of the Ariège River (about 538 km<sup>2</sup>) is an unconfined aquifer, the thin thickness of vadose zone making it vulnerable to contamination. The aquifer is in direct hydraulic connection with rivers Ariège and Hers Vif and feeds them during much of the year. As a result, the quality of surface water is highly influenced by groundwater quality. Due to intensive agriculture in this area, related to corn cultivation in particular, groundwater quality is affected by pesticides and some of their metabolites. Monthly monitoring of water quality at 16 water points in the plain was initiated in March 2009 and is still on-going. It includes some 50 organic molecules, major ions such as nitrate and other non-conservative parameters (pH, temperature, redox potential). A strong spatial variability of the contamination was demonstrated, with predominance of degradation products over parent molecules in some locations, and also showed a strong temporal variability. Besides aiming at a better understanding of the pesticides' fate, our study also addresses the effects of altered water quality on various aquatic organisms. Laboratory ecotoxicological tests calibrated with realistic environmental concentrations found in the aquifer and accounting for proportion of the different molecules were performed. In parallel, investigations of in situ exposures were better controlled. Spatial and temporal distribution of the microbial diversity in groundwater is followed to evaluate its relevance as bioindicators. Macro-organisms restricted to groundwater (hypogean organisms such as isopods or amphipods) are investigated in situ at the scale of species, populations and communities while acute and chronic toxicities are determined in laboratory. First results on amphibians, also studied, showed that the contamination of surface water affects populations (richness) in the environment while in most cases no toxicity has been demonstrated in *Xenopus* larvae. To our knowledge this project may be the first investigation of the effects of pesticides on these three types of organisms using both lab and aquifer studies.

**TU224 Environmental monitoring for the risk management of substances of high concern with the focus on PBT substances** C. Rauert, Umweltbundesamt / International Chemical Management; B. Baensch-Baltruschat, Federal Institute of Hydrology; E. Claus, Federal Institute of Hydrology (BfG); A. Coors, ECT Oekotoxologie GmbH; P. Heininger, Federal Institute of Hydrology; I. Prutz, Umweltbundesamt; U. Hommen, Fraunhofer IME; G. Reifferscheid, Federal Institute of Hydrology Federal Institute of Hydrology; H. Ruedel, Fraunhofer IME Institute for Molecular Biology and Applied Ecology / Molecular Biology & Applied Ecology; M. Keller, Federal

Institute of Hydrology; J. Schoenfeld, Federal Environment Agency. Besides providing the basis for assessments of environmental quality, findings of chemical environmental monitoring programmes (MP) can also contribute useful information for regulatory procedures on chemical substances. Thus, the enforcement of legislation regarding industrial chemicals (pursuant to REACH) as well as the registration or authorisation of medical products, biocides, and plant protection products uses experimental effect data and modelled exposure data within the prospective environmental risk assessment. Here, the detection of chemical substances in the environment through chemical MP may become a source of supplementary information to assess and to validate their environmental fate. The objective of the project presented here is to provide the scientific and technical prerequisites for closer strategic networking between the regulatory procedures for substances and the chemical environmental monitoring with a focus on the identification of potential PBT and POP substances. In a first step, an overview of ongoing chemical MP in Germany was compiled. It was found that mainly substances where production or use is already regulated (e.g. according to Stockholm Convention or REACH) are intensively investigated in MP. Substances as “new” industrial chemicals, currently authorised plant protection products and biocides are less often in the focus of MP. At the same time, the communication structures between the authorities concerned with MP and those responsible for the regulatory issues of chemicals in Germany and in five other EU-member states were investigated. So far, a formal information exchange is established only in exceptional cases: e.g. in Denmark a feedback process exists between the responsible authorities. In Germany, the use of environmental monitoring data within regulatory procedures is mainly practised for findings of plant protection products in groundwater. Possibilities and limitations of using monitoring programmes for regulatory purposes were identified. Based on these results, a concept is developed for a more efficient exchange between monitoring and regulatory authorities. A further concept will be elaborated to identify potentially problematic substances from monitoring results (with the focus on PBT substances). As a last step, concrete suggestions will be made how to organize the post-marketing monitoring of plant protection products, biocides, as well as human and veterinary pharmaceuticals.

#### **TU225 Occurrence of antifouling biocides in South Adriatic Sea: a comparison of Italian and Albanian scenario for coastal areas**

Di Landa, S. Manzo, G. Ansanelli, ENEA / Portici Research Centre; S. Chiavarini, C. Cremisini, P. Massanisso, ENEA / Casaccia Research Centre; C. Minopoli, E. Nardi, ENEA / Portici Research Centre; L. Parrella, University of Naples Federico II; A. Salluzzo, ENEA / Portici Research Centre; S. Schiavo, University of Naples Federico II; A. Tabaku, A. Pellumb, P. Lazo, Academy of Sciences of Albania. Antifouling (AF) paints are used to prevent the adhesion and growth of living organisms to submerged surfaces of ships, boats and aquatic structures, usually by release of AF biocides. The serious environmental problems caused in aquatic ecosystems by extensive use of organotin compounds (e.g. tributyltin, TBT) drove to a global ban of TBT-based paints for all vessels. Alternative products were developed by paint manufacturers and usually contained Cu(I) compounds as main biocide, but this component was ineffective against some algae and diatoms; hence AF properties were enhanced by the addition of “booster” biocides. With this aim a broad range of chemicals are used but they have quite different physico-chemical properties and also different key environmental properties and toxic effects to non-target organisms. Irgarol 1051 and diuron are typical AF biocides with adverse environmental profile but extensively used worldwide in AF paints. Both compounds do not undergo rapid degradation once released in seawater having half-lives longer than 100 days, hence they can be regarded as persistent organic pollutants. Consequently a large number of reports showed the occurrence of irgarol 1051 and diuron with significant concentrations in the coastal aquatic environment of several countries worldwide, especially in sensitive areas with intensive boating activity. Both Albania and Italy have coastal areas facing Adriatic Sea but they constitute a different scenario from the point of view of usage

of AF paints with co-biocides. Actually pleasure boating activities are still developing in Albania and we can expect a reduced environmental loading of AF biocides in comparison to Italian scenario. As far as we know no monitoring data of organic biocides are available for Albanian coastal areas whereas previous studies have been carried out in Italy. In this study seawater samples were collected in September 2012 in selected harbors and marinas located both in Italy and Albania and environmental concentrations of irgarol 1051 and diuron were determined. Low levels contamination of Albanian coastal water by irgarol 1051 (< 0.2-9 ng/l) was observed in comparison to Italy (Puglia region; 5.0-20 ng/l). In Albania diuron exhibited higher concentrations (2.0-93 ng/l) than irgarol 1051, but still lower than those detected in Italy (12-580 ng/l). Due to shortage of recreational craft diuron contamination is more likely attributable to agricultural run-off rather than AF usage.

#### **TU226 (Higher-tier) Risk assessment for birds and mammals under EFSA guidance: practical experience**

D. de Rooze, WIL Research Europe BV / Regulatory Affairs; U. Selditz, Wil Research. In 2009, EFSA published its Guidance document “Risk Assessment for Birds and Mammals” for plant protection products (PPPs). In this guidance document, the risk assessment starts with a screening tier, which is a calculation based on fictive so-called indicator species feeding on single diets, which makes them conservative for exposure (i.e. worst-case). If a risk cannot be excluded in the screening tier, a Tier 1 risk assessment is conducted. This assessment considers so-called generic focal species, which are still fictive, but more realistic as their foraging behaviour and ecology are based on those of real species. If the Tier 1 assessment indicates a potential risk, the assessment is refined in a so-called higher tier risk assessment. Before implementation of the EFSA guidance document, risk assessment of PPPs for birds and mammals was based on the SANCO guidance document (4145/2000). The risk assessment according to SANCO was considered too conservative and simplistic, and was therefore updated by EFSA. The EFSA guidance introduces more realism to the risk assessment. However, despite the fact that the new guidance was developed a.o. because the SANCO guidance was considered too conservative, we have seen many substances that were concluded to be of low risk to birds and mammals under SANCO and are now considered as potentially high risk substances, especially to mammals. Have we wrongly authorized plant protection productions in the past? The preparation of a refined risk assessment requires expert judgement. For each refinement, an uncertainty analysis should be provided. The way this uncertainty analysis is addressed by authorities is not straightforward, which challenges the higher tier risk assessment. In addition, the acceptability of refinement options varies between member states - despite the guidance provided in the EFSA guidance document. Some examples of differences between Member States will be discussed. We have seen that the effort put into the risk assessment for birds and mammals has increased. The question rises if these efforts are justified: has EFSA introduced more safety, or solely additional labour? The presentation aims at discussing issues encountered during risk assessment and what possibilities we may have to remediate and harmonize.

#### **TU227 Identification of community-level effects of toxicants – Trait-based taxa aggregation vs. multivariate statistics**

M.A. Bektov, UFZ - Helmholtz Centre for Environmental Research / Department of System Ecotoxicology; M. Kattwinkel, Eawag: Swiss Federal Institute of Aquatic Science and Technology / Department System Analysis, Integrated Assessment and Modelling; M. Liess, UFZ Center for Environmental Research / Department of System Ecotoxicology. Identification of toxicants effects on biological communities is challenged by complexity and variability of the communities. To overcome these challenges, the trait-based SPEAR approach has been developed. This approach is based on (i) using the relevant traits to identify the vulnerable species and (ii) aggregating these species into a group to reduce the between-replicate differences and scattered low-abundance distribution, which both are typical for biological communities. In previous studies this approach allowed to reduce the

noise and detect toxicants effects at low concentrations in both field and mesocosms studies. However, there is a need to quantitatively investigate its potential for the mesocosm data evaluations and application in the ecological risk assessment of toxicants. Therefore, in the present study we analyzed how aggregation of the sensitive species can facilitate identification of the effects. We used empirical data from a long-term mesocosm experiment with stream invertebrates and an insecticide, as well as a series of simulated datasets characterized by different data matrix saturation (correspond to different sampling efforts), number of replicates, and between-replicate differences. The analyses of both the empirical and simulated data sets revealed that species aggregation approach allows the effect detection at lower saturation of the data matrices, smaller number of replicates, and higher between-replicate differences when compared to multivariate statistical methods (Redundancy Analysis). This leads to the higher sensitivity of the analyzed systems, as the effects were detected at lower concentrations (up to 1000 times). These outcomes suggest that methods based on taxa aggregation have a good potential in the mesocosm data evaluations, as mesocosm studies are usually poorly replicated, have high between-replicate differences, and cannot be exhaustively sampled due to technical and financial constraints. The details can be found in the upcoming publication: Beketov M.A., Kattwinkel M., Liess M., Identification of community-level effects of toxicants – Trait-based taxa aggregation vs. multivariate statistics. Submitted.

**TU228 New developments in the conduct of laboratory and in-situ invertebrate bioassays for use in regulatory mesocosm studies** T. Bennett, Cambridge Environmental Assessments; E. Bateman; S. Taylor; S. Priestly, Cambridge Environmental Assessments. Mesocosm studies are a key part of the regulatory toolkit for refining effects endpoints in regulatory risk assessments. However, due to their closed system design and other practical limitations, a number of problems associated with microcosm and mesocosm studies are common. These include: The absence of biologically relevant recovery for some taxa, unable to re-colonise mesocosms by any means other than reproduction. Limited statistical power for some population endpoints due to the low abundance of larger, typically uni-voltinuous organisms. Seasonal limitations of recovery in line with recommended effect classification timescales (de Jong *et al.* 2008). In order to address these limitations, laboratory based and *in-situ* invertebrate bioassays are techniques that can be used as part of higher tier regulatory mesocosm studies to provide information on key taxa that would otherwise be unavailable for the reasons outlined above (HARAP, 1998). We will provide evidence of recent work carried out in this area as part of regulatory mesocosm testing conducted by Cambridge Environmental Assessments. The information we will present will include methods for the conduct of acute, chronic and reproduction assays for numerous different invertebrates including zooplankton, fly larvae, beetles, and crustacea. In addition, we will present the findings of our work and make recommendations for how these data can be used in support of mesocosm endpoints including by feeding the data into TK-TD models to address the limitations of these complex studies in regulatory risk assessments. Further relevant conclusions and recommendations will also be drawn to stimulate discussion in this area of regulatory research.

**TU229 Development of a chronic Lemna assay to support regulatory endpoints from mesocosm studies with herbicides** S. Priestly, J. Taylor, Cambridge Environmental Assessments; S. Taylor, . *Lemna* are free-floating macrophytes regularly used in tier 1 assessments of ecotoxicity for chemicals with herbicidal modes of action. However, recent experience has demonstrated that *Lemna* is not well suited for inclusion in higher tier static mesocosm studies due to its requirement to derive nutrients solely from the water column and its inability to grow in competition with macrophytes rooted in the sediment of experimental ditches when dissolved nutrients (principally soluble reactive phosphorous) are limiting. We will present the methodology behind the development of a laboratory assay in which the toxicity of a herbicide on the growth of *Lemna minor* could be assessed. This test which was based on existing OECD guidelines (OECD 221) was conducted

concurrently with a field mesocosm test, which provided water for the test which was conducted for 118 days using the same original culture throughout the exposure period. We will present the key findings relevant to the study in addition to guidance and recommendations for future work in this area based on our experience. For example, the original study showed that although recovery of affected populations could clearly be observed, some gibbosity (swollen, overlapping fronds) occurred occasionally in treatments and control replicates. A literature review highlighted ethylene, a component of the SIS nutrients added to the pond water as a possible cause of gibbosity along with low genetic diversity and a high growth rate. Based on the results obtained further tests were conducted to investigate whether the gibbosity observed during the test was a result of exposure to the test item or the findings highlighted from the literature review. To test this, duplicate cultures not previously exposed to any test item were maintained, one from original 'old' culture run alongside the chronic laboratory bioassay and one using 'fresh' *lemna* sourced from our mesocosm facility. Gibbosity, health and frond number were assessed. After comparison of the data from the two tests, the following recommendations can be made: 1) Laboratory bioassays should be used to inform on the recovery potential of *lemna* in higher tier mesocosm studies 2) The nutrient strength of growth media should be reduced for long term (>28 day) assays where effects of ethylene on gibbosity is likely 3) Long term endpoints should be based on frond number only 4) Similar techniques may aid higher tier testing of other macrophytes

**TU230 Exploring higher tier refinement options with fish.** E. Egan, ADAS; S. Taylor; S. Priestly, Cambridge Environmental Assessments. Current guidelines for evaluating the effects of pesticide exposure on fish are mainly limited to standard acute toxicity studies (OECD 203) and established sub chronic and chronic fish studies e.g. OECD 2010, OECD 2012. Despite the benefits these guidelines bring i.e. controlled conditions, high replication and validated methods they are limited in their application to real world situations due to the unrealistic constant exposure profiles and absence of realistic fate pathways typically found in laboratory studies. Consequently higher tier refinement options for fish are required to be developed. Areas for new research will be proposed which will explore options for conducting juvenile fish testing in mesocosms with scope for evaluating endocrine disrupting and toxicity end points under more realistic conditions. The overarching aim of this research is to address the challenges of improving ecological and contextual realism of effects assessment for fish. For this work a number of technical challenges are envisaged. These include the effects of seasonality and climate on the reproducibility of the results. Such challenges will be considered as part of this presentation in which we will propose options for full ecosystem and population based approaches for higher tier effects studies to be conducted on fish in mesocosms for regulatory risk assessments.

**TU231 The effect of applied abraded dusts from neonicotinoid treated seeds in large plots of *Phacelia tanacetifolia* on honeybees (*Apis mellifera* L.)** A. Wehner, M. Kriszan, Eurofins Agrosience Services EcoChem GmbH; S. Knaebe, EAS EcoChem GmbH; U. Heimbach, JKI; J. Pistorius, Julius KühnInstitut. Drift of abraded dust of insecticidal seed treatments resulted in bee poisoning incidents in the past. For risk assessment purposes, tests with realistic applications of defined amounts of dust are needed, e.g. to determine NOEC or LOEC values. However, tests with dusts are much more difficult than tests with liquid substances. Due to solid state and the varying particle size it is challenging to develop standard ways of applying dust in situ and in vitro. In the field it is even more problematic to apply the low rates required in a practical way over a larger area. For this purpose we developed a method to apply defined amounts of dusts together with a dilution material in the field, to determine the effects of exposure on honeybees (*Apis mellifera* L.) to dust from sowing of clothianidin-coated maize seeds. The dust was applied with a purpose-built dust applicator once during bee-flight to flowering *Phacelia tanacetifolia* in a field study in Germany. The study consisted of three treatment groups; two test item treatment groups T1 and T2 and an untreated control C.

The application rate of clothianidin was 0.25 g a.i./ha for the application in the treatment group T1 and 1 g a.i./ha for the application in the treatment group T2. Commercial bee colonies were placed at the edge of the test fields five days before the planned application. Mortality, foraging activity and behaviour of the bees were assessed over four days before and over seven days after the application. The condition of the colonies and the brood development of the colonies were checked once before and four times after application. The results are in line with test results of lower TIER studies.

**TU232 Practical experiences with the off-field non-target arthropod study design** S. Knaebe, EAS EcoChem GmbH; K. Liepold, Eurofins Agrosience Services EcoChem GmbH; G. Weyman, MakhteshimAgan. Structural protection is a main goal for the off-field habitat in the EU directive for the registration of plant protection products. In the past, impact on non-target arthropods (NTA) was assessed with in-field studies. In this study type, mainly the taxa where effects were found in lower tier studies were assessed with a clear focus on beneficials. Even off-field drift rates were tested in-field due to the lack of a better alternative. Since the publication of de Jong et al. (2010) and the ESCORT 3 (2011) a new study type for off-field NTA was introduced. The study runs in off-field habitat where the whole arthropod fauna is sampled and the impact of drift rates is investigated. Since there are a wide variety of off-field habitats (e.g. hedges, forest, shores, meadows, wetlands) a surrogate has to be used. The standard surrogate for the study would be a meadow, due to relevance and range of taxonomic groups found. The study is run for a maximum of 2 months with the aim to find a NOER (No Observed Effect Rate) and a NOEAER (No Observed Ecologically Adverse Effect Rate) for the drift rates tested; the latter allowing for some assessment of recovery potential. Since a large part of the arthropod community has to be sampled, several sampling methods are required. The sampling methods are given as well as the set-up. The field work as well as the reporting for two off-field studies taught a lot about the practicability of the study type. In order to further the standardization of the off-field studies the general set-up as well as the sampling methods will be discussed. Also the complexity of the community assessed results in open questions that should be discussed in a wider community.

**TU233 Honey bee risk assessment: Getting it right at Tier I** M. Miles, Bayer CropScience UK / Analytical Chemistry and Environmental Sciences. The European Food Safety Authority has recently published a draft guidance document on the risk assessment of plant protection products on bees (EFSA 2012). This document is intended to provide guidance for notifiers and authorities in the context of the review of plant protection products (PPPs) and their active substances under Regulation (EC) 1107/2009. The document suggests a tiered risk assessment scheme which claims to provide a simple and cost effective first tier to more complex higher tier studies under semi-field and field conditions. The draft guidance recommends the use of lower Hazard Quotient triggers for oral and contact exposure of 33 and 11 respectively to replace the existing Annex VI trigger of 50. In addition risk assessments which specifically cover the dietary risk for forager worker bees, nurse bees and worker larvae are also proposed. These assessments require new data and require that new test methods are developed and validated to achieve this. As the draft guidance document represents a significant change from existing data requirements and risk assessment the impact of such changes need to be evaluated to understand how these changes may affect future registrations and testing needs. The objective of this paper is to evaluate the impact of the proposed tier I risk assessment on the pass/fail rate of currently available active substances and make recommendations and changes to the EFSA proposal and presents an alternative risk assessment scheme using existing data and validated against the current hazard quotient trigger of 50.

**TU234 Increasing realism of effect trials: Example of a new evaluation of Honeybee brood trials** M. Wang, WSC Scientific GmbH / Dept. Efate & Modelling; J. Pistorius, Julius Kühn Institut. The

evaluation of effects on non target species after pesticide application relies on the availability and suitability of methods to investigate the potential effects. In some cases, the endpoints measured in such trials are selected based on feasibility and taking methodological constraints into account, rather than by biological relevance. In honeybees, for example, the recently formulated protection goal focuses on effects on the colony development (EFSA, 2010 and 2012). The currently used method to investigate effects on development on larvae determines the brood termination rate and compensation index, which are convenient measures due to their practicability. Nevertheless, it is not easy to directly translate termination rate to the effect on the colony level, since the time of brood termination, i.e. at the egg stage or later stages may result in a different impact on colony development. We therefore developed a new approach to include this information into the risk assessment. This new measure (RLI) is based on the time invested in the brood and it is intended to improve the characterization of potential effects on colony development and to address the recently formulated protection goal for honeybees more directly. Based on experimental data we show the differences of present methods and the new method.

**TU235 Comparison of current and new computer methods for honeybee colony assessments** M. Wang, WSC Scientific GmbH / Dept. Efate & Modelling. Colony assessments are an important method for the evaluation of the development and health of honeybee colonies. They are also routinely used to monitor the development in field trials focusing on effects by pesticides. Currently, colony strength is usually estimated visually, e.g. using the "Liebefelder" estimation method. While this method is quick and easy to conduct in the field, it is only a rough estimate of the colony size and individual variation between field staff can be rather strong. Due to practical reasons individual counting of honeybees is not feasible. However, recent computer methodologies based on digital images offer less time consuming evaluations. We therefore tested different computer methods for colony strength estimation and compared them to currently used methods.

**TU236 CALIBRATION OF TIER-2 EFFECT ASSESSMENTS (GEOMEAN APPROACH AND SSD APPROACH) FOR INSECTICIDES WITH RESULTS FROM MODEL ECOSYSTEM EXPERIMENTS** R. Van Wijngaarden, T. Brock, Wageningen University; L. Maltby, Sheffield University. Threshold concentrations for treatment related effects of insecticides, as derived from aquatic model ecosystem tests, were used to calibrate the predictive value of the European Tier-2 acute effect assessment (Geomean approach and SSD approach) on basis of laboratory toxicity tests with arthropods (i.e., the combined taxonomic groups of crustaceans and insects). Our calibration included the following types of insecticides: organophosphates, carbamates, pyrethroids, insect growth regulators, neonicotinoids, biopesticides and a remaining group of insecticides. The derivation of the Regulatory Acceptable Concentration by means of the Geomean approach and based on EC50 values for arthropods and the application of an AF of 100, was not protective in more than 20% of the insecticide cases available. However, in approximately 90% of the insecticide cases available, this Geomean approach was protective when the geomean EC50 was based on the lowest value from either the geomean for insects or the geomean for crustaceans. In the far majority of insecticide cases available, the SSD approach based on acute toxicity data from arthropods (acute HC5/3), was protective. It may be concluded that overall 1), the Tier-2 RAC (Geomean-AF approach) is protective when calculated for insects and crustaceans separately and 2), when applying the SSD approach the application of an AF of 3 to the median HC5 is protective.

**TU237 Public vs experiment data for extrapolation of IGR effect from lepidopteran to dipteran species: a case study** E. Beltran, B. Journeil, P. Adrian, C. Durou, CEHTRA SARL. Various Insect Growth Regulators molecules (IGRs) are currently developed by manufacturers for new Plant Protection Products. These compounds generally exhibit a specific mode of action and they may be proposed by manufacturers as candidates for Integrated Pest Management (IPM). The IGRs consist of

different classes of chemicals with specific modes of action, particularly ecdysteroid agonists and juvenile hormone analogs. Among these IGRs, the hydrazide family (to which chromafenozide, tebufenozide, methoxyfenozide belong) is known to act as moulting hormone on lepidopteran species. Some diacylhydrazine molecules are effective at controlling larvae stage of target lepidopteran however loss of effect is reported for some substituted derivatives of those molecules. Some aspects of the moulting enzymatic mechanism in Lepidoptera may also be observed in other insects, such as *Chironomus riparius* and other dipteran species, which species are non-target ones. Therefore comparing the mode of action of such compounds in Lepidoptera and in Diptera, a similar loss of effect of substituted derivatives may be hypothetically extrapolated for Diptera. Studies according to OECD test guideline No. 218 followed by a reproduction phase of 14 days (with relevant endpoints of the guideline OECD TG No. 233) are currently ongoing with an IGR active substance and its main metabolite (main difference with parent being substitution of chemical function) and the results expected in 2013 may confirm or not this assumption.

**TU238 ILUC impacts of biofuels in climate change impact category: to be, or not to be?** D. Garrain, C. de la Rúa, L. Herrera, R. SAEZ, Y. Lechon, CIEMAT / Energy Dpt. - Energy Systems Analysis Unit. Life Cycle Assessment (LCA) studies of biofuels still hold an advantage in terms of greenhouse gases (GHG) savings on fossil fuels. This statement is questionable if the effects that may have the feedstock crops for biofuels on commercial products from biomass are not counted in the whole life cycle. Induced tensions in the market for other products can cause changes in land use which, in turn, affect GHG emissions. This effect is called 'Indirect Land Use Change' (ILUC). Most of the methodologies for calculating these emissions (including Directives 2009/28/EC and 2009/30/EC) do not include ILUC. In this study we present the main issues addressed at this time within the scientific community, including: the concept of ILUC, the complex causes in order to estimate the effect on GHG emissions, the analysis of most relevant models, and a *pros & cons* list of the proposals of some studies on ILUC possible regulatory treatment (specifically in Europe) and those potentially included in EU directives.

**TU239 European characterization factors for damage to natural vegetation by ozone** T.v. van Goethem, Radboud University / Environmental Sciences; P. Preiss, Universität Stuttgart / Department for Technology Assessment and Environment; L.B. Azevedo, Radboud University Nijmegen / Department of Environmental Science; J. Roos, Universität Stuttgart; M.A. Huijbregts, Department of Environmental Science; R. Van Zelm, Radboud University. This study determined endpoint characterization factors (CF) for damage to natural vegetation caused by tropospheric ozone due to anthropogenic NO<sub>x</sub> and NMVOC emissions. Although endpoint CFs are available for human health damage caused by ozone, studies assessing the impact to natural ecosystems have yet only included regionalized fate and exposure modeling (mid-point level). Therefore, it is the first time these CFs for ozone were derived for actual damage to vegetation. The characterization factors were defined as the area-integrated increase in the potentially affected fraction (PAF) of trees and grassland species due to a change in emission of NO<sub>x</sub> and NMVOCs. The EMEP atmospheric fate model, which simulates emissions, transport and transformation of NO<sub>x</sub> and NMVOCs was used to estimate ground level ozone concentrations. The model divides Europe into 65 source regions. The relationships describing the ecological effects of a pollutant were based on a lognormal relationship between the PAF and ground level AOT40. We found higher CFs for NO<sub>x</sub> and these were largest in south European regions. Normalization factors for ozone exposure on natural vegetation were also determined to indicate relative magnitude of impact of ozone exposure. The normalization factor for ozone impact due to emissions of NO<sub>x</sub> and NMVOC in 2010 is  $1.6 \cdot 10^{-10}$  PAF.m<sup>2</sup>/capita. NO<sub>x</sub> contributed 81% and NMVOCs 19% to the total damage, implying that NO<sub>x</sub> is the main contributor to damage by ozone exposure in natural vegetation in Europe. The CFs found are in the same order as CFs for acidification and therefore it is important to also include ozone effects in

LCA.

**TU240 Modelling the premature deaths due to elevated ozone concentration in Europe** K. Drebszok, AGH University of Science and Technology / Faculty of Energy and Fuels; A. Wyrwa, AGH University of Science and Technology. This study estimates the annual cases of premature mortality (Mort) due to people exposure to elevated ground-level ozone concentrations. The analysis was carried out for the domain covering 33 European countries with a spatial resolution of 20 x 20 km. We used the methodology for estimating Mort based on a comprehensive meta-analysis of time series studies conducted for the WHO. We quantified premature mortality through an association with the so-called SOMO35 indicator for long-term ozone concentrations in ambient air. The calculation of Mort was made with the use of a code developed in Matlab. Three different years were considered: 1995, 2000, and 2005. Selection of years was conditioned by the availability of data: *Baseline mortality, Relative Risk, SOMO35, Gridded Population Data* needed to perform the calculation. Application of high resolution concentration and population data made it possible to indicate the areas with highest Mort values. The results show, there is difference between the number of cases of premature mortality in the period from 1995 to 2005 reaching up to 30%. This simply reflects the variations in ozone concentration in this period. Furthermore, as for: Czech Rep., Finland, France, Germany, Italy, Luxembourg, Portugal, Sweden, and United Kingdom the ozone concentration was higher in 2005 than in 1995, the number of premature deaths has increased. The results can contribute to better design and implementation of local strategies for combating air pollution and human health protection. Modelling the premature deaths due to elevated ozone concentration in Europe

**TU241 Development of a USEtox platform for the characterisation of substances used in cosmetic and detergent ingredients** J. Payet, E. Maillard, O. Hugonnot, C. Roussel, Cycleco. The USEtox model is the only consensual environmental model for the characterisation of ecotoxicological impacts in Life Cycle Assessment and for comparative assessment and ranking of chemicals according to their inherent hazard characteristics. This model has been developed by a team of researchers of the Task Force on toxic impacts under the UNEP-SETAC Life Cycle Initiative and has been chosen as the ecotoxicity method of choice for the ecotoxicological footprint calculation of cosmetic and detergent formulations. On the official internet site, USEtox is written in an excel file and is documented with a list of +3000 characterisation factors and a general guideline giving general recommendations for the user. In this form, USEtox is clearly a model designed for researchers with no efficient and easy use by industrial on their own. The development of an easy-to-use USEtox Web platform for the calculation of characterisation factors of cosmetic and detergent ingredients is presented. This platform is designed for a use by industries that want to use, develop and document characterisation factors for ingredients present in their formulations. With this platform, the cosmetic and detergent industries are provided with: - A guideline to use the model for the development of characterisation factors. This guideline is designed for non-expert industrial actors that need to collect data, calculate the input data value and develop characterisation factors. - A system of documentation on the input data of the model. One of the most important point is to provide the data sources and documentation allowing a continuous improvement of the characterisation factors. - Uncertainty calculation, analysis and management for each input parameter and for the characterisation factor itself. The platform is available online since the beginning of 2013.

**TU242 Integrating Human Indoor Air Pollutant Exposure in Life cycle Assessment of Wood Related Consumer Products** A. Abhishek, S. Hellweg, ETH Zurich. Wood is one of a multifunctional material whose use as a substitute to other non-renewable resources is expected to increase in coming decades. However, emission of toxic air contaminants like volatile organic compounds and formaldehyde from wood related products or heating/cooking systems into the air of buildings has been an environmental concern for several years.

Exposure to these indoor emissions causes adverse human health effects such as childhood asthma and chronic respiratory illnesses. Indoor concentrations of these chemicals can significantly exceed indoor air quality goals particularly in low ventilation settings. Moreover, people increasingly spend most of their time indoors when considering both time spent at home and at the workplace. Both aspects can result in high indoor emission intakes by human beings. Nevertheless, risk assessment and life cycle assessment of wood products generally neglects these health effects from indoor exposure. Such an omission is an important shortcoming, as it hinders a fair comparison with the environmental performance of competitive products and may result in product or process optimizations at the expense of inhabitant's health. The current study aims at - (i) gathering the emission rates of important toxic contaminants released indoors from wood related building materials and heating/cooking systems; (ii) review various decay models that quantify concentrations of emitted chemicals as a function of time (fate analysis) (iii) review existing indoor exposure models for households and occupational settings concerning their use in LCA (exposure analysis); (iv) assess the health effects resulting from intake of such emissions. Keywords: Life Cycle Assessment, Indoor air quality, Human health, Wood related emissions.

**TU243 A framework for assessing environmental damage from soil erosion in LCA** P.S. Quinteiro, University of Aveiro / Centre for Environmental and Marine Studies (CESAM), Department of Environment and Planning; B. Ridoutt, Commonwealth Scientific and Industrial Research Organisation (CSIRO); A. Dias, L. Arroja, University of Aveiro. Topsoil erosion by water and wind is a natural, inevitable and complex process that varies depending on local soil characteristics, ground slope, vegetation cover and climatic conditions. Rates of soil loss can be greatly amplified by agricultural and forestry production system practices, such as tillage. On average the erosion rate for cropland is in the order of  $7.5 \text{ t.ha}^{-1}.\text{yr}^{-1}$ , however rates can also exceed  $20 \text{ t.ha}^{-1}.\text{yr}^{-1}$  when land management practices are poor. The loss of topsoil can lead to a loss of productive capability of land and this has begun to be considered recently in LCA in the context of ecosystem services. However, soil which is displaced is itself a source of potential environmental harm, especially when it reaches water systems. This aspect is not presently considered in LCA and is a substantial gap considering the scale of the issue. This poster presents a framework for assessing environmental damages from soil erosion in LCA. The focus is on the Area of Protection natural environment. Impacts of sediments on human health, as a result of the degradation of drinking water quality, and on the built environment, such as the siltation of reservoirs, can generally be modeled in relation to the additional water filtration and engineering works that become necessary. The framework follows the fate, exposure and effects model: eroded soil transported to inland water bodies which leads to an increase in turbidity and sediment deposition affecting aquatic biota. Four environmental mechanisms for ecosystem quality impact are identified: Burial of invertebrates Decrease in phytoplankton due to reduction in light penetration Depletion of oxygen levels by suspended organic matter Direct impacts of sediments on fish These impacts are in addition to those associated with pesticides, nutrients and other compounds which may be transported to water along with the eroded soil. The development of operational characterization factors for soil erosion impacts on aquatic biodiversity is now the subject of ongoing research. **Keywords:** life cycle impact assessment, sedimentation, soil erosion, ecosystem quality impact **Acknowledgement**—Thanks are due to FCT (Science and Technology Foundation-Portugal) for scholarships granted to Paula Quinteiro (SFRH/BD/78690/2011) and Ana Cláudia Dias (SFRH/BPD/75788/2011).

**TU244 How and at which scale should we assess biodiversity? A comparison of three globally applicable land use LCIA methods in East Africa** L. de Baan, ETH Zurich IED / NSSI; C. Rondinini, Università di Roma La Sapienza / Department of Animal and Human Biology; P. Visconti, Microsoft Research - Computational Ecology; T. Koellner, University of Bayreuth; S. Hellweg, ETH Zurich / Institute of

Environmental Engineering. Land use is one of the main causes of global biodiversity loss. However, there is still no consensus on how the complex cause-effect chain of biodiversity loss can be meaningfully modeled within Life Cycle Assessment (LCA). Traditionally, land use life cycle impact assessment (LCIA) methods were developed based on regionally or nationally available European data sets and mostly restricted to vascular plants. Due to the inherent heterogeneity of biodiversity, these results are not easily transferable to other world regions or other taxonomic groups. In recent years, increasing research efforts have been made to provide comparable biodiversity data with global coverage for different taxonomic groups. This allowed the development of globally applicable land use LCIA methods. In this study, we develop a new LCIA method based on the Global Mammals Assessment (GMA). The results are compared to two other globally applicable land use LCIA methods, which have been recently developed. The first assesses land use impacts on *local* scales, using *relative* species richness as an indicator. The second method models *absolute* potential species extinction on *regional* and *global* scales. In contrast to these two methods, which assess local, regional or global reduction in *total species richness*, the new approach models land use impacts *species-specific*. For each mammal species, the GMA contains an expert based habitat suitability model, indicating the suitability of natural and human-modified habitats. To assess impacts of land use, we model the potential habitat area available to each species before human influence, based on maps of potential natural vegetation (PNV). We then model the habitat area lost per species due to various land use activities. To get the accumulated damage of all land use, we aggregate the individual habitat loss per species, weighted by the global rarity of each species. The habitat losses of rare species are thus associated with higher impacts than of common species. Additional weighing factors based on conservation status (Red List status) can be included. The comparison of the three globally applicable methods is done in the case of East Africa. We discuss the differences in model approaches and the strength and weaknesses of the approaches.

**TU245 The impact of crop cultivation on species richness: The influence of crop type, taxonomic group and location** P. Elshout, Radboud University Nijmegen; R. Van Zelm, Radboud University; R. Karupiah, I.J. Laurenzi, ExxonMobil; M.A. Huijbregts, Department of Environmental Science. Change of vegetation cover and increased land use intensity can directly affect the natural habitat and the wildlife it houses. The actual impact of agricultural land use is region specific as crops are grown under various climatic conditions and ways of cultivation and refining. Furthermore, growing a specific crop in a tropical region may require clearance of rainforest while the same crop may replace natural grasslands in temperate regions. Within life cycle impact assessment (LCIA), methods to address impacts of land use on a global scale are still in need of development. We aim to extend existing methods to improve the robustness of LCIA by allowing spatial differentiation of agricultural land use impacts. The goal of this study is to develop characterization factors for the direct impact of land use on biodiversity, which results from the replacement of natural habitat with farmland. The characterization factor expresses the change in species richness under crop cultivation compared to the species richness in the natural situation over a certain area. A second goal was to identify the differences in impacts caused by cultivation of different crop types, sensitivity of different taxonomic groups, and differences in natural land cover. Empirical data on species richness were collected from literature for both natural reference situations and agricultural land use situations. Reference situations were selected on an ecoregion basis. We calculated characterization factors for 15 crop types, 26 taxonomic groups and 43 ecoregions. A first analysis shows that there exist differences in the sensitivity of different taxonomic groups to land use change, with larger characterization factors (*i.e.* larger impact) for vascular plants than for animal groups.

**TU246 Characterization factors for freshwater eutrophication: sensitivity towards emission location, effect model and species group** L.B. Azevedo, Radboud University Nijmegen / Department of



Environmental Science; A.D. Henderson, University of Texas School of Public Health / Environmental Science; R. Van Zelm, Radboud University; O. Jolliet, University of Michigan / School of Public Health; M.A. Huijbregts, Department of Environmental Science. Different types of effect factors have been suggested in life cycle impact assessment [1-3]. These assume linear, marginal or average change in the effect with a change in the stressor of interest and specification or not of an optimum stressor concentration ( $C_{opt}$ ). The objective of this work is to assess the sensitivity of characterization factors for freshwater eutrophication towards the location of emission – including downstream freshwaters, the effect model selected, and the species group of interest. First, we tested how characterization factors for lakes and streams differ in a variety of European areas. Second, we tested how three different types of effect factors (based on linear, marginal, and average changes) differ for European freshwaters. Third, we compared characterization factors based on autotrophic and heterotrophic species. Our results show that characterization factors based for species living in lakes are usually higher than those based on species living in streams since lacustrine species in temperate regions tend to be more sensitive to phosphorus increases. The characterization factors based on the linear change were usually higher than those based on marginal or average changes since the concentration of phosphorus in freshwaters was usually below environmental targets. This points to the variability possible in characterization factors, as the species group, freshwater type, and effect factor type (linear, marginal, or average change) affect the characterization factors for freshwater eutrophication. Based on the analysis we present recommendations for characterization modeling choices. **References** [1] Rosenbaum RK, Bachmann TM, Gold LS, Huijbregts MAJ, Jolliet O, Juraske R, Koehler A, Larsen HF, MacLeod M, Margni M, McKone TE, Payet J, Schuhmacher M, van de Meent D, Hauschild MZ. 2008. USEtox-the UNEP-SETAC toxicity model: recommended characterisation factors for human toxicity and freshwater ecotoxicity in life cycle impact assessment. *Int J Life Cycle Assess* 13:532-546. [2] Pennington DW, Payet J, Hauschild M. 2004. Aquatic ecotoxicological indicators in life-cycle assessment. *Environmental Toxicology and Chemistry* 23:1796-1807. [3] Huijbregts MAJ, Hellweg S, Hertwich E. 2011. Do We Need a Paradigm Shift in Life Cycle Impact Assessment? *Environmental Science & Technology* 45:3833-3834.

#### **TU247 Implementing RA alongside LCA - a case study with veterinary medicines used in Asian pond aquaculture**

**J. Henriksson**, Leiden University / Institute of Environmental Sciences; A. Rico, Wageningen University / Aquatic Ecology and Water Quality Management Group; P.J. van den Brink, Alterra and Wageningen University; J. Guinee, University of Leiden / Institute of Environmental Sciences. The Life Cycle Assessment (LCA) and Risk Assessment (RA) frameworks are frequently used to evaluate the potential environmental and human health impacts of the use of chemicals in agricultural and industrial production processes. Both methods rely upon similar information, but at different levels of detail, for evaluating environmental toxicity impacts. However, LCA takes the product (which can also be a chemical) as a starting point and RA the environmental compartment. LCA evaluates the environmental consequences throughout a product's lifecycle, and emissions are scaled to a quantity of product or function, referred to as functional unit. As a result LCA focuses on mass loads that lack a time dimension, and also often, but not necessarily, lack a spatial perspective thereby neglecting the exposure concentrations in the receiving ecosystems, which are crucial parameters in RA. Moreover, the timing and characteristics of the exposure events, chemical dose-response effects, indirect effects, multiple exposure effects, bioaccumulation and biomagnification through trophic chains and ecosystem recovery, are largely neglected in LCA. As part of the on-going EU FP7 SEAT project ([www.seatglobal.eu](http://www.seatglobal.eu)), we tried and evaluated the potential of integrating LCA and RA results while evaluating the toxicological effects of veterinary medicines used in aquaculture in Asia. Building upon previous work, we here also aim to evaluate the strengths and weaknesses of each method by using both approaches alongside each

other when evaluating the potential environmental and human health impacts of veterinary medicines applied in Asian pond aquaculture. The assessments build upon a newly developed dataset containing information on chemical use and aquaculture management practices from more than 250 and 1600 farms, respectively, from Asia. The life cycle environmental impacts were assessed using the USEtox model, and the RA approach was performed using the mass-balance model ERA-AQUA. The ERA-AQUA model combines physico-chemical and toxicological information from the studied chemicals, as well as local characteristics of the aquaculture production systems and practices for the calculation of exposure concentrations and ecological risks ([www.era-aqua.wur.nl](http://www.era-aqua.wur.nl)). **Keywords:** Life Cycle Assessment, Risk Assessment, aquaculture, veterinary medicines

#### **TU248 Keeping USEtox up-to-date: What is coming and how you can contribute**

**R.K. Rosenbaum**, Technical University of Denmark / Management Engineering. It is essential that USEtox keeps up with scientific developments and user needs, while adhering to its development principles, remaining an interface between science and application. The USEtox team intends to publish updates to the model algorithms and inputs balancing frequency and stability. Therefore, updates not affecting published characterization factors (CFs) can be done anytime. Updates affecting published CFs may be published yearly at the maximum. The USEtox team has developed an update procedure aiming for an optimal mix of transparency, stability, and scientific quality of model and CFs. Everybody can submit an update proposal via <http://update.usetox.org> and all (worthwhile) proposals – including those coming from the USEtox team itself – have to go through this procedure. Three kinds of updates are distinguished: 1) Corrective updates affecting existing CFs found erroneous (e.g. algorithms, parameters). Correction proposals will be decided and implemented by the USEtox team without consulting external experts. 2) Updates based on data, scientific, and technical progress, further distinguished into updates of a) substance-specific data (affecting one or very few CFs) and b) model structure, parameters, and algorithms (affecting many CFs). Updates of type 2 are differentiated respectively into those affecting existing CFs, promoting “interim” CFs to “recommended”, and those adding new CFs. For any type 2 update judged worthwhile, the review chair of the UNEP/SETAC Life Cycle Initiative invites external experts to review the proposal and advice on implementation with respect to the USEtox development criteria (e.g. scientific quality and consensus, parsimony, evaluation, transparency). Based on that the USEtox team decides whether and how to implement the proposal. If the USEtox team refuses a proposal recommended for implementation by the review panel, the reasoning shall be published. The USEtox team documents the entire updating process, making all documents and decisions publicly accessible (website). The update procedure assures transparency, quality, and independence, avoiding biased influences. Several updates have been submitted for the 2013 USEtox update, pending peer review and final approval, e.g. improved modelling of freshwater metal toxicity, ionizing substances, indoor exposure, sub-continental-specific landscape parameter sets, exposure to pesticide residues in food and new substance input data.

#### **TU249 Biodiversity damage assessment for global warming considering extinction risk of species**

**L. Tang**, . According to Millennium Ecosystem Assessment (2005), climate change is recognized as one of the main factors of biodiversity damage. Though several damage assessment methods used in LCIA have been developed to quantify the damage of biodiversity caused by land use and chemical exposure, there are still few methods for climate change. Therefore the aim of this study is to develop a new method which can quantify the extinction risk per a unit of CO<sub>2</sub> emission for each species using EINES (expected increase in number of species). Procedures are shown below. Firstly, the current and future potential distribution area (up to 2100 year, with two CO<sub>2</sub> emission scenarios) of each species was predicted based on a niche based species distribution model. Secondly, extinction years of two emission scenarios were estimated by assuming that the decrease ratio of the potential distribution area every 100 years is

constant. Finally, extinction risk of each species per a unit of CO<sub>2</sub> emission was obtained by dividing the difference of EINES namely the change of inverse of life expectancy between two emission scenarios by additional CO<sub>2</sub> emissions. Based on this method, representative values of extinction risk of vascular plants in the temperate zone in Japan was obtained using climatic data and distribution data of 216 plant species. The annual damage of plant species diversity caused by global warming is bigger than that caused by toxic substance pollution, and smaller than that caused by land use change.

**TU250 A global comparison of mercury biomagnification in aquatic ecosystems**

R.A. Lavoie, Queens University / Biology; K.A. Kidd, University of New Brunswick; T. Jardine, University of Saskatchewan / Toxicology Center; M.M. Chumchal, Texas Christian University / <http://www.biocueduquaticcecolgylabindexhtml>; L.M. Campbell, Saint Marys University / School of Environmental Studies. The dietary habits of aquatic organisms, particularly those higher in the food web, are important determinants of their mercury (Hg). Over the past decade, many studies have used stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) to assess the relative positioning of biota within a food web and to quantify the "rate" of biomagnification of Hg from primary to tertiary consumers. Log-transformed concentrations of Hg are significantly predicted by  $\delta^{15}\text{N}$  [or trophic level (TL) using a per TL enrichment factor  $\sim 3.4\text{‰}$  for  $\delta^{15}\text{N}$ ] across diverse systems; the antilog of the Hg versus TL slope is a trophic magnification factor, or average biomagnification per TL through the food web, and can be used to assess factors affecting trophic transfer of Hg through aquatic food webs. It is well known that the biomagnification of Hg varies from one food web to another, but the factors underlying this variability remain poorly understood. Herein we compiled results from 65 studies and >240 sites around the world (published and unpublished) that measured Hg (total and/or methyl) trophic magnification in aquatic food webs using  $\delta^{15}\text{N}$ . Studies ranged from freshwater to marine and from tropical to Arctic ecosystems, with most sites in North America. The TMFs were compared against physical, chemical (i.e. productivity, pH, % wetlands in watershed, dissolved organic carbon, Hg deposition, latitude, longitude), and biological (species composition and food chain length) factors known or believed to affect Hg in aquatic systems. For methyl Hg, TMFs varied across all sites from 2 to 74 (median of 6.5), and were on average 1.8X higher than those for total Hg. Of all individual predictors examined, latitude was the strongest correlate with TMFs ( $r=0.38$ ) and, within groups, Arctic food webs had higher median TMFs than those for temperate and tropical food webs. In a multiple regression analysis, latitude and phosphorous loading were the best predictors of TMFs ( $r^2=0.19$ ). Results of this meta-analysis indicate that TMFs for methyl Hg are higher in colder, less productive systems; however, much of the among-system variability in TMFs remains to be explained.

**TU251 Methylmercury biomagnification in an Arctic food web – a sidelong glance across species, seasons and locations**

A. Ruus, NIVA / NIVA; I.B. Overjordet, SINTEF / Materials and Chemistry; H.V. Braaten, Norwegian Institute for Water Research / NIVA; A. Evenset; G. Gabrielsen, Norwegian Polar Institute; K. Borga, Norwegian Institute for Water Research. The Arctic is considered an important area for mercury accumulation, because of long-range atmospheric transport and deposition. Following transformation to organic mercury (e.g. methylmercury, MeHg) the toxicity and bioaccumulative potential is high. In the COPOL project, the aim was *inter alia* to study how prospective climate changes may affect the dynamics of environmental contaminants in Arctic marine food chains. We addressed this aim by scrutinising bioaccumulation of contaminants across years, seasons and locations (purely Arctic vs. Atlantic influenced location). Organisms of the pelagic food web (species of zooplankton, fish and sea birds) were collected from Svalbard (Norwegian Arctic) in 2007 and 2008. Samples were analysed for  $\delta^{15}\text{N}$  (for determination of trophic level), total mercury (TotHg) and methyl mercury. As expected, tissue concentrations of MeHg increased with higher trophic level in the food web (biomagnification) in an exponential manner, however, steeper than observed in several earlier studies (a trophic magnification factor,

TMF, of  $\sim 10$ ). Preliminary results indicate similar TMFs among seasons, with a trend towards slightly higher TMF in July, than in May and October. Highest TMFs in July were previously observed for organochlorine contaminants. There was good correlation between the MeHg and the TotHg content through the food web as a whole, showing an average proportion of  $\sim 65\%$  MeHg (of TotHg) in all organisms studied. Thus although MeHg has a much higher bioaccumulative potential than inorganic mercury, measures of MeHg and TotHg depict similar trends. The tissue selected for the analysis may, however, be of importance, as the amount of MeHg (relative to TotHg) was lower in kittiwake liver, compared with muscle.

**TU252 The role of chemical mode-of-entry and biotransformation on trophic magnification factors in the terrestrial environment**

J.A. Arnot, ARC Arnot Research Consulting / Department of Physical Environmental Science; L. Reid, ARC Arnot Research & Consulting; T.N. Brown, Helmholtz Centre for Environmental Research UFZ / Department of Analytical Environmental Chemistry; D. Mackay, Trent University. Biomagnification factors (BMFs) and trophic magnification factors (TMFs) can be used to assess chemical bioaccumulation in aquatic and terrestrial food webs. BMFs and TMFs greater than 1 are generally considered to be bioaccumulative hazards. Due to substantial data gaps in chemical properties and ecosystem conditions, uncertainties in TMFs in aquatic and terrestrial environments are extensive. In particular, fewer TMFs have been measured in terrestrial food webs than in aquatic food webs and measured chemical property information is limited compared to the large number of chemicals requiring evaluation. Field TMFs are expensive and alternative reliable methods for estimating TMFs are needed. The Risk Assessment IDentification And Ranking (RAIDAR) mass balance model is an evaluative screening-level tool that combines environmental fate and aquatic, terrestrial and agricultural food web bioaccumulation models. RAIDAR includes a terrestrial food chain model comprised of a plant, an herbivore, and a carnivore. Here we exploit this relatively simple linear food chain to examine the role of chemical emissions to the environment (i.e., mode-of-entry to air or to soil) on TMFs in the terrestrial environment for a set of hypothetical organic chemicals comprising a range of partitioning and degradation properties. The simulations include revisions to RAIDAR for improving dietary assimilation efficiency and urinary excretion in mammals and bioaccumulation in terrestrial plants. A case study compares predicted terrestrial TMFs with available measured terrestrial TMFs and includes uncertainty analyses for the chemical information used to parameterize the model. Several thousand organic commercial chemicals are screened to identify terrestrial TMFs greater than 1 using a novel quantitative structure-activity relationship (QSAR) for predicting biotransformation half-lives in mammals. The sensitivity and uncertainty analyses highlight the uncertainty in biotransformation half-lives on TMF calculations and the need to obtain more reliable measurements and estimates for biotransformation half-lives to improve TMF estimation.

**TU253 Importance of lipid analysis and its implications for metrics of bioaccumulation**

R.M. Seston, D.E. Powell, K.B. Woodburn, Dow Corning Corporation / Health & Environmental Sciences; H. Gandhi, Michigan State University / Biogeochemistry and Paleoproteomics Laboratory; P.W. Bradley, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science; M.J. Zwiernik, Michigan State University / Animal Science Vet Med. For the quantification of chemical residues in environmental matrices, many standardized methods have been developed. These methods commonly include rigorous quality control programs to ensure the accuracy and precision of the results. However, the accuracy and precision with which lipid determinations are performed are not held to the same level of rigor as that associated with analysis of chemical residues. Accurate measures of lipid content are of great importance as chemical residues are commonly normalized to lipid content so that results are more comparable between different tissues and different organisms. Additionally, lipid-normalized concentrations of chemical residues are required to calculate fugacity and may be used to calculate measures of

bioaccumulation, including bioconcentration factors, bioaccumulation factors, biomagnification factors (BMFs), biota-sediment accumulation factors, and trophic magnification factors (TMFs). Thus, the accuracy of these metrics is determined by the error associated with measurement of lipid content. In order to reduce error and increase the accuracy of these metrics, measurements of lipid content must be made with a greater deal of certainty than is currently in practice. To demonstrate the importance of this point, the lipid content of whole-body homogenates of aquatic organisms (zooplankton, macro invertebrates, and fish) collected from a temperate freshwater lake were determined using two different methods. The mean percent difference between whole-body lipid content measured using the two methods was approximately 28% across all organisms (n=52). Lipid contents measured by the two methods were used to normalize residue concentrations for select polychlorinated biphenyl (PCB) congeners measured in the same whole-body homogenates. Both sets of lipid-normalized residue concentrations were used to determine BMFs and TMFs for select PCB congeners. This presentation will discuss and expand upon the impacts that lipid content may have on these bioaccumulation metrics. This work was supported in part by the Centre Europeen des Silicones.

#### **TU254 Fugacity: The Rosetta Stone for Interpreting**

##### **Bioaccumulation and Biomagnification in Aquatic Environments**

D.E. Powell, Dow Corning Corporation / Health & Environmental Sciences; K.A. Kidd, University of New Brunswick; D. Mackay, Trent University; R.M. Seston, K.B. Woodburn, Dow Corning Corporation / Health & Environmental Sciences. The potential of a chemical to accumulate in living organisms (bioaccumulation) and increase in concentration with increasing trophic level within a food web (biomagnification) are important considerations for assessing ecological risk. Measures used to assess bioaccumulation and biomagnification take into consideration that pathways of exposure may occur through various sources (i.e., water, sediment, diet, and air). For aquatic organisms, bioconcentration factors (BCF; units of L/kg lipid) describe the uptake and accumulation of chemicals from water only. Bioaccumulation factors (BAF; units of L/kg lipid) describe uptake and accumulation from all sources relative to the amount of chemical stored in the water compartment. Similarly, biota-sediment accumulation factors (BSAF; units of kg OC/kg lipid) describe uptake and accumulation from all sources relative to the amount of chemical stored in the sediment compartment. Biomagnification factors (BMF; non-dimensional units of  $C_{\text{predator}}/C_{\text{prey}}$ ) describe the increase in concentration of chemicals in organisms that are separated by a single trophic level step on a food chain. Similarly, trophic magnification factors (TMF; units of  $C_{\text{organism}}/C_{\text{trophic level step}}$ ) describe the increase in concentration of chemicals in organisms that occupy successively higher trophic levels within a food web. Regulatory screening and assessment criteria used to identify potential bioaccumulative and biomagnifying substances are typically based on laboratory measurements of BCF or field measurements of BAF and BSAF. However, the field-based TMF is increasingly being used to assess bioaccumulation and biomagnification of chemicals in the environment. This has resulted in spirited debate on the utility and comparability of the various metrics for bioaccumulation (BCF, BAF, BSAF) and biomagnification (BMF, TMF). This presentation will show how these metrics are interrelated when expressed in equivalent terms of fugacity (units of Pa) and fugacity ratios (non-dimensional units of Pa/Pa). These interrelationships will be applied to field data for select contaminants and used to demonstrate how to minimize or eliminate bias and uncertainty associated with nitrogen-15 enrichment factors (used to estimate relative trophic level position). The field data will also be used to demonstrate how measures of bioaccumulation and bioconcentration, and their associated properties may be derived directly from the natural food web.

#### **TU255 Use of models to simulate uncertainty and statistical power of the TMF approach**

K.G. Drouillard, A. McLeod, D. Haffner, University of Windsor / Great Lakes Institute for Environmental Research; G. Paterson, SUNYESF / Department of Environmental

Forest Biology. Trophic magnification factors (TMFs) provide a method to assess food web biomagnification which integrates bioaccumulation processes occurring in individuals, in different species and across trophic levels in a given ecosystem. Recent reviews of the TMF approach focussed on sampling methodology to maximize statistical power for detection of contaminant/trophic level relationships and use of bioaccumulation models to establish TMF simulations as screening tools for emerging chemicals of interest. This study uses species optimized and food web bioaccumulation models coupled with Monte Carlo simulations to explore contributors to uncertainty in the TMF approach as influenced by variation in selected bioaccumulation parameters, animal bioenergetic performance and ecological characteristics. Simulations were performed for PCBs 28, 99 and 180 as model compounds which exhibit properties of negligible biotransformation in aquatic organisms, a range of hydrophobicities ( $\log K_{ow}$  5.7 – 7.4) and well established food web biomagnification behavior. Model simulations were established to assign uncertainty propagation related to individual and combined processes associated with 1) differences in AE by diet type across species, 2) differences in growth performance among individuals of a given species, 3) non-steady state in larger fish with indiscriminate environmental sampling of animals based on size rather than age, 4) uncertainty in feeding interactions, specifically contrasts of strong (fixed feeding relationships) and weak (high degree of omnivory) feeding interactions in the food web. Uncertainty propagation associated with the combined simulations above were subsequently used to provide further recommendations on sampling designs to maximize discriminatory power of empirical TMF assessments.

#### **TU257 Deriving environmental risk limits for secondary poisoning**

E. Verbruggen, RIVM Expertise Centre for Substance / Centre for Safety of Substances and Products; E. Smit, RIVM; C. Moermond, RIVM / Centre for Safety of Substances and Products. Secondary poisoning of birds and mammals is one the routes that should be considered in deriving environmental quality standards and performing risk assessments for chemicals. Due to the dependence of certain bird and mammal species for their food on single ecosystems, this route is a very relevant one. The different methodologies for including secondary poisoning in the derivation of environmental risk limits are studied here. It appears that both derivations on basis of diet concentrations and daily dosis have their disadvantages. Different diets may vary widely in energy content, which will cause the amount of diet consumed to vary widely, and as such diet concentrations may not be a good measure of exposure to a substance. On the other hand, daily doses are a good measure of acute exposure to a substance, but may not be adequate to describe body burdens after prolonged exposure, because these are dependent on other toxicokinetic parameters as well, such as metabolism and excretion. An alternative approach is studied, in which the body weight dependent energy expenditure of birds and mammals is used. After values have been set for different prey items, it could be considered to set the values for the environmental compartments (water, soil), instead of biota standards. As a matter of fact this will be necessary for risk assessment purposes to compare with predicted environmental concentrations in water and soil. For quality standard settings this may seem less robust than setting biota standards, but it could outweigh the disadvantages because of variability in monitoring biota. Therefore, reliable data on bioaccumulation are of utmost importance. Generally, the bioaccumulation data considered are a laboratory bioconcentration factor (BCF) and a biomagnification factor (BMF), which is often a default value. It has been suggested to use field derived trophic magnification factors instead of BMF. Recent research on hexachlorobenzene has shown that the field derived bioaccumulation factor (BAF) might be more appropriate than the product of BCF and TMF.

#### **TU258 Review and evaluation of exposure models in the framework of the 4FUN project**

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Barcelo, IIQABCSIC; E. Giubilato; A. Critto, University Ca Foscari of Venice. The assessment of risks to human health from chemicals is of major concern for policy and industry and ultimately benefits all citizens. In this process, exposure assessment is generally considered to be the weakest point, because currently available tools show major flaws such as lack of integrated approach for assessment of combined stressors (i.e. a number of potential pollutants), widespread use of 'worst-case' scenarios leading to over-conservative results and lack of uncertainty/sensitivity tools that allow identifying the important exposure drivers. To overcome these drawbacks, the FP6 project 2-FUN produced prototype software containing a library of models for exposure assessment, coupling environmental multimedia and pharmacokinetic models. The objective of the 4FUN project is to demonstrate, innovate and exploit this prototype software, to standardise it, to transfer it to stakeholders and guarantee its long term technical and economic viability. In order to optimize the prototype, the 2-Fun tool and other exposure tools were evaluated. First, a list of existing exposure tools, mainly multimedia models similar to 2-FUN, was composed based on literature review. The models were selected to cover a comprehensive range of exposure situations, in terms of the sources of exposure, the exposure pathways, and the receptor populations. Next, all exposure models were reviewed using a set of transparent and structured criteria, which were organised according to four lines of evidence: reliability, relevance, uncertainty and practical use. Each line of evidence is subdivided into categories, which are further subdivided into specific sub-categories. Finally, subcategories are composed of assessment criteria, which are evaluated through the use of questions. This approach makes it possible to put the 2-FUN tool in perspective and to identify the list of actions necessary for updating the design of the 2-FUN tool.

**TU259 Systematic prioritization of existing chemicals and exposure pathways under China REACH** S. Zhao, Lancaster Environment Centre; O. Price, Unilever / Colworth Science Park; H. Wang, Z. Liu, Chinese Research Academy of Environmental Sciences; K.C. Jones, Lancaster University / Lancaster Environment Centre; A.J. Sweetman, Lancaster University. As a developing industrialized country, China is evolving a progressive approach in the development of chemicals risk assessment regulations. The Ministry of Environmental Protection (MEP) of China released the revised version of the Provisions on Environmental Administration of New Chemical Substances in 2010. This new regulation, which replaced the previous hazard based approach issued in 2003, describes a risk based approach to chemicals management and has been referred to as "China REACH". This regulation currently applies only to 'new' previously unregistered chemicals. As part of this process the Chinese government also released a new version of the existing chemical substances inventory, the Inventory of Existing Chemical Substances Produced or Imported in China (IECSC), which records about 45,000 chemicals. The systematic prioritization of chemicals on a list such as the IECSC is a complex task and requires a clear definition of the protection goals. This study has reviewed a range of current approaches which are used to identify priority chemicals based on risks to humans with the main objective of identifying suitable screening methods applicable to the Chinese situation. Using an initial training set of chemicals and models such as The European Union System for the Evaluation of Substances (EUSES) and Risk Assessment Identification And Ranking model (RAIDAR), key human exposure pathways have been identified and quantified. Further evaluation on selected pathways has been carried out to determine the most appropriate models/algorithms for consumption of key food groups such as fish and root vegetables. The applicability of these algorithms, QSARs and modeling approaches has been assessed with respect to the chemical distribution routes and human exposure pathways appropriate to China and the variability of these pathways within such as diverse country. As dietary structure of Chinese residents and human characteristics of the population vary significantly from Europe and North America where most of the models were developed, the structure of the exposure models has been reviewed. Predominant indirect human exposure pathways for a broad range of chemicals with a wide range of physicochemical properties have been identified and

evaluated for the Chinese population. A refined modelling approach suitable for assessing human exposure to the Chinese population has subsequently used to prioritize a subset of the substances contained in the IECSC.

**TU260 Global environmental exposure model applicable to any countries and regions for predicting river concentrations of down-the-drain chemicals** M. Yamane, Y. Honda, Kao Corporation / Safety Science; T. Kawaguchi, Nihon Suido Consultants Co., Ltd.; N. Nishiyama, Kao Corporation. Global environmental exposure model which can estimate the river concentration of down-the-drain chemicals has been developed. This new model is distributed hydrological model applicable to river basins both in developed and developing countries. Model is constructed mainly by two parts. One is the Hydro-BEAM (Hydrological River Basin Environment Assessment Model) to estimate river discharge and the other is multi media environmental fate model to estimate chemical concentrations. The model provides river basin data required for analyses and usable by specific latitude and longitude of objective area. The data include altitude, land use, water flow direction, population density and meteorological elements such as precipitation, air temperature, which are freely downloaded from websites. The model can predict daily chemical concentrations with spatial resolution of approximately 1 km grid. Now, we are trying to evaluate the exposure analysis in Java Island, Indonesia as the first targeted country by this model. The number of grid covering to Java Island is 132791 and divided into 2190 river zones. It is confirmed that accuracy of estimated discharge is very good through results of application of this model. Chemical concentrations of major surfactant based on some scenarios have calculated though information of waste water treatment plants (WWTPs) and usage volume of substance per person are limited. This model will be very useful tool for evaluating the detailed environmental exposure analysis of down-the-drain chemicals in any countries and regions, especially, for countries where a high tier model does not exist.

**TU261 COMPARAISON AND ANALYSE OF ENVIRONMENTAL EMISSION/EXPOSURE SCENARIO ON COATING INDUSTRY** N. Pucheux, M. Guiot, S. Andres, INERIS. An Emission Scenario Documents (ESD) describes the sources, production processes, pathways and use patterns of a chemical. Considering the whole life of the substance, an ESD aims to provide a method of estimating emission quantity in the absence of sufficient empirical data and to provide data on each parameter. In July 2009, OECD has developed an ESD about coating industry (ESD number 22). For this ESD, the industry category relevant is IC 14 and includes paints, lacquers and varnishes in industrial uses. In September 2009, Japan authorities provided an addendum intended as a complement to the method used in the ESD No.22, it treats about solvent substances and focus on industrial coating processes but contains also wood products on document. It should be noted that ESD number 22 doesn't include specific uses such as in wood preservation treatment. However, an emission scenario dedicated to wood preservatives has been developed by the OECD Task Force on biocides (revised in 2012). In the context of REACH, Specific Environmental Release Categories (SpERCs) have been developed by the industry to comply with the REACH requirements for ERC (Environmental Release Categories) associated with paint application in certain industries. We propose to compare the different exposure scenarios quoted previously each other in a practical case. Pros and cons will be discussed.

**TU262 Estimating and Evaluating Bioaccumulation of Hydrophobic Compounds in the Aquatic Environment Using Linked SimpleTreat+QWASI+AQUAWEB models** J. Kim, Dow Corning Corporation / Dow Corning Corporation; D.E. Powell, K.B. Woodburn, R.M. Seston, Dow Corning Corporation / Health & Environmental Sciences; F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment). Assessment of the potential for chemicals to accumulate in living organisms (bioaccumulation) and to increase in concentration with increasing trophic level within aquatic food webs (biomagnification) are integral

aspects of the risk assessment process. Bioaccumulative substances are defined as those substances that bioaccumulate in organisms and biomagnify in food webs. The trophic magnification factor (TMF) describes the accumulation and increase in concentration of a chemical that occurs in organisms that occupy successively higher trophic levels within a food web. It has been proposed that  $TMF > 1.0$  may be the best indicator of a bioaccumulative substance. Models developed to evaluate bioaccumulation and biomagnification of chemicals in defined aquatic food chains are used in the United States and Canada for risk assessment and to identify potential bioaccumulative substances. However, these models rely on static concentrations to estimate chemical exposure in water and sediment, which does not take into account dynamic and variable conditions of exposure that may occur in aquatic systems. TMF values may be skewed by variable inputs of a chemical into a food web, concentration gradients, localized hot spots of chemical exposure, or by migrating species that are exposed to different conditions than organisms in the local food web. These are especially relevant considerations when evaluating the TMF of chemicals having point-source emissions. To demonstrate and correct for these biases, three models were coupled together on the same spreadsheet platform: the SimpleTreat model for chemical release from wastewater treatment, the multimedia fugacity model QWASI for chemical fate and transport, and the AQUAWEB model for bioaccumulation in aquatic food webs. The linked models calculate chemical concentrations in the wastewater effluent, the distribution and concentrations of the chemical in sediment and water for each compartment or segment of the defined system, and chemical concentrations in aquatic organisms of the defined food web for each compartment. The linked SimpleTreat+QWASI+AQUAWEB models provide a collective understanding of complex behaviors of a chemical in the aquatic environment. Especially they are useful for evaluating bioaccumulation in food webs of heterogeneous systems where migration or variable exposures may occur or that are confounded by concentration gradients—e.g., concentration gradients across the sediment/water interface.

**TU264 A novel approach to physico-chemical prediction increases accuracy and applicability domain** P. Thomas, CEHTRA SAS; R. SAMSERA, CEHTRA; F. SAHIGARA, Milano Chemometrics and QSAR Research Group; P. Frizet, J. Berlusconi, C. Durou, V. Burosse, CEHTRA. According to the REACH regulation, QSAR results can be used on their own if testing does not appear necessary and if QSAR results are considered relevant, reliable and adequate for the purpose (Chapter R.6 ECHA). The quality of QSAR predictions resides on two factors: the experimental dataset they are based on and the quality of the algorithm used. As data are traditionally divided into training and validation sets, without high quality data to begin with, the algorithm cannot be validated. Much experimental data also have their shortcomings which make the decision on data to be used even more difficult. For example, experimental values may differ depending on technicians, protocol followed or other laboratory conditions. We have therefore concentrated on the production of high accuracy QSARs by initially validating each datapoint selected and then applying a set of internal rules to develop predictions for the applicability domains which are as good as the experimental data used. This approach also allows us to expand to new applicability domains very easily with just a limited (but equally high quality) dataset. Keywords: QSARs, log Kow

**TU265 A spatially explicit dynamic multimedia fate model to investigate pesticide fate in mountain river basins** M. Morselli, University of Insubria / Department of Science and High Technology; A. Ippolito, R. Giacchini, University of Milano-Bicocca / Department of Earth and Environmental Sciences; A. Di Guardo, University of Insubria / Department of Science and High Technology. The use of pesticides in agricultural areas generally implies chemical loadings to surface waters, which can pose a risk to aquatic ecosystems. Due to the episodic nature of pesticide applications and to the spatial and temporal heterogeneity of the processes regulating chemical environmental fate, aquatic organisms are often exposed to fluctuating concentrations or sequential pulses of contaminants. In this context, a modelling approach capable of capturing

such dynamics becomes necessary for an accurate assessment of exposure concentrations. In the present study, a new spatially explicit dynamic multimedia fate model (DynANet) was developed and applied to investigate the fate of some pesticides in an illustrative scenario including agricultural areas located in a mountain river basin. DynANet is based on the DynA model, previously developed by our research group to investigate the fate of chemicals in a dynamic surface water-sediment system. In DynANet, the implementation of geographic information system (GIS) tools allows the simulation of a river network, composed of different links classified according to the Strahler method. Water flux between the river links is described using a water routing procedure, according to which links receive water from their upstream links and from the overland flow occurring in their drainage basins after precipitation events. In the present version of the model, interactions with groundwater are neglected. Runoff contributions of chemicals are estimated coupling DynANet to the existing dynamic model SoilPlus, developed to simulate the fate of organic chemicals in the air/litter/soil system at the local scale. In the present study, DynANet was applied to investigate the fate of a series of pesticides in an illustrative scenario located in the Non Valley (Northern Italy), where apple orchards surround the Novella River and its tributaries. A whole-year simulation was performed for each chemical, using realistic meteorological and application information. Results showed the potential magnitude of exposure peaks, which are particularly important after intense rain events, and highlight the role of such a dynamic modelling tool in understanding the fate of non-point source pollutants such as pesticides.

**TU266 Development of method for estimating emission of metals from waste treatment stage** K. Tsunemi, National Institute of Advanced Industrial Science and Technology / Research Institute of Science for Safety and Sustainability. Four metals—lead, cadmium, mercury and hexavalent chrome—have had their use in products restricted by RoHS directive as specified toxic substances used in electric and electronic equipment. Industries and companies have complied and responded by substituting for substances. However, there is also a possibility that risks from substituted substances are increasing and that there is a risk tradeoff between the substituted and substituting substances. Some issues must be overcome, including the difficulty of risk assessment due to lack of information on exposure and hazardousness of the substituting substances and differences in the endpoints of the substituted and substituting substances which prevents risk comparisons. Thus the development of methodology and implementation of risk tradeoff assessment is needed. This study covers environmental emissions of metals used in various products such as electric and electronic equipment, cars and industrial machines from waste treatment stage such as recycling, incineration and landfill. Focused on incineration, emission factor of each metal into the air was derived using vapor pressure ratio based on the relationship between the emission factor and the vapor pressure of each metal at its refining. This method is applicable to recycling of metals in which emission factor was derived using vapor pressure ratio and removal efficiency.

**TU267 Demonstration of a modular computational tool for human health risk assessment due to contaminated sites or emissions from facilities** R. Bonnard, INERIS / Chronic Risks. In France, risk assessment studies have to be conducted before the implementation or the enlargement of some new facilities and before the reuse of some contaminated sites. These studies have to be performed in accordance to four principles: precautionary, specificity, proportionality, transparency. Until now, no devoted software has been developed in France to assess future exposure and risks for such studies. Because of that, many problems have been reported in exposure assessment studies leading to inconsistency between risk assessment studies and a lack of confidence in results provided. To improve the practices and the transparency of the estimates obtained in these studies, INERIS develops and diffuses modeling tools in the framework of one's missions for the Ministry in charge of the Environment. A peer-reviewed handbook, entitled "Sets of equations for modeling exposure linked to soil contamination or emissions from an industrial facility", has been published and is available

on INERIS' website. This document presents the equations used at INERIS for estimating the media concentrations, the exposure and risk levels. It also describes the origin of these equations and underlines the hypotheses on which they are built and their limits. A modeling and simulation platform (MODUL'ERS) based on the equations presented in this handbook has also been developed. Our main objectives during its conception were to provide a tool (1) suited to different site conditions and tier studies, (2) transparent for any stakeholders and helpful to perform uncertainties analysis. MODUL'ERS consists in a library of preset modules enabling the users to build models in accordance with the site conceptual model (that is to say the pathways from the source to the receptor), by downloading modules and connecting them, to create an exposure matrix. Many options are also available to create a customized application. To improve transparency of studies all the equations, parameters can be viewed by the users, as well all the intermediate calculations performed. Especially, hyperlinks enable to browse among variables and their equations. The first version of the tool will be available at soon. Coupling with a GIS is forecast. Some reports will be also published to make more transparent the assignment of values to input parameters, by describing the values collected and the choices made (best-estimate, ranges, probabilistic distributions).

**TU268 Developing and evaluating exposure models for use in chemical risk assessment in China** O. Price, Unilever / Colworth Science Park. As a developing industrialized country, China is evolving a progressive approach in the development of chemicals risk assessment regulations. The Ministry of Environmental Protection of China released in 2010 the revised version of the Provisions on Environmental Administration of New Chemical Substances. This new regulation, which replaced the previous hazard based approach issued in 2003, describes a risk based approach to chemicals management. As part of this process a new version of the existing chemical substances inventory, the Inventory of Existing Chemical Substances Produced or Imported in China (IECSC) was released, which records approximately 45,000 chemicals. The systematic prioritisation of chemicals on the IECSC is a complex task and requires a clear definition of the protection goals. However, to date no exposure models have been developed to enable a systematic prioritisation and currently the Authorities are using models that have been developed in Europe and the US, the relevance of which remains unknown (Liu et al., 2012). This project is tasked with developing a risk assessment framework, specific for the Chinese environment and population. Four work streams were initiated to develop: a regionalised multimedia fate model for China to provide an assessment of key source, transport and fate pathways; an understanding of the key human exposure pathways to determine the appropriate algorithms for consumption of key food groups to estimate indirect exposure to humans via the environment; methods to quantify exposure through sewage treatment plants (STPs) using a model that is representative of the technology, performance and connectivity in China; passive sampling technologies to enable the wide scale evaluation of surface water quality and STP performance in China. Here we provide an update on the research activities and future integration of the modules into a framework that could be used (1) to prioritise chemicals of concern in China, (2) in risk-based assessments and (3) to guide future National monitoring programmes.

**TU269 Apportionment of sources of PCDD/Fs in Baltic Sea sediment cores** K. Wiberg, . The Baltic Sea is one of the water bodies where high levels of dioxins are observed. In this study, historical contributions of responsible sources to offshore and coastal sediment cores of the Baltic Sea are presented. The source tracing was conducted using positive matrix factorization (PMF). Five factors (F) (potential source types) were obtained, then the chemical fingerprints of these were compared with real source fingerprints. The identification of the sources is summarized in Table 1. **Factor Source Remark on model fingerprint** F1 Atmospheric background or pentachlorophenol Complete dominance of Hp- and O- CDDs F2 Incinerator or High temperature processes Higher fractions of PCDFs F3 Tetrachlorophenol Dominance of 1234678-, 1234689- HpCDFs and OCDF F4 HxCDD /

kraft pulp Dominance of 123679- and other HxCDDs F5 Chlorine (e.g. chlorine bleach, chlor-alkali prod.) Higher fraction of 2378- and other TCDFs Table 1: The five source profiles (Factors) identified by receptor modelling Generally, both in coastal and offshore areas the contributions of all sources have been decreasing from peak years (PkY) (Table 2). **Coastal Periods PkY F1 F2 F3 F4 F5 Residual** Seskarö Bay 1944-2009 1966 5-14 0-9 21-50 26-36 0-2 7-41 Gussö Bay 1972-2008 1992 17-18 20-21 20-24 28-31 1-2 8-9 Kallholm Bay 1928-2008 1984 12-15 61-63 4-7 3-6 3-4 10-15 Nordmaling Bay 1969-2008 1982 64-83 2-13 12-18 1-7 0-1 0-4 Nätra Bay 1961-2009 1976 17-28 3-15 15-23 2-11 18-28 9-35 Svartvik Bay 1960-2009 1970 30-43 0-15 19-35 4-11 9-24 5-11 Sandarne Bay 1925-2009 1967 8-20 0-3 4-19 49-70 0-2 7-19 Lövsta Bay 01 0cm-40cm 40cm 54-65 0 24-32 0 0 9-19 Lövsta Bay 02 0cm-47cm 40cm 52-67 0 26-44 0-3 0-1 0-11 Table 2: Percentage contributions of sources to coastal sediment cores after peak year

**TU270 Accuracy in measured values or robustness in (Q)SAR predictions ! Is it the shift of a paradigm?** R. SAMSER, CEHTRA SAS; F. Sahigara, Milano Chemometrics QSAR Research Group / Environmental Sciences, University Milano-Bicocca; J. Berlusconi, P. Thomas, CEHTRA SAS. Undoubtedly, the recent years have seen a growing awareness of (Q)SARs and their possible applications. This quantitative way to relate structures and their activities has considerably built a bridge to overcome several existing gaps in the scientific domain! Their reliability and robustness has now also found possible applications in the regulatory world! Experimentation on the other hand, can surely be a good way to assure accuracy in results, but the limitations of experimental techniques are not unknown to the mankind! At least, uncertainties in measured values can be the simplest way to reflect this fact! Building a trend on the sum of, at least, experimental uncertainties, can lead invariably to a false description and therefore induce false predictions. Based on this scenario, it is normal that robustness of a (Q)SAR is always doubtful. In other words, the impact of uncertainty in measured values can easily be observed on the predictive ability of the resulting (Q)SAR model. Irrespective of such uncertainties with measured or predicted values, in theory there does exist an exact reference value for any property of a given structure. If a (Q)SAR could be built based on an equation that describes an absolute mechanism, not submitted to biological variations – such as those described, for example, by Mackay et al. (2009)[1] for activities, and if the parameters that define that (Q)SAR are determined efficiently, then that (Q)SAR will be independent to experimental uncertainties and can provide an absolute theoretically true value. Thus, it would lead to a shift of the paradigm: the true reference would be the mathematical value (as accepted in physics for example), and experiments would tried to reach that reference. Of course, we are aware that there would be no value to have a “perfect theoretical value impossible to reach”. But regarding some endpoints such as Log P and Water solubility, some complex studies give near perfect results (by slow stirring for example), and that is our goal: reaching the same “near perfect results” in a less complex way, but as robust. We will discuss the impacts of such approach, from a regulatory and an experimental point of view. <br clear="all" /> [1] D. Mackay et al., The physicochemical basis of QSARs for baseline toxicity, *SAR and QSAR in Environmental Research*, Vol. 20, Nos. 3-4, April-June 2009, 393-414.

**TU271 The Role of Uncertain Substance Property Predictions in the Chemical Hazard Ranking of Triazoles** L. Golsteijn, Radboud University; M. Iqbal, Linnaeus University, School of Natural Sciences; S. Cassani, University of Insubria / QSAR Research Unit / Department of Theoretical and Applied Sciences; H. Hendriks, Radboud University Nijmegen, Department of Applied Stochastics; S. Kovarich, University of Insubria; E. Papa, QSAR Res. Unit Environ. Chem/Dep. Structural Functional Biology; E. Rorije, National Institute for Public Health and the Environment (RIVM); U. Sahlin, Linnaeus University, School of Natural Sciences; M.A. Huijbregts, Department of Environmental Science. Comparative Toxicity Potentials (CTPs) quantify the potential ecotoxicological impacts of chemicals per unit of emission. They are the product of a substance's environmental fate, exposure, and hazardous

concentration. When empirical data are lacking, substance properties can be predicted. The goal of this study was to assess the influence of uncertainty in substance property predictions on the CTPs of triazoles. Uncertainty in predicted physicochemical and toxic properties was quantified from the data underlying the applied quantitative structure-activity relationships (QSARs). Uncertainty in aquatic biodegradation half-life categories based on chemical structure, and in water-soil and water-sediment extrapolation factors, was derived from empirical data ranges. Uncertainty in the prediction of the hazardous concentration related to the species' sample size was also included. Parameter uncertainties were treated as probability distributions, and propagated by Monte Carlo simulations. The 90% confidence interval of the CTPs spanned typically over 4 orders of magnitude. CTP uncertainty was mainly determined by uncertainty in biodegradation rates and soil sorption, together with the small number of species sampled. Our findings imply that the reliability of CTP predictions for triazoles can particularly be improved by including experimental data for biodegradation and soil sorption, and developing toxicity QSARs for more species.

**TU272 Comparison of Predicted vs Experimental Partition Coefficient Results** D. White, S. Woolley, Harlan Laboratories Ltd; C. Mead, Harlan Laboratories Ltd / Ecotoxicology dept. With the ever increasing importance of the environmental impact of partition coefficient data and the implications on REACH testing and other regulatory requirements, the reliability of data obtained for partition coefficient is of increasing concern. The use of QSAR's to predict Physico-Chemical data is widely recognised. However, due to the number of experimental method choices available for partition coefficient assessment and the varying applicability of these methods to different substances, the QSAR prediction is now scrutinised more widely. Additionally, the accuracy of QSAR's has become more important when initial method considerations are performed. In this assessment we compare the accuracy of the results obtained from the predictive software against experimentally determined values from various methodology.

**TU273 Acute and chronic ecotoxicity QSARs for narcotics based on water solubility** P. Thomas, CEHTRA SAS; J. Dawick, Shell Health - Environment and Product Health; R. van Egmond, Unilever / Safety & Environmental Assurance Centre; M. Lampi, ExxonMobil Petroleum and Chemical / EMBSI; S. Presow, Euro Chlor; P. Lemaire, Total - Special Fluids Division; M. Galay-Burgos, ECETOC. The relationship between Chemical Activities (as defined by phase equilibrium thermodynamics) and toxicity of narcotic chemicals was originally hypothesised at the end of the 1930s but has only recently been reanimated by Mackay *et al.* (2009). These authors demonstrated that chemical activities can be used to determine toxicity for narcotics for any species (mammals, fish, invertebrates...) regardless of the exposure medium (air, water...) as the toxic effect is hypothesized to occur at a specific activity in the organisms (estimated by the above authors at around 0.01). Thus, toxicity as described by concentration (i.e. LC50) can be substituted for by the ratio between concentration and the subcooled liquid solubility) and the regression slope of LC50 and water solubility is expected to be 1. Nevertheless, the authors found that in practice the slope for this relationship is actually closer to 0.8. ECETOC set out to explore this relationship further using a high quality dataset and consider its potential for use as a QSAR to estimate acute and chronic toxicity for non-polar narcotics. Keywaords: Activities, Phase-equilibrium-thermodynamics, QSAR, effects,

**TU274 Modelling physicochemical properties, degradation, bioconcentration and ecotoxicity of alkanes** P. Fisk, A. Girling, L. McLaughlin, R. Wildey, Peter Fisk Associates; D. Livingstone, ChemQuest Consultancy; G. Whale, Shell Technology Centre. Quantitative structure-activity relationships (QSARs) for property prediction are widely available 'off the shelf'. The use of such models is well-known but this suffers from the drawback that for the majority of cases the models are not trained for specific chemical classes. In this

poster we demonstrate that higher accuracy and reliability can be achieved by focussing on the class of concern, and that development of new QSARs need not be too demanding. We concentrated on alkanes in the range 4 to 50 carbon atoms; they were branched, linear and cyclic. Unsaturated and aromatic substances were not included. In summary, the developments achieved in this work were: **Physicochemical properties:** new fragment-based methods and new property correlation methods for boiling, vapour pressure, octanol-water partition coefficient and solubility in water have been developed. **Bioconcentration:** a standard bilinear approach was developed, showing that alkanes bioconcentrate less than might be expected from their octanol-water partition coefficient, and less than other hydrocarbons **Biodegradation in water:** predicted from a new fragment method based on structural elements present. **Ecotoxicity** of pure substances and complex multi-constituent substances must be considered in terms of the predicted effect and the amount of substance that can dissolve, taking into account the volume of substance added. The results have enabled assessment of persistence, bioaccumulation and toxicity potential for a wide range of complex alkanes without the need for costly experiments.

**TU275 Spatial distribution and risk of cyclic volatile methylsiloxanes (cVMS) in surface sediments of Tokyo Bay, Japan** D.E. Powell, Dow Corning Corporation / Health & Environmental Sciences; N. Sukanuma, M. Itai, K. Kobayashi, T. Nakamura, K. Ninomiya, Silicone Industry Association of Japan (SIAJ); S. Ushioka, Environmental Control Center (ECC). The spatial distribution and environmental risk of three cyclic volatile methylsiloxanes (cVMS) were evaluated in surface sediments of Tokyo Bay, Japan. The three cVMS materials were octamethylcyclotetrasiloxane (D4; CAS No. 556-67-2), decamethylcyclopentasiloxane (D5; CAS No. 541-02-6) and dodecamethylcyclohexasiloxane (D6; CAS No. 540-97-6). The cVMS materials are widely used in industrial and consumer applications and the wastewater stream represents a major post-use disposal route. Generally, the cVMS materials are relatively volatile, have very low water solubility, have intermediate values for  $K_{OA}$  (octanol/air partition coefficient), and have very high values for  $K_{OW}^{SA}$  (octanol/water partition coefficient) and  $K_{AW}^{SA}$  (air/water partition coefficient). When released to water, the cVMS materials are removed from the water column by volatilization, deposition to sediment, and hydrolytic degradation. Because of the very low water solubility and high affinity for organic matter, cVMS materials that deposit to sediment appear to be confined to the general vicinity where released. Surface sediments (surface 1-cm of sediment) were collected from 20 locations in Tokyo Bay following a systematic stratified random sampling design based on a 5-km mesh grid that extended across the bay and about 30 km seaward from the head to the foot of the bay (defined study area of 500 km<sup>2</sup>). Concentrations of the cVMS materials in surface sediments were log-normally distributed over the study area, with concentrations decreasing with increasing distance from point-source discharges at the head of the bay. Except for D4 in some samples, concentrations of the cVMS materials were greater than the method detection limit of 0.6 ng/g wet weight (ww). Mean concentrations for the study area were 1.61 ng/g ww for D4 (range 0.155 to 6.07), 45.9 ng/g ww for D5 (range 4.79 to 113), and 9.13 ng/g ww for D6 (range 1.70 to 18.9). When normalized to total organic carbon (OC) content, mean concentrations for the study area were 201 ng/g OC for D4 (range 59.9 to 671), 5420 ng/g OC for D5 (range 1350 to 13300), and 1240 ng/g OC for D6 (range 341 to 2230). Probabilistic risk assessments demonstrated that there was >99.9% certainty that exposure concentrations in surface sediments of Tokyo Bay *did not* exceed chronic no-observed effect concentrations (NOECs) that were protective for 99.9% of sediment dwelling organisms.

**TU276 Heavy metal and organic carbon distribution in brackish tidal marsh soils along the Elbe estuary** K. Hansen, A. Eschenbach, A. Groengroeft, E. Pfeiffer, University of Hamburg / Institute of Soil Science. The tidal marshes of the Elbe estuary in northern Germany form a transition zone between land and sea. Marsh soils are an important component of this highly dynamic ecosystem, as they fulfil various ecosystem functions. Amongst others they act as a source or



sink for carbon and as a filter and buffer for contaminants. Today the estuary is more and more affected by the consequences of climate change and anthropogenic interference (e.g. deepening of the waterway). However, the pollution of sediments and marsh soils of the Elbe estuary, resulting mainly from industrial activities in former decades, is still present today. The central subjects of this research project are the ecosystem functions of the tidal marsh soils. Particularly the distribution of heavy metals within the soil and its link to the organic carbon content was investigated. Furthermore the suitability of heavy metals as a proxy for the sedimentation dynamics of the sites was examined. The distribution of carbon and pollutants were determined at sites within different salinity zones (oligo-, meso- and polyhaline). Therefore we examined study sites in the inner, mid- and outer estuary. Preliminary results show positive correlations between heavy metal and organic carbon concentrations as well as the amount of the fine grain-size fraction (< 20µm). When looking at the respective soil profiles, this correlation does not become especially obvious at all sites, as topsoil horizons contain high amounts of organic carbon, but low amounts of heavy metals. Within the subsoil the relations become more pronounced. The heavy metal distribution and its connection to the sedimentation dynamics will be discussed in this presentation on selected profiles.

**TU277 An integrative toolbox to evaluate the environmental quality of transboundary transitional waters: the case study of Minho**  
**Lopes**, University of Aveiro / CESAM; C.M. Cardoso, University of Aveiro & CESAM / Biology & CESAM; M.E. Pereira, CESAM, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal / Department of Chemistry; M. Caetano, IPMA I.P.; P. Coelho, CESAM, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal / Chemistry; F. Laranjeiro, A. Lillebo, University of Aveiro / Biology & CESAM; R. Pereira, University of Aveiro / CESAM, Center of Environmental and Marine Studies, University of Aveiro; P. Pereira, Portuguese Institute of Sea and Atmosphere; J. Raimundo, IPMA I.P. / Biogeochemistry & Environmental Impact; S. Rodrigues, CESAM and Aveiro University; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; R. Beiras, Universidade de Vigo / Ecology Faculty. The Directive 2000/60/CE of the European Parliament and of the Council (Water Framework Directive-WFD) aims at maintaining and improving the aquatic environment in the Community. According with this, each member state has to protect, improve and recover the conditions of all aquatic ecosystems to achieve, by 2015, a good chemical and ecological status for all water bodies, including transitional waters. These are bodies of surface water in the vicinity of river mouths (estuaries) which are partly saline but substantially influenced by freshwater flows. To achieve the WFD objectives, further specific measures for pollution control and for establishing environmental quality standards will be required. Meeting these needs, the Team-Minho project aimed at establishing harmonized scientific criteria, which would allow typing, referencing and classifying the ecological status of transitional water bodies in southern Galicia and northern Portugal, including the transboundary Minho river estuary, and the transfer of results to the relevant public institutions, stakeholders and society in general, in order to assist in the implementation of the WFD by providing a framework for the effective protection of transitional waters. This presentation will introduce the results obtained within the Team-Minho project regarding the application of chemical, biological and ecotoxicological indicators to establish reference conditions and for the evaluation and classification of the ecological status. Chemical analysis and ecotoxicological assays, with species representative of different taxonomic and functional groups, from different trophic levels, were carried out for water and sediment samples collected along the Minho rivers. The obtained results point to a different status of contamination of the water and sediment compartments along this water course. The integration of the ecotoxicological indicators, as a complement to chemical and biological indicators, in the classification of the two water bodies within the WFD will be discussed.

**TU278 Reconstruction of Contaminant Profiles in Marine**

**Ecosystems using Whale Earplugs** **E.M. Robinson**, Baylor University / The Institute of Ecological, Earth, and Environmental Sciences; S.J. Trumble, Baylor University; M. Berman-Kowalewski, Santa Barbara Museum of Natural History; B. Subedi, Baylor University / Department of Chemistry; S. Usenko, Baylor University. Contaminant profiles were reconstructed for 12 organic contaminants and mercury from a blue whale earplug with 24 chronological layers at 6-month resolution. Contaminant trends and profiles have been reconstructed from matrices, such as sediment and ice cores, have provided a wealth of information regarding contaminant behavior and environmental fate. Similar to all mammals, whales excrete wax into their ear canals; however, select whale species, such as blue whales, accumulate their earwax (cerumen) over their lifetime (~20 to 100 years) forming an earplug. Over the past 60 years, many earplugs have been archived in some of the world's most prestigious natural history museums. Earplug layers were used to estimate the organism's age similar to aging tree rings. Whale earplugs represent a unique mammalian matrix capable of recording and archiving lipophilic contaminants. An analytical method was developed and validated for the analysis of lipophilic organic contaminants (22 pesticides, 14 PCBs, and 7 PBDEs) in whale cerumen was developed using an enhanced pressurized liquid extraction and gas chromatography-mass spectrometry electron capture negative ionization and electron impact modes. This method was utilized for the analysis of a historically archived gray whale (*Eschrichtius robustus*) earplug (harvested in 1969) and a recently harvested blue whale (*Balaenoptera musculus*) earplug (harvested in 2007). The blue whale earplug was also analyzed for mercury following EPA method 1631. Blue whale blubber (from the sample organism) was also analyzed for pesticides, PCBs, and PBDEs. The blue whale earplug and blubber had similar contaminant profiles; although, contaminant concentrations were an order of magnitude higher in the blubber than the earplug. Reconstructions of the blue whale's lifetime contaminant burden demonstrated similarities to a previously published pharmacokinetic model for PCBs in a beluga whale. Concentrations ranged from 0.070

**TU279 Integrative assessment of water quality in transitional waters zones: The Lima river as a study case.** S. Rodrigues, CESAM and Aveiro University; C. Barroso, Department of Biology & CESAM, University of Aveiro; M. Pereira, J. Coelho, Department of Chemistry & CESAM, University of Aveiro; F. Laranjeiro, A. Lillebo, Department of Biology & CESAM, University of Aveiro; R. Pereira, Department of Biology of Faculty of Science, University of Porto; A. Soares, Department of Biology & CESAM, University of Aveiro; R. Beiras, ECIMAT, University of de Vigo; **I. Lopes**, Department of Biology & CESAM, University of Aveiro. The Water Framework Directive 2000/60/EC (WFD), adopted on 22 December 2000, intends to establish a framework for the protection of inland surface waters, transitional and coastal waters, and groundwater masses. The overall objective of the WFD is to establish harmonized scientific criteria allowing the characterization and determination of reference conditions and classification of ecological status of all water bodies. The ultimate objective of the WFD is to achieve a good ecological status of all water masses by 2015. To attain these purposes, further specific measures for pollution control and for establishing environmental quality standards will be required. This work aimed at assessing the water quality of the Lima river estuary by integrating chemical, biological and ecotoxicological indicators. This estuary was selected as a case study because it is an especially productive and ecologically sensitive area, with high population density and socio-economic importance, therefore being subject to numerous anthropogenic pressures. This presentation will introduce the results obtained regarding ecotoxicological assays (performed with species representative of different trophic and functional groups), monitoring imposex in key indicator species and chemical analyses, performed for water and sediment samples, to evaluate and classify the water quality of the Lima river estuary. The results showed different degrees of contamination in the sediment and water compartments of the Lima river, in terms of chemical and ecotoxicological assessment. High levels of mercury (though within regulatory guidelines), toxicity effects and imposex were registered in

the harbour area, being highest at the sampling site near the shipyard. A weigh-of-evidence approach was used to assess the ecological status of the aquatic system of the Lima river.

**TU280 Evaluation of the use of sediment bioassays with *Nassarius reticulatus* within a TBT pollution monitoring framework**

Laranjeiro, CESAM & Biology Department; S. Perez, Universidade de Vigo / ECIMAT; P. Navarro, Universidad del País Vasco/ Euskal Herriko Unibertsitatea / Zientzi eta Teknologia Fakultatea; R. Beiras, Universidade de Vigo / ECIMAT; C. Miguez, University of Aveiro / CESAM & Biology Department. Due to intense anthropogenic activities, estuarine areas are often outbreaks of pollution. An example is the pollution by tributyltin (TBT), a biocide present in antifouling paints applied to ship hulls that causes the imposex phenomenon in gastropods. Despite the use of TBT in antifouling paints had been totally banned from European waters in 2008, the persistence of this compound in sediments may cause a slow decline in pollution levels over time. Using Vigo harbour (Spain) as a case study, this work presents a methodology to assess the sediment quality regarding the contamination by TBT in estuarine areas that are subject to intense naval traffic. To accomplish this purpose, females of *Nassarius reticulatus* (Gastropod) were exposed during 28 days to sediment collected from several sites around Vigo harbour. The penis growth in females was used as the endpoint to assess the level and bioavailability of TBT in sediments, since this is a specific dose dependent response to TBT. The concentration of TBT and its debutylated forms (DBT and MBT) in sediments were determined. Also, an imposex survey was carried out at the same sites where sediments were collected. The percentage of imposex (%I), the vas deferens index (VDSI) and the relative penis length index (RPLI) were used to assess the levels at each site. The laboratory bioassays showed a significant penis increase in females exposed to sediments collected from two sites around an area of intense naval traffic (fishing port and small marinas) with nearby dockyards. The field survey corroborated the results obtained in the bioassays, i.e., the highest levels were observed in the same sites that gave a significant increase of penis length: percentage of females with imposex, VDSI and RPLI levels attained 100%, 4.8 and 88.3 respectively. Interestingly, the TBT concentration in sediments obtained from chemical analyses didn't correlate well with the above results, which points out for the importance of the bioavailability of TBT in sediments that may be determined by the nature of the sediment itself (e.g. grain size and organic matter content). This work thus provides an integrated methodology to assess the status of TBT contamination of sediments and their impact to ecosystems, as well as it asserts the need for assessing the levels/bioavailability of contaminants in sediments by measuring their biological effects through laboratory bioassays, instead of relying on chemical assessments solely.

**TU281 Analysis of Marine Environmental Assessment approaches regarding contaminants within the PERSEUS project**

D. Gonzalez Fernandez, IESJRC; G. Hanke, N. Zampoukas, Institute for Environment and Sustainability - Joint Research Centre; M. Giani, Nazionale di Oceanografia e di Geofisica Sperimentale; B. Andral, M. Bouchoucha, S. Laroche, Lab. Environment and Ressource Provence Azur Corse, IFREMER. France; M. Marini, A. Campanelli, Institute of Marine Science - CNR; A. Oros, National Institute for Marine Research and Development; M. Pantazi, C. Vassilopoulou, Institute of Marine Biological Resources - HCMR. Policy-orientated marine Environmental Research for the Southern European Seas (PERSEUS) is a research project that, based on the objectives and principles of the Marine Strategy Framework Directive (MSFD, 2008/56/EC), assesses the impact of natural and human-derived pressures on marine ecosystems in the Mediterranean and Black Seas in order to design an effective and innovative research governance framework based on solid scientific knowledge. One of the focal points of the project is the policy cluster, which aims to identify, develop and promote tools and methods to ensure consistency in the assessment of marine environmental status across EU and non-EU countries, according to the criteria for Good Environmental Status (GES) relevant to the 11 MSFD Descriptors (Commission Decision 2010/477/EU). The information presented herein

is focused on the analysis of assessment elements used by Regional Sea Conventions and the EU countries in Descriptor 8: Concentrations of contaminants are at levels not giving rise to pollution effects. Analysis included a review of criteria and methodologies applied in the MSFD Initial Assessments to help identification of diverging strategies and to highlight their differences. Initial Assessments for Descriptor 8 varied greatly in their shape, organization and volume. Data sources ranged from transboundary level to national-regional monitoring programs. Criteria and methodologies included EU regulation, Regional Seas Conventions and national approaches. Main gaps and needs related to frequent lack of data for concentration of contaminants in different matrices (water, sediment and biota) or biological effects. The outcome of this analysis will be used in further steps to harmonize coordination among EU countries and Regional Seas Conventions approaches, and to develop in a collaborative approach assessment elements for the application in non-EU countries.

**TU282 Bivalve shells reuse and recovery: bioinertisation and engineering applications**

A. Zuin, Ca Foscari University of Venice / Molecular Sciences and Nanosystems Department; S.-. Manente, Ca Foscari University of Venice / Department of Molecular Sciences and Nanosystems; R. Ruggeri, G.R.A.L. Lagoon Fishery Resources Management; G. Ravagnan, Ca' Foscari University of Venice. The disposal of waste material constituted by shells resulting from processing subsequent to Bivalve Molluscs harvesting for sale purpose constitutes a big problem for the Venice Lagoon ecosystem from the environmental point of view both because of the large volumes involved and its specific characteristics. In some areas this material, which is currently discarded at sea, comes up to cover the lagoon bed with a thick layer that prevents the benthic communities natural development. A previous work highlighted that as shells are bio-minerals constituting the exoskeleton of organisms known for their ability to accumulate pollutants, they are able to block these pollutants in the crystal structure of the valve thus acting as bio-inerting tools. So we assessed the possibility of a differing shell waste management studying the opportunity to use it in the construction of protective barriers for hydraulic consolidation such as gabions. This would offer the double advantage, on one hand, to provide a sustainable solution to the shell waste disposal problem, with the aim of an integrated closed-loop (the material produced in the lagoon of Venice will be reused in the same place), and, secondly, to remove inert material that contains pollutants that would, in this way, subtracted from the system. After the product implementation we evaluated the evolution of its biological and mechanical characteristics with the aim of highlighting its stability, strength and effectiveness, as well as the opportunity to extend the use of this technology in all erosion risk areas located in the Venice Lagoon and, in general, throughout the Northern Adriatic.

**TU283 Approaches for calculating environmental exposures of aquaculture medicines**

J.P. Staveley, Exponent; R. Endris, Merck Animal Health; G. Scheef, MSD Animal Health Innovation GmbH / Preclinical Development. Potential risks to the environment from the use of veterinary pharmaceuticals are required to be evaluated in a number of jurisdictions including the European Union, the United States, Japan and Australia. Harmonized guidance (VICH, 2000; VICH, 2004) as well as more recent guidance from the European Medicines Agency (EMA, 2008) provides a process for conducting these environmental assessments which essentially involves comparing the Predicted Environmental Concentration (PEC) to the Predicted No-Effect Concentration (PNEC) for key receptors. However, available guidance is much more developed for drugs used on terrestrial animals than for aquatic animals. In particular, guidance is limited on approaches for derivation of the PEC for aquaculture drugs. In this presentation, case studies are used to illustrate approaches for a variety of aquaculture systems. In the environmental assessment of the use of Aquaflor® (florfenicol) for freshwater-reared finfish, PECs were derived for pond, flow-through raceway, and recirculating aquaculture systems. In the environmental assessment for the use of SLICE® (emamectin benzoate) for saltwater salmonids, approaches were developed to derive PECs for

net pen aquaculture systems. The assumptions and calculations used in these case studies to derive the initial PECs and refined PECs, for Tier A (acute) and Tier B (chronic) exposures, are discussed. In most instances, a typical case as well as a worst-case scenario is used. Derived PECs also reflected factors considered important by regional authorities. The PECs were then compared to the PNECs to determine the risk quotients.

**TU285 New characterization factors for PCDD/Fs including oil influence on their fate in a life cycle assessment context** E. Taing, CIRAIQ / Polytechnique; C. Bulle, CIRAIQ Polytechnique Montreal / Chemical Engineering; L. Deschenes, Ecole Polytechnique de Montreal / Genie Chimique. Is the (eco)toxic impact of a single contaminant the same when it is in interaction with other contaminants in a mixture? Life Cycle Impacts Assessment (LCIA) methods do consider so far that it is the same, whereas studies showed influence on contaminants fate in mixtures. The project aims to check to what extent (eco)toxic potential impacts would change significantly (with regard to model uncertainty) when including interactions between contaminants. In LCIA Characterization Factors (CFs) calculate the amount of potential impacts by amount of emitted pollutant, and (eco)toxic CFs include the contaminant fate in the environment (Fate Factor FF), exposure (Exposure Factor XF, set to 1 for ecotoxic impacts) and effects (Effect Factor EF). This study only considers the influence of contaminant interactions on fate. This project is based on the specific case study of the pentachlorophenol (PCP) pole-treating oil which contains contaminants in interaction (oil, traces of dioxins and furans (PCDD/Fs) and PCP). PCP has been neglected so far because of lack of information. As pole-treating oil is a significant source of PCDD/Fs to soil in the Canadian inventory of release (47% of total emissions in 2003), it may be of interest to see if the interactions significantly influence the overall potential impact of Canadian emissions of PCDD/Fs. The method is based on the assumption that the PCDD/Fs are entirely "transported" by the oil emitted in the environmental compartments in which the oil is degraded. This approach leads to significant different CF's: for an emission of PCDD/Fs (and oil) to soil, the aquatic ecotoxic and the toxic carcinogenic CF's are respectively over 16 times and 51 times higher than the original USEtox CFs (PCDD/Fs without oil). For an emission to air, the new aquatic ecotoxic and toxic carcinogenic CF's is 12 times and 6 times lower than the original CFs. Consequently results obtained by applying new CF's to the PCDD/F emitted in pole-treating oil in the Canadian pollutant release inventory increase by 10% for the aquatic ecotoxicity and by 34% for the carcinogen toxicity. However differences between original USEtox CF and new one remain small compared to USEtox uncertainty (around two orders of magnitude). A strong assumption was made on the transport of PCDD/Fs in oil, which probably overestimates oil influence and needs to be refined (influence of oil in PCDD/Fs volatilization).

**WE001 Alternative Aquatic Macrophyte Species for Improved Aquatic Ecotoxicity Assessment** J. Newman, Centre for Ecology and Hydrology / CEH Wallingford; J. van Valkenburg, Plant Protection Organisation. The continued use of *Lemna* species as the standard aquatic macrophyte test species provides little relevant data for adequate ecological impact assessment of pesticides tested using this system. The adoption of a *Myriophyllum* test protocol is a big step forward in making ecotoxicological tests valuable in terms of what effects a pesticide might have in aquatic systems. However, there are many different life forms, life cycle strategies, reproductive strategies, physiologies, carbon acquisition mechanisms, carbon dioxide uptake capacities, epiphyte interactions, root zone physiologies and various other morphological and physiological adaptations to the aquatic environment adopted by other important aquatic species which make reliance on single species tests virtually worthless in an ecological context. In addition to morphological variability between environments, which can also be accompanied by physiological responses to water temperature, light intensity and pH (which is also linked to carbon availability and alkalinity), plants grow in different sediment types. Any aquatic toxicology protocol or assessment without sediment will produce artificially elevated toxicity levels and prolonged persistence

data. Aquatic ecosystems are robust and recovery of macrophytes perceived as sensitive is often rapid, and almost always within one growing season, even after application of herbicides formulated specifically to control aquatic macrophytes. We believe that selection of a wider range of aquatic macrophyte species with a range of tolerances to temperature, light, sediment preference, and carbon physiologies will provide a more robust ecotoxicological assessment framework for assessment of the impacts of pesticides in the aquatic and riparian environment. We will propose a list of species suitable for growth in the laboratory with defined measureable responses to pesticide stress. We will propose further developments of aquatic plant testing include ecophysiological and molecular marker techniques that could be adopted to make such tests much more rapid, reliable and robust without the need for extensive large scale mesocosm studies with adequate recovery periods.

**WE002 The selection and evaluation of appropriate macrophyte taxa for regulatory mesocosm studies with herbicides** R. Bromley, H. Walton, Cambridge Environmental Assessments; S. Taylor; S. Priestly, Cambridge Environmental Assessments. A number of new developments in mesocosm testing have taken place since the most recent revisions to guidance for these complex studies (e.g. HARAP, 1998 & CLASSIC, 2001). Much of the progress in this area has been in the design and conduct of freshwater mesocosm studies to evaluate the toxicity of herbicides. For example, recent research has shown that mesocosms should incorporate marginal zones to provide habitats for key taxa not normally included in mesocosm studies in order for them to more accurately resemble natural ponds (Maltby L et al., 2008). In addition, our research has shown that emergent macrophytes comprise a major component of edge of field water bodies and this important group of plants are often underrepresented in mesocosm studies. We will show how we have implemented this research using examples drawn from recent state of the art regulatory mesocosm studies. We will also provide recommendations for macrophyte species selection for different functional groups and embryonic classes. Finally, we will provide examples of assessment methods for evaluating the effects of pesticide exposure to macrophytes for regulatory assessment. It is hoped that this information will advance mesocosm design for herbicide studies and offer advice to those considering conducting these studies, whilst stimulating discussion in this developing area of higher tier aquatic ecotoxicology.

**WE003 Species Sensitivity Distribution Tests with Macrophytes in Outdoor Mesocosms** L. Doeren, Institut für Gewässerschutz Mesocosm GmbH; H. Christl, Tier Solutions GmbH; U. Hommen, Fraunhofer IME; L. Doeren, Institut für Gewässerschutz Mesocosm GmbH; P. Ebke, MESOCOSM GmbH. In herbicide risk assessment species sensitivity distributions (SSD) for macrophytes reduce the uncertainty in extrapolating from the standard test species (*Lemna* spec.) to other macrophyte species that may be exposed in the field. Usually single species tests are performed on seven or more additional macrophyte species to ensure that different types of macrophytes regarding taxonomy, physiology and growth form are considered. An alternative to an SSD based on tests with macrophytes in the laboratory is the use of macrophytes tested in bioassays in outdoor mesocosms. These pond studies combine a number of advantages: Growth performance of plants in semi-natural pond systems is generally good (provided good coordination of test systems and selected species), and realistic fate processes affecting the exposure of the plants are factored in. The focus on macrophytes (and algae) in these study means less effort and costs compared to a typical mesocosm study and also saves analytical costs compared to e.g. seven single species test in the laboratory. On the one hand the familiar environment of the macrophytes in outdoor mesocosms supports realistic growth conditions. On the other hand those studies provide a realistic exposure scenario and the opportunity to assess recovery potential or long time effects. This poster shows design and selected results of such an outdoor SSD-study in mesocosms with 12 different macrophytes. Eight submerged or emergent species were planted in pots and hooked into mesocosm enclosures at depths suitable

to the corresponding species. Four pots with two plants were used per species in each enclosure. Three of these four pots were used for destructive sampling on different dates to gain results for the endpoints fresh weight, dry weight, shoot length and root length during the study, while the remaining pot was used for measurements of shoot length at regular time intervals until the destructive sampling at the end of the study. Four species (free-floating) were regularly assessed by counting leaf/frond number or measuring fresh weight by using micro scales.

**WE004 The effects of sulfonyle-urea herbicides on aquatic macrophytes – species sensitivity distribution compared to Lemna** P. Sowig, Bayer CropScience / Ecotoxicology; J.M. Giddings, Compliance Services International; M. Dollinger, Bayer CropScience; A. Solga, Ecology. The 7-day duckweed growth inhibition study usually conducted with *Lemna gibba* according to OECD 221 is a regulatory requirement for the registration of herbicides. For many herbicidal compounds duckweed is the most sensitive aquatic organism. The EC50-figure obtained from the standard *Lemna* study determines the risk assessment. Sulfonyle-urea (SU)-herbicides inhibit acetolactate synthesis. Five SU-herbicides were tested in artificial outdoor ponds with potted aquatic plants over a period of six weeks. These dose-response studies resulted in EC50-figures for nine or ten macrophyte species. The species sensitivity distribution (SSD) of these EC50 figures was fit to a lognormal regression. Greater-than figures were excluded from the regression analysis, but included in the calculation of the rank of each species. The standard *Lemna* EC50-figure was not included in the regression. For three SU-herbicides *Lemna gibba* was the most sensitive species, in case of the other two compounds one macrophyte species was more sensitive than *Lemna*. The position of *Lemna* within the SSD was quantified by the percentile of its EC50-figure. In cases where *Lemna* was the most sensitive species the percentile was between 1.7 and 6.8 %. In the two other cases the percentiles were 13.7 and 15.5 %, and the *Lemna* EC50 was within a factor of two of the EC50 of the most sensitive species. It can be concluded that for SU-herbicides the risk assessment will be over-conservative with a *Lemna* EC50 combined with an assessment factor of 10.

**WE005 Mercury accumulation in the submersed plant *Elodea nuttallii*** R. Flueck, Institut F.-A. Forel; V.I. Slaveykova, University of Geneva / Institute Forel, Earth and Environmental Sciences; C. Cosio, Geneva University. Aquatic primary producers are key organisms for ecosystems because they represent a source of oxygen and food at the basis of trophic chains. Not only algae but also aquatic plants can be a source of pollutants to higher consumers as they can accumulate toxic metals from water or sediment. Mercury (Hg) is a global concern for water pollution and is frequently studied for its high biomagnification rate. Understanding Hg accumulation in aquatic primary producers is essential for predicting probable impact on higher trophic levels and understanding Hg uptake mechanisms in organisms of the aquatic environment. Previous studies suggested that *Elodea nuttallii* and Hg are strongly linked by different aspects, namely (i) this macrophyte has been shown to accumulate Hg in the environment compared to other species [1] and (ii) its associated microbial communities can have impact on Hg biogeochemical cycle, increasing Hg methylation [2]. *Elodea nuttallii* is a rooted submerged macrophyte found in freshwaters. Originally from North America it is invasive in European waters. Its colony gives a shelter to many invertebrate and fish species. Tolerant to changing environmental conditions, this plant can bioaccumulate different metals (e.g. Cu, Zn and Cd [3]) and may be a precise bioindicator of pollution [3]. We exposed the macrophyte *Elodea nuttallii* to Hg spiked artificial water in the laboratory. We found that *Elodea nuttallii* bioconcentrates Hg quickly from the water without showing toxic effect (PSII, growth). Accumulated Hg concentration in aquatic plants could be used in water monitoring to predict environmental concentrations. It could then complete Hg exposure assessments as chemical analyses of Hg in liquids can be costly and difficult because of the low environmental concentrations. Its application for phytoremediation has also been several times underlined. [1] Regier, N. *et al.* (2013). "Mercury bioaccumulation in the aquatic plant *Elodea nuttallii* in the field and in

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**WE006 Ecotoxic effect of Organic Contaminants on Aquatic Rhizosphere: A Metabolomics approach** M.K. Chakravarthy, Singapore-Delft Water Alliance; A. Wijdeveld, DELTARES; S. Reuben, Singapore-Delft Water Alliance, National University of Singapore; L. Samavedham, Department of Chemical and Biomolecular Engineering, Natioanl Univeristy of Singapore. About 300 million tons of chemical compounds annually used in industrial and consumer products enter into natural fresh water resources. They include pharmaceuticals, pesticides and industrial chemicals. These contaminants are taken up by aquatic organisms or humans via contaminated water or food, and are transported to different tissues within the organism. Depending on their chemical properties and the biology of the target species, they either bio-accumulate or cause adverse effects on the metabolism. We applied metabolomics to study ecotoxicity of organic contaminants in urban stormwater runoff on plants and microbes around the root zone (rhizosphere) of *Pandanus amaryllifolius*. A novel bioreactor was designed for *on-line* extraction of metabolites released in the rhizosphere. The metabolic profiles from the dose-time experiments were analyzed for short term (5 min) exposure as well as long term (72 hour) exposure responses. Results showed that the stress response of the rhizosphere was immediate in both the exposure studies. In the short term exposure study, 65% of metabolites extracted from the contaminant exposed rhizosphere showed a change of greater than two fold over control, in the first two hours post exposure. In contrast, 82% of metabolites had the higher fold change in the long term exposure study. This indicates that longer exposure of contaminants positively up regulates more metabolites in the rhizosphere. In both studies, 20% of the metabolites showed a strong positive correlation (Pearson correlation coefficient > 0.95). We implemented piecewise multivariate modelling of the metabolic profiles to interpret time related variation in the data, including small and local changes in the time domain. Such data driven characterization provided us detailed information of the temporal effects on aquatic rhizosphere exposed to organic contaminants.

**WE007 Implementation of a water plant test system using *Ceratophyllum demersum*** H. Granse, University of Applied Sciences and Arts Northwestern Switzerland; S. Hoeger, Harlan Laboratories Ltd.; A. Liedtke, Harlan Laboratories Ltd / Ecotoxicology. Herbicides from agriculturally used land can have negative effects on the aquatic ecosystems via drift. As part of this ecosystem aquatic macrophytes can be affected by these herbicides as non-target organisms. Therefore the registration of herbicides requires ecotoxicological testing using an aquatic macrophyte species. The required macrophyte test species is the monocotyledonous duckweed, *Lemna* spec. It is under discussion that *Lemna* spec. might not be sufficient for herbicides with distinct mode of actions, as the sensitivity of this species might not be adequate for the substance tested. The establishment of guidelines using dicotyledonous macrophyte species is in preparation since years. In light of this development we established a growth inhibition test with *Ceratophyllum demersum* to investigate the possibility of *C. demersum* as an alternative species for the testing of herbicides. *C. demersum* is a dicotyledonous, not rooted, submerged aquatic macrophyte. Due to these characteristics there is no need for sediment and thus the focus is on one absorption route, which leads to a distinctly easier test system and clearer results. The use of only one compartment in a test results in an easier handling during both, the biological and analytical part. These characteristics are seen as an advantage compared to the tests with rooted dicotyledonous plants. On the other hand the two compartment tests, e.g. water sediment test with *Myriophyllum spec.* includes two absorption routes, which might be

important for substances mainly absorbed through the roots. Advantages and disadvantages of the *C. demersum* growth inhibition test compared to already established tests will be identified and presented.

**WE008 A proposed OECD test guideline for the submerged macrophyte, *Myriophyllum*, in a water-sediment system.** J. Davies, Syngenta / Environmental Safety; M. Dollinger, Bayer CropScience; M. Ratte, ToxRat Solutions GmbH. Under current EU pesticide regulation, regulatory tests are required for the aquatic macrophyte, *Lemna*, and two algal species for herbicides. In 2008, participants of the SETAC – AMRAP (Aquatic Macrophyte Risk Assessment for Pesticides) workshop identified the need for regulatory tests with submerged, rooted macrophyte species for some herbicidal compounds where root uptake from sediment is considered an issue or where the sensitivity of standard algae and *Lemna* species is believed not to be representative of other macrophyte species. In light of existing experience, *Myriophyllum* species were selected as the preferred test species to represent submerged and dicotyledonous species. A work group was established to develop an appropriate test method and the suitability of the proposed method has since been evaluated in 51 tests conducted in 15 laboratories using two test species (*M. aquaticum* and *M. spicatum*) and three test substances (3,5-dichlorophenol, isoproturon and trifluralin). Statistical analyses of the resulting data have demonstrated that tests with both species are practical, with coefficients of variation for repeatability within laboratories falling between 10 and 30% in many cases. Coefficients of variation for reproducibility between laboratories were higher than for repeatability while assessments of a variety of growth parameters revealed that the lowest coefficients of variation were typically associated with growth rate endpoints derived from shoot length or weight measurements. Results also revealed that minimum detectable differences were lower for *M. spicatum* than *M. aquaticum*, indicating that some modification of the test method and/or validity criteria may be necessary for *M. aquaticum*. The existing protocol will be updated to reflect these findings and make recommendations for validity criteria. These recommendations will be described in this presentation.

**WE009 Testing chemicals for herbicide activity by *Myriophyllum* Toxicity Test** P. Gebala, Faculty of Computer Science and Material Science, University of Silesia / X-ray Microtomography Lab, Department of Biomedical Computer Systems; H. Rzodeczko; K. Winiarska, M. Wojcik, Institute of Industrial Organic Chemistry Branch Pzszczyna. Chemicals with herbicide activity are assessed for undesired impacts on aquatic plants based on growth inhibition tests with algae and duckweed *Lemna* sp. (OECD Guidelines for Testing of Chemicals No 201 and No 221). The growth inhibition test with water milfoils *Myriophyllum* sp. in sediment-water system is recognized as a promising assay to evaluate toxicity for rooted submerged aquatic macrophytes. Shoot tips of parrot's feather water milfoil were planted into sediment for 3 days rooting phase and then exposed for 7 days to water spiked with chemicals: 3,5-dichlorophenol, isoproturon and trifluralin. Concentration of each chemical in a static design was determined by chromatography methods. In water the concentration of 3,5-dichlorophenol, isoproturon and trifluralin decreased during exposure. In sediment (with porous water) the concentration of isoproturon increased during exposure. Changes of plant morphology were observed. For plants exposed to 3,5-dichlorophenol (reference substance) progressive chlorosis and changes in root development were observed. In plants exposed to isoproturon (photosynthesis inhibitor) chlorotic parts recovered but roots were shorter and very few. Only minor effects were for plants exposed to trifluralin (microtubule assembly inhibitor). Total shoot length, fresh weight and dry weight were variables measured in tests performed in Institute of Industrial Organic Chemistry in 2011 within the Ringtest organized under the auspices of the SETAC AMEG. P. Gebala is beneficent of "Silesian Cooperation: Innovations For Efficient Development (SWIDER) " project realized within Human Capital Operational Programme, Priority VIII: Regional human resources for the economy, 8.2 Transfer of knowledge, Sub-measure 8.2.1 Support to cooperation of scientific environment and enterprises.

Project partially funded from European Union Project based on European Social Funds.

**WE010 Modified water/sediment *Myriophyllum* biotest** G. Gonsior, . Some plant protection products and industrial chemicals showed an unavoidable high risk for submersed aquatic plants. Tests with *Myriophyllum* spec. should be performed to reduce uncertainties. *Myriophyllum spicatum* represents such a submerged macrophyte. Up to know ring-tests with *Myriophyllum spicatum* in an unsterile water-sediment or axenic sediment-free system were performed. Here we present data of a water / sediment test design with the focus on variability and repeatability.

**WE011 Synthetic auxins revisited - Is the risk really low?** V. Knezevic, Faculty of science; T. Tunic, M. Rodic, Faculty of Sciences; S. Lazic, Faculty of Agriculture; D. Brkic, Institute for Pesticides and Environmental Protection; I. Teodorovic, Faculty of Sciences. One of the groups of pesticides with incomplete toxicity data to aquatic plants are auxin simulators. In order to contribute to scarce data, we tested auxin simulators with different chemical structures (2,4 D and dicamba) on a monocotyledonous and dicotyledonous aquatic plant species. Even though there is a vast amount of published data concerning the effect of chemicals on *Lemna* sp., some of the endpoints apart from the most frequently reported ones (e.g. relative growth inhibition based on biomass) are being neglected. Experience shows that the choice of endpoint can affect test or species relative sensitivity, especially in case of substances with specific mode of action. Another issue is being addressed by the scientific community: is *Lemna* sp. as a floating monocotyledonous plant, being protective enough of other aquatic plant species when assessing pesticides with specific modes of action? In regard to these concerns, a novel guideline has been proposed to evaluate the toxicity of substances to submerged, rooted, dicotile aquatic plants with *Myriophyllum aquaticum* as one of the species suggested. The effect of selected auxins in this study was assessed using relative growth rate as well yield based endpoints: frond number, fresh weight and total frond area for *L. minor*; and shoot length, plant fresh and dry weight for *M. aquaticum*. Aims of the presented study were (i) to evaluate whether current risk assessment procedure (based on duckweed species) is protective enough of other aquatic macrophyte species (ii) to assess the impact of auxin simulators to *M. aquaticum* as additional macrophyte species (iii) to assess whether the choice of endpoints in both types of tests effects the outcome for the risk assessment of auxin simulator plant protection products. Results question the current opinion about auxin simulators as low risk pesticides for non-target aquatic plants and provide some ecologically relevant information which might contribute to refinement of risk assessment of pesticides.

**WE012 Field-derived periphyton communities recover from an acute herbicide exposure** R. Prosser, School of Environmental Sciences, University of Guelph; R. Brain, Syngenta Crop Protection Inc / Department of Environmental Risk Characterization; A.J. Hosmer, Syngenta Crop Protection Inc; K.R. Solomon, University of Guelph / School of Environmental Sciences; M.L. Hanson, University of Manitoba / Department of Environment and Geography. Periphyton communities are an integral component of freshwater ecosystems, and in recognition of their foundational role, it is becoming more common to include periphyton in toxicity testing and risk assessment. This study characterized the acute response and recovery of field-derived periphyton to the herbicide atrazine by measuring the quantum yield of photosystem II (PSII) with pulse amplitude modulated fluorometry. Periphyton samples were collected from three and six agricultural stream sites from across the Midwestern United States in the summer of 2011 and 2012, respectively. Periphyton was exposed under laboratory conditions to atrazine (concentrations ranging from 10 to 320 µg/L) while inhibition of PSII yield was measured at different time intervals (from 2h up to 48h). Subsequently, the periphyton exposure media was replenished (with control media) in order to assess recovery upon atrazine removal at 24h to 48h post-exposure. Sensitivity to atrazine varied with site and date of sampling but EC10 and EC50 values for

PSII quantum yield did not differ significantly with exposure interval. Only the highest test concentration (320 µg/L) still demonstrated greater than ~5% inhibition at 48h after removal of atrazine, however all other test concentrations exhibited recovery within ~5% of control levels, typically within 24h. The rapid physiological recovery of the periphyton community upon atrazine removal implies that acute effects will not likely result in significant or sustained impacts on either periphyton structure or function in a lotic ecosystem. For ecological risk assessment, this means relying on acute direct effects data alone, without considering recovery, may result in overly conservative estimates of toxicity, especially for primary producers.

**WE013 A ring study of a new rapid alga test estimating chemical influence on algal growth, algal luminescence toxicity test** M. Katsumata, Hamamatsu Photonics / Central Research Laboratory; Y. Kobayashi, Hamamatsu Photonics K.K.; N. Tatarazako, National Institute for Environmental Studies / Environmental Risk; K. Arizono, Prefectural University of Kumamoto / Faculty of Environmental and Symbiotic Sciences; M. Kikuchi, Kanagawa Institute of Technology; T. Tanaka, Kumiai chemical industry Co., LTD.; N. Oishi, Public Interest Incorporated Foundation BioSafety Research Center; M. Kawanishi, Osaka Prefecture University / Graduate School of Science; N. Yokobori, Sumika Chemical Analysis Service, Ltd. / Ehime Laboratory; H. Yamamoto, University of Tokushima / Faculty of Integrated Art & Science; Y. Tsuboi, Chemicals Evaluation and Research Institute; T. Mizuno, Unitika Environmental Technical Center, LTD.; T. Niino, Mitsubishi Chemical Medience Corporation / Environmental Risk Assessment Center; Y. Shimasaki, Kyushu University / Faculty of Agriculture; Y. Sugaya, National Institute for Environmental Studies / Research Center for Environmental Risk; T. Hakamata, Hamamatsu Photonics K.K.. Rapid bioassays for various species are a promising development to improve hazard assessment. However, when considering practical use of new methods, it is necessary to validate not only scientific reliability but also reproducibility and precision of the test. We have conducted a ring study of a new rapid bioassay, algal luminescence toxicity test. Here, we will share our investigation on validation of the new method. The algal luminescence toxicity test is based on luminescence from alga that is termed delayed fluorescence (DF). Since the DF originates from a reverse reaction of photosynthetic electron transfer, the DF inhibition can estimate growth inhibition in a shorter time than the conventional test (e.g. OECD test guideline 201). This test provides a rapid and easy protocol; the test algae (*Pseudokirchneriella subcapitata*) are prepared from frozen algae in a 1 hour pre-incubation and can then be immediately exposed to the test sample prepared in a range of concentration in small tubes (10 ml volume). The DF from algae in the tube is directly measured by the high sensitivity luminometer (HAMAMATSU, type-7100). Eleven institutes consisting of GLP labs, chemical analysis companies, universities and chemical industry companies participated in the ring study. A factorial design experiment with five factors, i.e., laboratories (11), test chemicals (2: DCP, DCMU), exposure concentrations of chemicals (6 levels each), exposure duration (4: 0, 1, 6, 24hr), and different types of measuring devices (two: a 6 channels type, and a single channel type) was conducted to evaluate repeatability (within-lab precision) and reproducibility (between-lab precision) through ANOVA. The repeatability is defined as a square root of error variance of ANOVA. Reproducibility is defined as a square root of sum of error variance and laboratory factor. Repeatability in each lab had a wide variation in 3.9% to 33.1%. Reproducibility of our whole test design was 28.2 % for entire dose-response, 15.2% for EC<sub>50</sub> of DCP, 18.4% for EC<sub>50</sub> of DCMU. The ring study provides us important bottleneck of current protocol. Low precision labs seem have some strange data that may relate to sample preparation or incubation or measurement. In the presentation, we will discuss more details of precision analysis, and the possibility to improve the precision by solving current problems in the protocol.

**WE014 Modeling uptake and elimination dynamics of herbicides in *Myriophyllum spicatum*** S. Heine, RWTH Aachen University / Institute for Environmental Research; A. Schaeffer, RWTH Aachen

University / Chair of Environmental Biology and Chemodynamics; G. Goerlitz, Bayer CropScience AG / Environmental Safety; W. Schmitt, Bayer CropScience AG / Environmental Modelling; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research. The toxicological effects of chemicals are predominantly influenced by internal concentrations within an organism which depend on uptake and elimination kinetics. These processes might be a key component for assessing the effects of time variable exposure of chemicals which occur in aquatic systems regularly. Despite their potential importance, these transport processes are hardly considered in risk assessment. Reasons for neglecting kinetic processes in risk assessment are difficulties establishing appropriate experimental settings and the time and labor experiments demand. Mechanistic kinetic models have the ability to evaluate effects of time variable exposure requiring only some experimental data for substance specific calibration, thus, offering a beneficial alternative for analyzing time variable exposure. In this work, a previously developed toxicokinetic/toxicodynamic (TK/TD) growth model of *M. spicatum* is used to simulate the uptake and elimination dynamics of some herbicides. The TK/TD growth model dynamically calculates internal concentrations of the respective chemicals depending on external concentrations and some physicochemical substance properties, as well as plant characteristics. Model predictions have been validated with experimental data considering uptake and elimination processes of the respective chemicals to show the applicability of the model to simulate time dependent kinetic pattern. This information might be useful for evaluating effects of time variable exposure of these chemicals.

**WE015 Measured plant bioaccumulation data and screening-level models for organic chemicals** J.A. Arnot, ARC Arnot Research Consulting / Department of Physical Environmental Science; C. Shunthirasingham, Departments of Chemistry and Physical and Environmental Sciences; E.M. Dettenmaier, Utah State University; W.J. Doucette, Utah State University / Utah Water Research Laboratory; D. Mackay, Trent University. Plants form the basis of food webs in aquatic and terrestrial ecosystems and comprise a major proportion of the total biomass on the planet. Anthropogenic chemicals released to the environment may pose potential risks to plants and higher trophic level organisms. The relationship between chemical exposures to plants and concentrations in or on plants is the result of competing rates of chemical uptake and elimination in plants (i.e. bioaccumulation). Plants have the potential to biodegrade many chemicals and biotransformation in or on plants may be an important process for overall chemical fate in the environment. Plant bioaccumulation and biotransformation rates are thus critical aspects related to multimedia fate, exposure and risk assessment and bioremediation. To better understand the state of the science for quantifying the bioaccumulation and biotransformation of organic chemicals in plants we conducted a literature review for measured plant bioaccumulation metrics using key-word searches such as bioconcentration factor (BCF), root concentration factor (RCF), transpiration stream concentration factors (TSCF), biotransformation rate and several others. Approximately 500 published papers were collected and reviewed resulting in a database of 3,600 unique entries for 360 chemicals from approximately 170 published sources. The database includes plant bioaccumulation metrics for polycyclic aromatic hydrocarbons (PAHs), legacy pesticides, current use pesticides (CUPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs), as well as pharmaceuticals and personal care products (PPCPs), and veterinary chemicals. This review highlights that improvements in the reporting frequency of key test parameters are required to better quantify and understand plant bioaccumulation processes. Due to the general paucity of measured plant bioaccumulation data compared to the tens of thousands of chemicals requiring assessments, there is a need to develop and evaluate plant bioaccumulation models. Plant bioaccumulation models used in screening-level exposure and risk assessment models such as EUSES and RAIDAR are compared with the measured database. To improve plant bioaccumulation models to address uncertainty in exposure and risk assessment, there is a need to improve plant bioaccumulation testing

and the reporting of key information.

**WE016 Phytotoxicity of lead to the aquatic plant *Lemna minor*** D. Mohammed, Plymouth University / School of Marine Science and Engineering (Faculty of Science and Technology); A. Turner, University of Plymouth / School of Earth, Ocean and Environmental Sciences; M. Brown, University of Plymouth / School of Biological Sciences. The toxicity and accumulation of lead in the aquatic plant *Lemna minor* was investigated, using a modification of the Organisation for Economic Co-operation and Development (OECD) standard growth inhibition test. Plants were cultured in a modified Swedish Institute Standard (SIS) at pH 6.5 under  $85 \mu\text{mol m}^{-2} \text{S}^{-1}$  at  $25^\circ\text{C}$  and exposed to a wide range of lead concentrations from environmentally realistic to very high concentrations (0.1, 1, 10, 100, 1000 and 10 000  $\mu\text{mol/L}$ ) for seven days. Various physico-biochemical endpoints were measured after seven days of exposure. The concentrations of dissolved lead remaining in the residual solutions, and accumulation of lead in fronds and roots were assessed using Inductively Couple Plasma – Mass Spectroscopy (ICP-MS). Over the first four days of exposure, lead concentrations in solution decreased rapidly and chlorosis was observed in fronds exposed to the three highest lead concentrations. After seven days of exposure, there were significant decreases in the relative growth rate (RGR), relative frond area (RFA), pigment content (chlorophyll a, b and total carotenoid) and activity of photosystem II (Fv/Fm) at concentrations of 100, 1000 and 10 000  $\mu\text{mol/L}$ . Cellular concentrations of Pb were higher in roots than fronds, whereas more Pb was adsorbed to the extracellular matrix of fronds than roots. The bio - concentration factor (BCF; i.e. lead concentration in plant tissue at day seven relative to residual lead concentration in the growth medium at day seven) indicates that *L. minor* is a good accumulator of Pb but the physiological data shows that it is toxic at concentrations that can be encountered in wastewater treatment facilities. Therefore additional studies are required to evaluate the potential of *L. minor* for phytoremediation of wastewater contaminated by lead, and of other metals. **Keywords:** *Lemna minor*, Lead (Pb); toxicity; phytoremediation.

**WE017 Toxicity tests of gold, silver, antimony and arsenic in *Fontinalis* spp.** F. Pratas, J. Canhoto, J. Pratas, University of Coimbra; P. Favas, University of Trás-os-Montes e Alto Douro / Geology. This study aimed to evaluating the response capacity of species *Fontinalis antipyretica* and *Fontinalis squamosa* (water mosses) in toxicity tests, as well as the potential of these plants to the accumulation of heavy metals in aquatic environment. Stems of both species were collected in a stream in the centre region of Portugal and brought to the laboratory where attempts to obtain aseptic cultures were performed. The results showed that a 5% calcium hypochlorite treatment for 3 min. gave the best rate of sterilization without harmful effects. Best results for plant establishment were achieved in a Knop modified solution containing the minor salts of the MS medium and the Fe of the MS medium reduced at half strength. The effect of different concentrations of the benzyladenine (0.5–4.0mg/L) on plant growth was evaluated. The results showed that BA has an inhibitory effect on plant growth since in all the concentrations tested shoot length was lower than that observed in the control (medium without plant growth regulators). Following these experiments we have tested the ability of both species to growth in the presence of gold added to the growth medium as sodium tetrachloroaurate (III) hydrate ( $\text{NaAuCl}_4$ ) in concentrations ranging from 0.5 to 20mM. The results indicated that *F. antipyretica* showed a growth rate similar to the control on media containing until 3.75mM. Higher concentrations impaired plant growth. *F. squamosa* showed better adaptation to higher concentrations. We also made similar studies with elements often associated with Au, As, Sb and Ag with the same concentrations range. Experiments made in aquarium over 30 days tested the behavior of the plants in the presence of gold iodide (AuI), gold cyanide (AuCN), sodium tetrachloroaurate (III) hydrate ( $\text{NaAuCl}_4$ ), antimonium tartaricum ( $\text{C}_4\text{H}_4\text{O}_4\text{Sb}_2\text{Na}_2$ ), arsenic trioxide ( $\text{As}_2\text{O}_3$ ) and silver nitrate ( $\text{AgNO}_3$ ) in three different concentrations. *F. antipyretica* showed morphological changes at concentrations above 1.25 $\mu\text{M}$  of As, and above 3.75 $\mu\text{M}$  of Au and Ag and unchanged for Sb.

Morphological changes were pronounced at 5 and 20 $\mu\text{M}$  of As, at 5 $\mu\text{M}$  of Au and at 20 $\mu\text{M}$  of Ag, and there is the highest levels of toxicity with marked necrosis on all explants. For the specific case of 20 $\mu\text{M}$  of As was clearly visible to the fact that high levels of toxicity is associated with the formation of a greater number of buds, suggesting an attempt to plant expansion in other directions with improved growth conditions.

**WE018 Cell membrane integrity of the macrophyte *Ceratophyllum demersum*, introduced to a metal-polluted South African river** D. Erasmus, R. Snyman, J. Odendaal, Cape Peninsula University of Technology; P. Ndakidemi, The Nelson Mandela African Institute of Science and Technology. Cell membrane integrity was measured in the submerged macrophyte *Ceratophyllum demersum*, introduced to the Diep River, Cape Town, South Africa. Previous studies on this river have shown high levels of metal contamination, however, studies on metal bioaccumulation and toxicity in aquatic macrophytes in this river is very limited. Plants were obtained from a reference site and placed in baskets at two sites within the river, one upstream (site 1) and one downstream (site 2) from urban pollution sources. Plants were collected every fortnight for twelve weeks. Cell membrane integrity was determined by placing plants in deionised water and measuring electrical conductivity and solute (sodium, calcium, potassium and magnesium) concentrations after 24 hours. Results showed that for sodium and potassium, the ions generally leaked out of the experimental plants, whereas calcium and magnesium were mostly absorbed from the deionised water. When studying overall solute loss (as indicated by the EC readings), plants from both sites showed significantly greater solute loss, compared to the reference plants (not exposed to the river environment), which actually gained solutes. This indicates a loss of cell membrane integrity of all the plants exposed to the Diep River conditions, probably as a result of metal bioaccumulation. The most solute loss overall was experienced by plants at site 2. As these plants had lower metal bioaccumulation than plants at site 1, these results can possibly be ascribed to the higher salinity measured at this site. It was concluded that membrane integrity of *C. demersum* can potentially be used as biomarker of metal exposure but needs further research as present results were inconclusive.

**WE019 Comparative ecotoxicity study of solvents from biomass: levulinate family** L. Lomba, Universidad San Jorge-B. Giner; E. Zuriaga, R. Pino, Universidad San Jorge. One of the most important targets of green chemistry is the use of renewable raw materials because materials such as natural gas, coal or petroleum are irrevocably decreasing. Chemicals from sustainable sources, including those from biomass, which are usually obtained by fermentation, enzymatic, or esterification processes, can be used for a number of industrial processes. Biomass is a good alternative because it is the only renewable resource of fixed carbon, which is essential for the production of conventional hydrocarbon liquid transportation fuel. This is the case of the chemicals studied in this work: methyl levulinate, ethyl levulinate and butyl levulinate. These compounds can be used in the manufacturing of synthetic fibres, pesticides, pharmaceuticals, plastics and rubber, as an acidulant in food, as plasticizers, odorous substances and solvents in polymers, textiles and coatings. [1]. These chemicals have been characterised from the point of view of their physicochemical properties [2-3], however, we have found that there is not a comprehensive and rigorous study about the ecotoxicology of these solvents and their environmental risk. In this work, we present a part of our research project which is based on the physicochemical and ecotoxicological characterization of several products such as solvents or drugs. In this case, we are focusing on the ecotoxicity of several solvents from biomass (methyl levulinate, ethyl levulinate and butyl levulinate). The standardized toxicity test using *Vibrio fischeri* (UNE-EN ISO 11348-3) has been used to test the environmental risk of these solvents. Furthermore, a critical micellar concentration, *cmc*, of the studied solvents has been obtained through densities and speed of sound properties [4]. Finally, their solubility has been measured to complete the study. All this data has been analysed and interesting information about the ecotoxicological risk, molecular structure and behaviour in



water of the studied chemicals from biomass has been achieved. The results obtained show that the cmc depends on the structure and the solubility of them. The values of cmc increase as the length of alkyl chain decrease, and as expected, the same trend is obtained in solubility. On the other hand, the ecotoxicity of these chemicals increase as the length of alkyl chain does, i.e the most toxicity chemical is the butyl levulinate followed by ethyl and methyl levulinate.

**WE020 Chlorophyll concentrations in the macrophyte *Ceratophyllum demersum*, introduced to a metal-polluted South African river** D.V. Erasmus, Cape Peninsula University of Technology / Horticultural Sciences; R.G. Snyman, Cape Peninsula University of Technology / Biodiversity and Conservation; J. Odendaal, Cape Peninsula University of Technology; P.A. Ndakidemi, The Nelson Mandela African Institute of Science and Technology. Chlorophyll a, b and total chlorophyll contents were measured in the submerged macrophyte *Ceratophyllum demersum*, introduced to the Diep River, Cape Town, South Africa. Previous studies on this river have shown high levels of metal contamination, however, studies on metal bioaccumulation and toxicity in aquatic macrophytes in this river is very limited. Plants were obtained from a reference site and placed in baskets at two sites within the river, one upstream (site 1) and one downstream (site 2) from urban pollution sources. Plants were collected every fortnight for twelve weeks. Leaf samples were measured for chlorophyll a, b and total chlorophyll contents using a spectrophotometer. Results showed that plants at site 2 had significant increases in all chlorophyll groups over the experimental period, possibly due to higher nutrient content in the water, originating mainly from the nearby sewage works and industries. When data was pooled, chlorophyll contents of plants at site 1 were overall significantly lower, compared to plants at site 2, probably due to increased metal bioaccumulation in the leaves. This may have been as a result of metal pollution from surrounding agricultural areas and an increased metal bioavailability. It was concluded that chlorophyll contents of *C. demersum* can potentially be used as biomarker of metal exposure but needs further research as present results were inconclusive.

**WE021 Pollutants induce changes in polyphenols of *Myriophyllum*, resulting in modification of its allelochemical capacities** A. Nuttens, UMR CNRS; J. Masfaraud, UMR CNRS 7360 LIEC; E.M. Gross, University of Lorraine / Laboratoire interdisciplinaire des environnements continentaux (LIEC), CNRS UMR 7360. Agricultural and industrial activities introduce pollutants into the environment, which often remain as complex mixtures in soil and water. Aquatic systems are often the target of leaching and runoff processes, and they may receive considerable cocktails of pesticides, heavy metals or other pollutants, thus creating multipollution scenarios in ecosystems. One current challenge is to estimate impact of such mixture of pollutants at the ecosystem level. Photosynthetic organisms, as primary producers, are the first impacted at the ecosystem level, which makes them a key compartment to study. Our study uses axenic (*in-vitro*) cultures of the submerged aquatic dicotyleous *Myriophyllum spicatum* L., currently under discussion as future test organism. The advantage of this plant is that it propagates well *in vitro*, but can also be used in non axenic, larger scale micro- to mesocosm approaches up to studies *in situ*. In a first approach, we will test the impact of arsenic or cadmium on the plant's performance, but also its interaction with other organisms mediated by specific secondary metabolites. Polyphenols may be particularly useful in this context because they affect allelochemical interactions with competitors and herbivores, and thus may allow to assessing pollutant effects in an ecologically relevant context. However, due to the anti- or pro-oxidant activity of the ellagitannins, one group of polyphenols present in this plant, the effects of pollutants on *M. spicatum* might either be diminished or enhanced. We will thus investigate not only standard endpoints such as plant growth and performance, but also the direct and indirect effect of pollutants on this group of secondary metabolites. We expect that arsenic and cadmium act very differently on *M. spicatum* since the metalloid and the heavy metal are taken up by different transporters. **Keywords** : polyphenol, ecotoxicology, allelochemical

interaction, watermilfoil

**WE022 Toxic Effects Of Copper And Aluminum On Photosynthetic Capacity Of Microalgae** C.H. Soares, Universidade Federal de Santa Catarina / Bioquímica; I. Baptista, A. Weiler, Universidade Federal de Santa Catarina. Several studies have demonstrated toxic effects of metals for microalgae. The aim of this study was to assess the photosynthetic capacity of microalgae *Scenedesmus subspicatus* in medium containing different concentrations of copper and aluminum (from 0.1 to 100 ppm). It was evaluated the rate of algal growth, the kinetics of oxygen production, changes in concentration of chlorophyll a and b, quantum yield and maximum fluorescence intensity, rate of quenching photochemical and non-photochemical quenching. The algal growth rate was evaluated during 72 h under continuous illumination (1500 lux) at a temperature of 25 °C and constant air flow. Photosynthetic parameters were measured using a PAN fluorometer, in samples collected after 48 and 72 h of exposure to the metals (0, 6, 12, 25, 50 and 100%, 0% used as control). The toxic effects were indicated by the reduction of algal growth rate correlated to increasing metal concentration. The alteration of photosynthetic capacity was shown by the reduced maximal fluorescence intensity, slower production of oxygen as well as lower values of oxygen concentration in the stationary phase. We also observed a reduction in the concentration of chlorophyll depending on the metal concentration used. The results indicated significant changes in the photosynthetic system of microalgae, especially in their PR system. **Key words**: microalgae; copper; aluminum, photosynthetic capacity

**WE023 Cellular Changes In Microalgae Exposed To Copper And Aluminum** C.H. Soares, Universidade Federal de Santa Catarina / Bioquímica; I. Baptista, A. Weiler, Universidade Federal de Santa Catarina. Toxicological effects of metals have been extensively studied in organisms such as algae, fish and micro crustaceans. The aim of this study was to characterize the possible changes in the ultrastructure of unicellular microalgae - *Scenedesmus subspicatus* - using transmission electron microscopy (TEM). The microalgae were grown in different concentrations of metal (from 0.1 to 100 ppm and 0% as control) for 72 h under constant aeration, at 25 °C and under continuous illumination (1500 lux). Samples containing algae were fixed with glutaraldehyde (2.5%) and washed with phosphate buffer - pH = 7.1. Post-fixation and washes, the samples were treated with osmium tetroxide, diluted with 1% sodium phosphate buffer 0.1 M for 3 hours and then washed with phosphate buffer - pH = 7.1. Dehydration was performed with acetone (30-100%) and infiltration with Spurr resin. Algae cells exposed to the metal showed significant changes in the plasma membrane and cell wall, chloroplast morphology and increased concentration of the starch stored. Additionally, morphological cell alterations were observed such as the cell elongation. The observed changes are more intense in samples containing higher concentrations of metal. **Key words**: microalgae; ultra structure; copper; aluminum, morphological alterations

**WE024 Mixed stress: phosphate (nutrient) limitation enhances the sensitivity of freshwater algae *Pseudokirchneriella subcapitata* to toxic metals** C. Gao, L. Versieren, Department of Earth and Environmental Sciences; E.E. Smolders, Katholieke Universiteit Leuven. It has repeatedly been reported that increased phosphate (P) supply to algae decreases uptake and toxicity of metals in algae. Conversely, increased metal stress might also induce P deficiency. The formation of intracellular polyphosphate bodies at high P supply might explain this interaction since these bodies can bind metal ions and decrease metal availability. As such, increasing the concentration of either P or metal will depress the functionality of the other component. Nevertheless, contrasting results have been found, i.e. some studies report that the uptake of Zn and Cd is promoted by increasing P supply. We postulate that the inconsistencies across different studies are primarily due to methodological issues regarding the experiments, i.e. the lack of steady state P supply in the cells and lack of control of the free metal ion that may interact with phosphate outside the cell. In order to unravel the mechanism of the P-metal interaction, a series of

experiments were designed. First, batch systems with different initial P concentrations (0.1-10  $\mu$ M P) were set-up in a factorial of metal supply. In such a system, P-deficiency is only induced once P is depleted from solution. Hence, phosphate deficiency in this scenario is time dependent. The law of minimum predicts that biomass yield primarily depends on the limiting factor, i.e. P, suggesting that metal stress may be smaller at low P supply than at adequate P supply given sufficient time after P depletion, in contrast with the physiological hypothesis given above. This will be tested by time dependent analysis of metal toxicity. Practically, cells in exponential phase were grown in a factorial of metal supply and initial P. Radioactive tracer of P isotope is added to monitor the uptake of P. The metal ion activity in solution is buffered by resins following procedure developed before. Cell growth was monitored over time along with solution composition, i.e. before and after solution P is depleted. The growth response is related to the doses (metal ion activity and initial P in solution), revealing the interactions, i.e. the change in toxic limits (EC10; EC50) with initial P and, conversely, the effect of P supply on growth in absence and presence of metal stress. These results will be communicated at the conference. Future work will entail steady-state culturing of algae to ensure constant cell-P content and assess effects on metals on growth of these cultures.

**WE025 Lead accumulation and tolerance in plants growing on contaminated soils: implications for phytoremediation** J. Pratas, University of Coimbra; P. Favas, University of Trás-os-Montes e Alto Douro / Geology; R. D. Souza, M. Varun, M. Paul, St. John's College. The present investigation is an effort to assess the phytoremediation potential of the flora found growing on Pb enriched soils in an abandoned Pb mine in Central Portugal. Samples of soil and 49 species of plants were collected from two line transects in the area surrounding the Barbadalhos mine. Line transect 1 is perpendicular to the mineralized veins. Line transect 2 was in nearby non mineralized zone. Most plants could tolerate soil Pb concentrations averaging 2380 mg/kg and reaching 9330 mg/kg. Pb concentrations ranged from 1.11 to 548 mg/kg. This is far above the 100-400 mg/kg Pb content considered toxic for most plants. Along line transect 1 significant accumulation of Pb was seen in *Cistus salvifolius* (548 mg/kg), *Lonicera periclymenum* (318 mg/kg), *Anarrhinum bellidifolium*, *Phytolacca americana*, *Digitalis purpurea*, *Mentha suaveolens* (217-255 mg/kg). Pteridophytes like *Polystichum setiferum*, *Pteridium aquilinum*, and *Asplenium onopteris* also showed 117-251 mg/kg Pb in aerial parts. In line transect 2, Pb content was not significant, ranging from 0.94 to 11.6 mg/kg. However, concentrations higher than toxic level in some species like *C. salvifolius*, *D. purpurea*, *L. periclymenum*, *A. bellidifolium*, *P. americana* indicate that internal detoxification metal tolerance mechanisms might also exist; therefore, their utility for phytoremediation is possible. Though at first glance maximum Pb content observed in trees like *Acacia dealbata* (84 mg/kg: leaf), *Olea europaea* (62 mg/kg: stem), and *Quercus suber* (58 mg/kg: stem) from line transect 1 is not very promising compared to that of smaller plants mentioned above, nevertheless these trees can be very effective due to their enormous biomass. Also, *A. dealbata* covers more than 75% of the arboreal and 30% of arbustive strata in the area. When combined with the hardy nature, biomass and abundance of this species, the moderate accumulation indicates immense potential for phytoextraction of Pb in the area.

**WE026 The toxic consequences of high level soil contamination by glyphosate-based herbicides** M. Sihtmae, Laboratory of Environmental Toxicology; K. Kunis-Beres, L. Kanarbik, I. Blinova, National Institute of Chemical Physics and Biophysics. Glyphosate and glyphosate-based herbicides have been extensively studied for their properties to produce adverse human health and ecological effects. The manufacturers of glyphosate-based herbicides claim their products have low toxicity and are environment-friendly. Nevertheless, questions regarding their safety are periodically raised and recent independent studies have indicated that glyphosate may have adverse effects on human and the environment. The aim of the current study was to evaluate the possible long-term effects of two glyphosate formulations, Roundup Quick™ and Roundup Max™, to soil microbial community and

terrestrial plants. Effects of high concentrations were studied, to simulate large-scale spills in case of transportation accidents. Natural soil samples were spiked with different doses (up to 1000 times higher than recommended by the producer for the weed control) of herbicides and incubated outdoors during four months (April-August 2012). The impact of the Roundup formulations on soil microbial community was measured at different time points and the residual toxicity of soils was evaluated by the seedling emergence and growth of the red radish (*Raphanus sativus*) and barley (*Hordeum vulgare*). High doses of Roundup Quick noticeably increased the total number of heterotrophic bacteria compared to the clean control soil. At the same time the diversity of the soil microbes was drastically decreased. In general, the barley was more sensitive to both Roundup formulations compared to the red radish. However, in 110 days after the contamination there was no residual phytotoxicity also to the barley. Ten days after spiking, the aqueous eluates of the soils (1:10) were not toxic to the crustacean *Daphnia magna* and bacteria *Vibrio fischeri* even in the case of highest dose of herbicides. The current study showed that the investigated Roundup formulations had a profound impact on soil microbial community structure and tested plant species when applied in high concentrations. This research is supported by the Central Baltic INTERREG IV A Finnish-Estonian project: Risk Management and Remediation of Chemical Accidents (RIMA) and basic funding of NICPB.

**WE027 Assessment of the effect of detergents and shampoo in germination of seeds of lentil *Lens esculenta* L.** A.S. Sobrino-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia, Laboratorio Alejandro Villalobos. The use of seeds as test organism to evaluate the toxicity of water and sediment, is limited because it is only the use of 1 normalized species *Lactuca sativa*. Because the seeds are important in the food web, the aim of this study was to evaluate the response of seeds of lentil (*Lens esculenta*) exposed to 10 commercial detergent formulations and 5 types of shampoo and propose the use of these seeds as an alternative method of for assessing the toxicity of effluent and sediment from water systems. bioassays which tested five concentrations of each toxic for quintuplicate, plus a control without-toxic were performed. After 5 days of incubation, we evaluated the percentage of inhibition of the germination of the seeds was measured and radicle length (cm) to determine the degree of inhibition of growth. Significant difference was observed between the organisms exposed to xenobiotics and controls. The toxicity of detergents was higher in products containing enzymes in its formulation. The most toxic shampoo products were used as antidandruff. It was observed that the response in lentil seeds is similar to that obtained with other test organisms like *Daphnia magna* and lettuce seeds. Tests with lentil seeds could be an alternative method for assessing the toxicity of water and sediment from aquatic systems.

**WE028 Seawater in low-lying coastal ecosystems: differential sensitivity among soil primary producers** C. Venancio, University of Aveiro / Biology & CESAM; R. Pereira, University of Aveiro / CESAM, Center of Environmental and Marine Studies, University of Aveiro; V. Teixeira, University of Porto / Biology; I. Lopes, University of Aveiro / CESAM. Sea level rise is expected to happen as a consequence of global climate changes. Concomitantly, the intrusion of seawater (either through surface flooding or groundwater) into low-lying coastal areas may occur, which, in turn, will cause an increased salinity in soils. Several works have been carried out to address the effects of such increased salinity in terrestrial biota, namely by using sodium chloride (NaCl) as a surrogate. The present study intended to assess the sensitivity of four species of plants to an increased salinity, by comparing the toxicity of NaCl (which is the salt present at highest concentrations in seawater) and natural seawater (from the Atlantic Ocean). Effects on plant germination and growth of the monocotyledonous *Lolium perenne* and *Festuca arundinacea* and of the dicotyledonous *Vicia sativa* and *Trifolium pratense* were monitored, as these species are very common in forage/livestock systems in several parts of the world. Standard growth toxicity tests were performed by

exposing each plant species to serial dilutions of filtered seawater (from 6.5 to 46.6 mScm<sup>-1</sup>; using adilution factor of 1.2) and of NaCl (ranging from 2.6 to 22.8mScm<sup>-1</sup>; using a dilution factor of 1.2) for a period of 14 to 21 days. The obtained results, for dry weight, revealed that, in general, seawater exerted lower adverse effects on the tested species than NaCl. As an example, the EC<sub>50</sub> for *T. pratense* exposed to NaCl was 4.32 (2.95-5.68) mScm<sup>-1</sup> while the EC<sub>50</sub> for this same species exposed to seawater was 2.8-fold higher: 12.06 (10.29-13.84) mScm<sup>-1</sup>. Furthermore, results showed differential sensitivity to increased salinity among the tested species. Growth of *T. pratense* was the most affected by NaCl with an EC<sub>50</sub> of 4.32 (2.95-5.68) mScm<sup>-1</sup> while the two monocotyledonous tested species (*L. perenne* and *F. arundinacea*) where the species exhibiting a higher resistance to increased salinity with EC<sub>50</sub>s of 11.50 mScm<sup>-1</sup> and 11.82 mScm<sup>-1</sup>, respectively. In conclusion<sup>50</sup>, NaCl demonstrated to exert higher toxic effects than seawater of similar salinity, thus suggesting that the use of NaCl as a surrogate to assess the toxic effects of increased salinity due to sea level rise is a protective approach, as it simulates a worst-case scenario of salinisation.

#### **WE029 Effects of different TiO<sub>2</sub> nanoparticles on rhizobia and their symbiosis with clover** J. Moll, Agroscope ReckenholzTänikon

Research Station / Ecological Farming Systems; A. Gogos, Agroscope / Analytical Chemistry; M. Van der Heijden, Agroscope Reckenholz-Tänikon Research Station; K. Knauer, Federal Office for Agriculture / Section Plant Protection Products; T. Bucheli, Agroscope ART / Analytical Chemistry; F. Widmer, Agroscope Reckenholz-Tänikon Research Station. The symbiosis between leguminosae and rhizobia are of high importance in agricultural systems. For example clover and rhizobia can produce up to 200 kg N ha<sup>-1</sup> y<sup>-1</sup> that is available for plants. This sensitive ecosystem function has for example been shown to be negatively affected by CeO<sub>2</sub> nanoparticles (NPs) in soybeans. Several patents and studies discuss the application of NPs as part in plant protection products (PPP) as well as in fertilizers. Thus, it should be investigated if these nano-PPP and -fertilizer might affect rhizobia and their possibility to form symbiosis with legumes. In addition, TiO<sub>2</sub> is the NP with the currently highest production rate, resulting in a high likelihood of this NP to enter the environment. Thus, the effects of this NP on growth of rhizobia and the symbiosis between rhizobia and clover were tested. *Rhizobium trifolii* was grown in liquid cultures and exposed to four different concentrations of TiO<sub>2</sub> NPs. Different TiO<sub>2</sub> NPs were tested consisting of rutile (M262, P25)<sup>2</sup> as well as anatase particles (E171, P25) and an anatase bulk control. E171 decreased the growth of *R. trifolii* at a concentration of 23 mg/l by 33 %. P25 and M262 showed no effect at the same concentration but with M262 a tendency to decrease growth was found. The bulk particle reduced growth at a lower concentration of 18 mg/l. TEM pictures showed that the bulk TiO<sub>2</sub> contained The influence of NP on the nodules formation is currently tested in a second experiment. Therefore, clover is grown in a hydroponic system. The TiO<sub>2</sub> NPs are added together with the bacteria to the liquid medium in which clover is growing. The formation of nodules will be quantified. We evaluate if plants treated with E171, M262 and bulk TiO<sub>2</sub> build less nodules than the control while the ones treated with P25 are not different from the control.

#### **WE030 Concentration of Carbamazepine, Ofloxacin, and Trimethoprim in Wheat Straw and Grain Grown on Wastewater Irrigated Soils** E. Woodward, The Pennsylvania State University /

Ecosystem Science and Management; D. Andrews, The Pennsylvania State University; C.F. Williams, USDAARS / Lead Research Soil Scientist; J. Watson, Penn State Univ / Ecosystem Science and Management. The use of effluent to irrigate crops has benefits and drawbacks. While it provides an additional source of nutrients and water to plants, it also contains emerging contaminants such as pharmaceuticals and endocrine disrupting hormones. The crops grown at an irrigation site are often used as animal feed. Before these crops can be introduced into the food chain, it's important to determine whether or not they contain concentrations of pharmaceuticals that might be of concern. Wheat grain and straw samples from Penn State's Living Filter,

a wastewater irrigation site, were analyzed for three common pharmaceuticals: Carbamazepine, Trimethoprim and Ofloxacin. The environmental concentrations of these chemicals are low, making extraction and analytical methodology tedious. The irrigated grain and straw were dried and ground after harvest. One gram of each material was sampled and extracted through a pressurized liquid extraction procedure (PLE). The remaining extract was concentrated using a series of evaporation and reconstitution steps. The final extract volume, 0.5 ml of MeOH, was mixed with nanopure water until the organic solvent content was less than 5%. This primarily aqueous solution was cleaned up and concentrated through a solid phase extraction procedure (SPE). The eluent from the SPE process had a solvent composition of approximately 50% acetonitrile/50% methanol. This was concentrated to 200 µl under Nitrogen and then brought to a 1 ml volume comprised of 80% nanopure water/20% solvent that remained from the evaporation step. This solution was quantified for each pharmaceutical using LC-MS/MS. For all three pharmaceuticals, initial results indicated that concentrations in the wheat ranged from 0.1-10 ng/g wheat grain or wheat straw. This initial data suggested two things: (1) total plant uptake of pharmaceuticals varies between chemicals, and (2) the location within the plant where pharmaceuticals concentrate varies between chemicals.

#### **WE031 Uptake of 20 different human drugs by nasturtium irrigated with water containing emerging contaminants**C.F. Williams,

USDAARS / Lead Research Soil Scientist; D. Roll, USDA-ARS; C. Ray, P. Moravcik, University of Hawaii; J. Watson, Penn State Univ / Ecosystem Science and Management. The use of reclaimed municipal sewage for irrigation can be seen as a potential new source of water in arid and semi-arid regions of the world. One potential obstacle to reusing reclaimed water for irrigation of food crops is the uptake of emerging contaminants and the introduction of these compounds into the human food chain. Nasturtiums were irrigated with solutions containing 20 different drugs for 90 days and harvested to determine the mass of drug accumulated. Plants were sectioned into leaves, flowers, small stems, large stems and roots. Plant parts were extracted using a mixture of ethyl acetate, dichloromethane and methanol (35:35:30). Extraction was carried out by placing a known quantity of plant material into a bottle containing solvent, sonicated for 1 hr then allowed to soak for 12 hr. The solution was filtered followed by a second identical extraction. The filtered solvent was combined and concentrated through evaporation. Drug concentrations were measured using LC-MS-MS. Nasturtiums were found to accumulate the drugs applied in irrigation water. In general it was found that the highest drug concentrations were found in the roots and leaves. Drug concentration in the stems and flowers were similar but lower than the roots and leaves. The high concentrations in the roots can be attributed to being in contact with the drug in the soil solution. This contact would lead to sorption of the drug on the root as well as transport through the root. High concentration of the drugs in the leaves would also be expected due to evaporation of water taken up. The drugs are carried through the plant in flowing water and then would be left behind and accumulated in the leaves as the water carrying them is evaporated. The accumulation of emerging contaminants in leaves has implications for irrigating leafy vegetables, like lettuce, for human consumption.

#### **WE032 Carbon nanoparticles mobilize hydrophobic organic contaminants in groundwater: Linking aggregation properties and contaminant-mobilizing capabilities** W. Chen, College of

Environmental Science and Engineering / College of Environmental Science and Engineering; L. Wang, L. Hou, F. Wang, Nankai University; J. Fortner, Washington University in St. Louis; M. Tomson, Rice University / Department of Civil and Environmental Engineering. Engineered carbon nanomaterials are produced and used increasingly in many areas of applications, and their eventual release to the environment is inevitable. Carbon nanomaterials can form stable colloidal suspensions in aqueous solution, and we found that such stable colloidal suspensions—for examples, C<sub>60</sub> fullerene nanoparticles (nC<sub>60</sub>)—in aqueous environments can significantly mobilize hydrophobic organic contaminants by serving as a contaminant carrier. We investigated

facilitated transport of 2,2',5,5'-polychlorinated biphenyl (PCB) and phenanthrene by  $nC_{60}$  through sandy soil columns, and found that the capability of  $nC_{60}$  to enhance the transport of hydrophobic organic compounds is far greater than that of a corresponding amount of conventional dissolved organic matter (DOM) – a few mg/L  $nC_{60}$  significantly enhanced the transport of PCB and phenanthrene through saturated porous media, whereas DOMs (e.g., humic acid, fulvic acid, and bovine serum albumin) at similar carbon-based concentrations had minimal effects. The surprisingly high contaminant-mobilizing capability of  $nC_{60}$  compared to conventional DOMs is likely attributable to the unique porous structures of  $nC_{60}$ , which result in both enhanced adsorption affinity and desorption irreversibility. We also compared the contaminant-mobilizing capabilities of a variety of  $nC_{60}$  samples, prepared with different methods (e.g., solvent exchange from toluene or tetrahydrofuran (THF); long-term stirring or sonication; alternating detailed aggregation formation processes by involving a secondary solvent; etc.). Interestingly, different  $nC_{60}$  samples exhibited vastly different contaminant-mobilizing capabilities, likely attributable to the differences in aggregation properties—particularly, pore volume and pore geometry—among the samples, as controlled by the specific water chemistry and detailed aggregation formation routes. Findings in our studies indicate that colloidal carbon nanoparticles in the subsurface environment can greatly enhance the mobility of highly hydrophobic organic contaminants, which typically exhibit very low mobility. Furthermore, carbon nanoparticles from different sources or formed in the environment under different aquatic conditions might have vastly different effects on contaminant fate and transport. These should be taken into account when assessing the potential environmental risks of engineered carbonaceous nanomaterials.

**WE033 CuO nanoparticles – algae interactions: dissolution, aggregation and effects to green microalga *Chlamydomonas reinhardtii*** Y.I. Slaveykova, L. Maillard, E. Marti, G. Cheloni, University of Geneva / Institute Forel, Earth and Environmental Sciences. Interactions between engineered nanoparticles (ENPs) and aquatic microorganisms, such as algae are central in understanding the potential toxicity of ENPs at the cellular level and the biological hazards associated with their increasing use. These interactions are determined by the ENPs, microorganism and exposure medium characteristics. The present study aims therefore to study in a systematic way the influence of exposure medium on the interaction of CuO nanoparticles (CuO-NPs) and microalga *Chlamydomonas reinhardtii* with emphasis on the CuO-NPs dissolution, aggregation and toxic effects induced in microalgae. Interactions between CuO and *C. reinhardtii* were studied in the biological buffers  $10^{-3}$  M 2-(N-morpholino)ethanesulfonic acid (MES, pH=5.5) and 3-(N-morpholino)propanesulfonic acid (MOPS, pH=7), TAP and OECD as well as water sampled from Geneva Lake. CuO-NPs concentrations in different media were varied from 0.1 mg/L to 10 mg/L. To distinguish the contribution of CuO-NPs from that of the released Cu, bioassays were also performed in the presence of CuSO<sub>4</sub>. Growth inhibition, chlorophyll fluorescence, membrane damage and lipid peroxidation in a short (up to 2h) and long term (24 and 48h) exposure experiments were studied. The results show that the dispersions of CuO in all tested media contained dissolved Cu and Cu complexes, and different size of CuO-NPs aggregates of 3 to 5 particles. The proportion of different fractions was dependent on the medium composition. The amount of the dissolved copper in the CuO-NPs dispersions increased following exponential rise function over the time. However the degree of dissolution varied significantly in different media. Hydrodynamic size was around 130-150 nm with no significant changes over the time and even a decrease in the hydrodynamic size in the media with high degree of CuO-NPs dissolution was observed. The growth inhibition, the decrease of algal autofluorescence and increase of the percentage of cells with lipid peroxidation after 24h exposure to 10 mg CuO/L decrease in the order MES ~ MOPS > Lake >> OECD > TAP. This tendency reflects both the release of Cu from CuO-NPs and the differences in the composition of the medium, in particular the presence of phosphates and EDTA in TAP and OECD.

**WE034 A TIE Approach to Determine Particle Interactions and Modes of Uptake** R.R. Garner, Clemson University / Institute of Environmental Toxicology (CU-ENTOX); W.S. Baldwin, Clemson University / Department of Biological Sciences; A.M. Rao, Clemson University; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX). There are currently over 1000 consumer products on the market that contain or utilize nanomaterials, and while these materials have been shown to cross cell membranes, little research has examined the processes by which this occurs. The goal of this project was to characterize the means by which particle-serum protein complexes interact with membrane surfaces and are subsequently endocytosed. A549 carcinomic human alveolar cells were utilized to describe the movement of gold nanoparticles across cellular membranes. Particles were characterized in both their stock solutions as well as their exposure media, and uptake was quantified by inductively coupled plasma-mass spectrometry (ICP-MS). Particle uptake decreased as cells were exposed in media supplemented with increasing concentrations of fetal bovine serum (FBS) ( $R^2=0.87$ ,  $p < 0.0001$ ). Subsequently, particle uptake increased as cells were exposed in media supplemented with increasing concentrations of bovine serum albumin (BSA) ( $R^2=0.89$ ,  $p < 0.0001$ ). These data suggest that albumin, one of a mere vast array on components in FBS, is integral in the transport of particles across membranes. A toxicity identification evaluation (TIE) approach was then utilized to elucidate what components of FBS assist in particle uptake through first determining those that deter uptake. Following methods similar to a Phase II TIE, certain classes of compounds were removed through the purchase of specialty FBS. Specifically, cells were exposed to particles in media supplemented with charcoal stripped FBS to remove lipophilic non-polar materials such as viruses, growth factors, hormones, cytokines, and steroids. Cells were further exposed to particles in media supplemented with dialyzed FBS to remove hormones, cytokines, and amino acids, as well as with ultra-low IgG FBS to examine their effect on uptake. A Phase III TIE was then utilized to examine the effect of fetuin, transferrin, apolipoprotein, and hemoglobin on uptake through their addition to media supplemented with particles and BSA. This research provides a fundamental foundation upon which to accurately understand biological interactions with nanoparticles and will facilitate future risk assessment endeavors through the utilization of this rapid high throughput assay that may be used to predict particle-cell interactions.

**WE035 Developing a comprehensive test system for nanoparticle toxicity towards relevant activated sludge organisms** C. Burkart, Technische Universität Dresden / Institute of Hydrobiology; P. Obert-Rausser, Technische Universität Dresden / Institute of Hydrobiology; N. Scheibe, W.v. Tuempling, Helmholtz Centre for Environmental Research; T.U. Berendonk, D. Jungmann, Technische Universität Dresden / Institute of Hydrobiology. Nanotechnology is the most promising and innovative technology of the 21<sup>st</sup> century. It is known, that waste water treatment plants receive nanomaterials (NM) via sewage water which possibly have negative impact on communities of activated sludge organisms. Only few studies have addressed bioaccumulation effects of NMs in food chains. Effects on relevant eukaryotic organisms and whole communities in waste water treatment plants (WWTPs) were neglected so far. Due to specific fate and behaviour of NM in different media, an adjusted approach is necessary to provide comparability within a tiered approach. Therefore we developed a test system based on single species tests with bacterial and eukaryotic species *Raoultella planticola* and *Paramecium tetraurelia* under considerations of the special demands for a subsequent integrated assay. Nanosilver (nAg) was used as a model NM. nAg was characterized in the respective media. The fate and behaviour in the test systems was analyzed by ICP-OES. In order to distinguish between toxicity caused by ionic silver ( $Ag^+$ ) and by nanoparticles themselves,  $AgNO_3$  was applied as ionic control and analysed respectively. Effective concentrations of total Ag were shown to be significantly different from nominal concentrations in *Paramecium* tests. Therefore, effective concentrations were used to calculate ECx values. For *Paramecium* nAg EC<sub>50</sub> values were between 2.1 and 2.2 mg/L and for

$\text{Ag}^+$  1.4 and 1.9 mg/L, respectively. For *R. planticola* EC<sub>50</sub> derived from nominal concentrations for nAg were 0.14 to 0.16 mg/L and 0.01 for  $\text{Ag}^+$ . Furthermore, the results implicated that ionic silver, originating from both  $\text{AgNO}_3$  and nAg, are the toxic silver species in the single species tests instead of nAg itself. However, this might change at different environmental conditions in a WWTP. Hence, it is essential to characterise and analyse NM as properly as possible at all levels of the toxicity test system.

#### **WE036 Does one AgNP represent another AgNP in hazard testing and does it just come down to ions release** N.P. Rodrigues,

University of Aveiro Department of Biology CESAM / department of Biology & CESAM; M.J. Amorim, Universidade de Aveiro / Department of Biology and CESAM; J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience – Terrestrial Ecology. Enchytraeids are key sentinel organisms playing an important role in improving the soil structure. Here we tested the toxicity of silver nanoparticles (Ag NPs) to worms, *Enchytraeus crypticus*. Silver nanoparticles are widely used in a range of consumer products mainly as antibacterial agents and thus cause potential risk once these NPs are released into the environment. We tested the differences in toxicity for a range of AgNP materials, including OECD-AgNPs, Ag (PVP Coated), Ag (Non Coated) and a soluble salt  $\text{AgNO}_3$ . The standard parameters adult and juvenile numbers were measured following standard exposure periods. The particles were characterised with the standard techniques e.g. TEM, DLS, Purity etc. The exposure was measured as total concentrations and as released ions. The various particles caused differences in toxicity i.e. the mass based EC<sub>50</sub> values differed, with coated particles having the lowest (mass based) toxicity. These differences could not be explained based on the measured release of ions (into soil solution) from the particles, although a low amount of ions in soil solution may be due to a rapid binding (of ions) within the soil matrix. It was not possible to relate other parameters to the toxicity differences. It was not possible to predict the toxicity of one particle based on another particle, and ions could not be shown as the explanatory parameters.

#### **WE037 Ecotoxicological implications of the interactions between single-walled carbon nanotubes with metals** m. alshaerj,

HeriotWatt University / Centre for Marine Biodiversity & Biotechnology, School of Life Sciences; L. Paterson, Heriot-Watt University / 3SUPA, Institute of Biological Chemistry, Biophysics and Bioengineering; M. Hartl, HeriotWatt University / Centre for Marine Biodiversity and Biotechnology, School of Life Sciences. The present study explores the ecotoxicology of single-walled carbon nanotubes (SWCNTs) and their likely interaction with metal co-contaminants, with a focus on the effect of *in vivo* exposure in marine mussels. The nano scale effect was negated by the tendency of uncoated SWCNTs to agglomerate in water, particularly with high ionic strength as is the case in estuarine and seawater, which lead to the formation of aggregates of several millimetres in diameter. Despite this, SWCNTs, in combination with natural organic matter (SRNOM), stay suspended in seawater for considerably longer than might be expected and thereby become available to filter-feeding mussels, leading to their concentration on and increased contact with gill epithelia during exposure. SWCNTs in combination with either  $\text{Cd}^{2+}$  or  $\text{Zn}^{2+}$ , or  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$ , led to a 62%, 96% and 129% increase, respectively, in DNA strand breaks obtained using the Comet assay above background and threshold levels for individual constituents. We attribute this phenomenon to the large negatively charged surface area of the SWCNT agglomerates, that, under exposure conditions, attract metals and caused a higher than expected toxicological response than either nominal water concentrations or total body burden would normally suggest. The observed toxicological responses are incompatible with either an additive or synergistic effect, but rather suggestive of potentiation. If these laboratory experiments are confirmed in the natural environment, the observations will have implications for the understanding of the role of carbon nanotubes in environmental metal dynamics, and toxicology, and consequently, regulatory requirements.

#### **WE038 Effect of titanium dioxide (TiO<sub>2</sub>) nanoparticles on Mountainous Star Coral (*Montastraea faveolata*) stress responses** B. Jovanovic,

Ludwig Maximilians University of Munich / Chair for Fisheries Biology and Fish Diseases; D. Palic, Ludwig Maximilians University of Munich; H. Guzman, Smithsonian Tropical Research Institute. Increased presence of titanium dioxide nanoparticles in the environment suggests a rising need for the monitoring and evaluation of potential toxicity, stress induction, and bleaching in corals. Mountainous Star Coral (*Montastraea faveolata*) has frequently been used as model species in coral studies on gene expression, stress, and bleaching. In this study specimens of *M. faveolata*, collected in Panama, were exposed to 0.1 mg L<sup>-1</sup>, and 10 mg L<sup>-1</sup> of nano-TiO<sub>2</sub> as suspension in aquariums with seawater for 17 days. The exposure to nano-TiO<sub>2</sub> caused partial zooxanthellae expulsion in all colonies, without mortality. Dose dependent induction of the gene for heat shock protein 70 was observed at the early stage of exposure, indicating acute stress; followed by return to the basal level of transcription at later exposure, indicating potential coral adaptation and recovery from stress. ICP-MS analysis revealed that nano-TiO<sub>2</sub> was predominantly trapped and stored within the posterior layer of coral fragment (burrowing sponges, bacterial, and fungal mats), rather than being absorbed/adsorbed by coral tissues. Bioconcentration index in posterior layer was close to 600 after exposure to 10 mg L<sup>-1</sup> of nano-TiO<sub>2</sub> for 17 days. According to the theory of multiple stressors it is expected that the localized stressors will have additive or synergistic values on global stressors. In fact, mass bleaching of coral reefs has been shown to occur more frequently nowadays than in the past, and the extent can not be fully justified by ocean warming alone. Other effects such as ocean acidification, pollution, overfishing, UV light, etc. must be taken into consideration in addition to temperature changes alone. The modest ability of nano-TiO<sub>2</sub> to cause short term stress and partial zooxanthellae expulsion should not be disregarded.

#### **WE039 Effects of TiO<sub>2</sub> Particles on *Caenorhabditis elegans*: Impact of Size, Light Exposure and PAH Interaction.** J. Angelstorf,

Hamburg University of Applied Sciences; W. Ahlf, TU HamburgHarburg / Environmental Technology & Energy Economics; S. Heise, Hamburg University of Applied Sciences. Engineered nanoparticles (ENPs) are used in a variety of industrial and consumer products and the number of applications and innovations is steadily increasing. Production and consumption of nanoparticle containing goods will inevitably lead to emissions of ENPs to the environment with the aquatic ecosystem as a main sink. At the same time, even though research on nano-ecotoxicology has been emerging rapidly within the last decade, potential effects of ENPs on human health and the environment are still poorly understood. In comparison to their bulk scale counterparts, nanoparticles pose a higher risk to the environment for several reasons. Their small size enables ENPs to penetrate tissues and cells and to interact with cellular processes. Due to their large surface area, nanoparticles have the potential to affect the bioavailability and toxicity of co-contaminants. In order to assess those nanoscale specific effects and the potential impacts of environmental conditions, this study addresses the following aspects: 1) Does the primary particle size affect the toxicity of TiO<sub>2</sub> particles? 2) Can toxic effects be assigned to the photocatalytic activity and thus increased by sunlight? 3) How does nano-TiO<sub>2</sub> interact with PAHs as common co-contaminants in sediments? Effects of nano-TiO<sub>2</sub> (P25, 21nm) are investigated in comparison to bulk TiO<sub>2</sub> (NM100, 100 to 200nm), by using the nematode *Caenorhabditis elegans*. In addition to chronic *in vivo* endpoints, molecular biological indicators are considered. The results of this investigation stress the importance of primary particle size and environmental conditions for the ecotoxicity of TiO<sub>2</sub> materials. In contrast to bulk-TiO<sub>2</sub>, nano-TiO<sub>2</sub> significantly inhibits *C.elegans* reproduction and growth with a LOEC of 10mg/L and 30mg/L respectively, while mean particle sizes of aggregated bulk- and nano-TiO<sub>2</sub> are similar and both materials are ingested by *C.elegans*. There is evidence for photocatalytic activity and phototoxic effects of nano-TiO<sub>2</sub> induced by exposure to simulated sunlight at ambient intensity, while no

phototoxic effects are evoked by bulk TiO<sub>2</sub>. Regarding the expected accumulation of nTiO<sub>2</sub> in sediments with alarming rates of up to 1,4 µg/kg annually [1], even moderate ecotoxicological effects, as observed within this study, point to a serious environmental risk posed by nanoscale TiO<sub>2</sub>. [1] Gottschalk et al. *Environmental Science & Technology* 2009, 43(24):9216-9222.

**WE040 From cell lines to organisms: the role of reactive oxygen species at the end of the life cycle assessment of Baytubes A. Simon,** RWTH Aachen University / Inst. for Environmental Research; U. Sarnow, H. Maes, Inst. for Environmental Research; A. Meyer-Plath, BAM – Federal Institute for Materials Research and Testing; H. Hollert, A. Schaeffer, Inst. for Environmental Research. It is predicted that the worldwide production of carbon nanotubes (CNT) will scale up in the future to the range of tons per year. To date, only a few reports about studies on toxic effects of CNT are available and their results are often controversial. Hence, further evaluation and characterization of the toxic potential of CNT is necessary. In this study, we investigated whether multiwalled CNT (MWCNT) cause oxidative stress in cells and organisms. Therefore, both, *in vitro* and acute and chronic *in vivo* experiments were performed. Three different cell lines (rainbow trout liver cells (RTL-W1), human adrenocortical carcinoma cells (T47Dluc) and human breast adenocarcinoma cells (H295R)) were exposed to MWCNT (1-100 mg/L), as well as to graphene and graphite reference particles. The possible induction of oxidative stress in fish (*Danio rerio*) after exposure to MWCNT was investigated in whole body homogenates using an enzymatic assay (Amplex® Red). Acute toxic potential of MWCNT was determined by exposing daphnids (*Daphnia magna*) according to OECD-guideline 202. At last, to evaluate the possible chronic toxicity of MWCNT, an additional population assay with these organisms was performed. The production of ROS could be observed in RTL-W1 as well as in T47Dluc cells. The experimental setup did not allow the determination of an effect concentration (EC) because the production of ROS was still increasing upon the highest used concentration. Hence, a no observed effect concentration (NOEC) was calculated. In each experiment, the NOEC was lower than 3.125 mg/L. No acute toxicity of MWCNT to *D. magna* as well as to *D. rerio* was observed. However, first indications of oxidative stress in these organisms were obtained from *in vivo* experiments. Since we measured uptake of the material in both species using <sup>14</sup>C-CNT in previous studies, long term experiments are performed at the moment to figure out whether relevant chronic effects of this nanomaterial in dispersion to aquatic organisms may occur. In the present study, it was shown that ROS can be produced in organisms after exposure to MWCNT. Nevertheless, no acute toxicity could be observed even though exposure to concentrations much higher than those expected in the environment. Therefore, the results of chronic experiments will be presented in order to discuss whether observed MWCNT-cell interactions have relevant long termed consequences for aquatic organisms.

**WE041 Insights on the toxicity of TiO<sub>2</sub> nanoparticles and nanomaterials on the green algae *Pseudokirchneriella subcapitata*** J. SANTIAGO-MORALES, Chemistry I; D. Vignati, Laboratoire des Interactions Ecotoxicologie, Biodiversité, Ecosystèmes (LIEBE), UMR 7146, CNRS-UPV-M, Université de Lorraine, LIEBE, CNRS UMR 7146; C. Pagnout, Laboratoire des Interactions Ecotoxicologie, Biodiversité, Ecosystèmes (LIEBE), UMR 7146, CNRS-UPV-M, Université de Lorraine. 3. International Consortium for the Environmental Implications of Nanotechnology (iCEINT); J. Ferard, Laboratoire des Interactions Ecotoxicologie, Biodiversité, Ecosystèmes (LIEBE), UMR 7146, CNRS-UPV-M, Université de Lorraine; R. Rosal, University of Alcalá / Chemistry I. **Keywords:** nanoTiO<sub>2</sub>, green alga, agglomeration, esterase activity The use of nanoparticles (NPs) and nanomaterials (NMs) has increased rapidly over the last years, with TiO<sub>2</sub>-based NPs/NMs being among the most abundantly used. Concerns about adverse environmental effects of TiO<sub>2</sub> NPs/NMs are diverse. TiO<sub>2</sub> NMs leaching from house facades was reported by Kaegi et al. [1], while sunscreens contain TiO<sub>2</sub> nanoparticles that are likely to end up in bathing waters and wastewaters. Westerhoff et al. [2] detected

TiO<sub>2</sub> NPs in wastewater treatment plant influents and effluents at concentrations from 141 to 615 µg/L Ti and from 2 to 20 µg/L respectively. The presence of TiO<sub>2</sub> in WWTP effluent confirms that NPs/NMs can find their way to natural ecosystems through sludge or wastewater. The actual mechanisms by which TiO<sub>2</sub> NPs/NMs exert ecotoxicity to algae are not completely understood yet. This work focuses on the assessment of the ecotoxicity of TiO<sub>2</sub> NPs of different forms (cubic or elongated) and crystal phases (anatase, rutile or a mixture), and a TiO<sub>2</sub>-based NM commonly used in sunscreens (T-Lite<sup>®</sup>, BASF) to the freshwater alga *Pseudokirchneriella subcapitata*. Because the tendency of NPs/NMs to form aggregates can influence NP interaction with living organisms, the time and extent of the formation of aggregates in algal medium were also investigated. Ecotoxicological endpoints include biomass growth after 72h, esterase activity and ROS production determined using high-throughput devices such as flow cytometer. The results showed that aggregation of native NPs/NMs was clearly observed already after 1 hour of their introduction in ISO medium and tended to increase with time. This agglomeration might influence in the exposure-metrics and the fate of nanomaterials once released. The esterase activity decreased according to a concentration relationship. T-lite was more ecotoxic than elongated rutile meaning that derived NM can be more toxic than native NP. References [1] Kaegi R., Ulrich A., Sinnet B., Vonbank R., Wichser A., Zuleeg S., Simmler H., Brunner S., Vonmont H., Burkhardt M., Boller M. 2008. Synthetic TiO<sub>2</sub> nanoparticle emission from exterior facades into the aquatic environment. *Environ. Pollut* 156: 233-239. [2] Westerhoff P., Song G., Hristovski K., Kise MA. 2011. Occurrence and removal of titanium at full scale wastewater treatment plants: implications for TiO<sub>2</sub> nanomaterials. *J. Environ. Monit* 13: 1195-1203.

**WE042 Interactions between (fluorescent) silica nanoparticles and developing zebrafish embryos** J. Lacave, A. Retuerto, University of the Basque country (UPV/EHU); D. Gilliland, Institute for Health and Consumer Protection, European Commission - DG JRC; M. Cajaraville, University of the Basque country (UPV/EHU); A. Orbea, University of the Basque Country. Silicon is the most common element in the earth's crust behind the oxygen. Its use in solar panels is very extended and the multicrystalline silicon solar cells are the 50% of the worldwide photovoltaic cells production. In the field of nanotechnology, silica nanoparticles (SiO<sub>2</sub> NPs) have additional utility in a wide range of applications such as biologic delivery platforms, imaging and diagnostic agents, and targeted therapeutics. The aim of this study was to test the toxicity of arginine-capped SiO<sub>2</sub> NPs of different sizes (15, 30 and 70 nm) to zebrafish (*Danio rerio*) embryos in comparison to bulk and aqueous forms, as well as to investigate the interactions between fluorescent SiO<sub>2</sub> NPs (15 nm) and developing embryos. LC50 values for all tested forms were above 100 mg Si/L, the highest concentration tested. Significant effects on the embryo survival rate were observed only in the case of 70 nm NPs at the two highest exposure concentrations (50 and 100 mg Si/L). Results for hatching rate showed a similar pattern, except in the case of embryos exposed to sodium trisilicate at 100 mg Si/L, which caused a delay or inhibition in hatching. It also produced an increased in malformation prevalence causing cardiac malformation, spinal cord flexure and yolk sac edema. Control embryos did not present fluorescence signal at any time. In exposed embryos, fluorescence signal intensity was time- and concentration-dependent. Fluorescent NPs were seen attached to the chorion surface from the beginning of the exposure (6 h) until hatching at approximately 48-72 hours. Thus, it was clear that during the first days of development, the chorion played a protective role acting as a barrier for the NP entrance. After hatching, fluorescence was observed on the surface of the fish body, but also under the gill opercle covering the surface of the gill lamellae, in the gut tract and in the cloacal chamber of the posterior intestine. In conclusion, compared to other metal-containing NPs, SiO<sub>2</sub> NPs do not show acute toxicity to zebrafish embryos, but the effects of prolonged exposures deserve further investigations. Acknowledgements: Work funded by EU 7th FP (Nanoretox Project), Spanish MICINN (CTM2009-13477), UPV/EHU (UFI 11/37), and Basque Government (grant to consolidated research groups GIC07/26-IT-393-07).

#### **WE043 Interactions between polycyclic aromatic hydrocarbons and carbon nanotubes in aquatic environments** B. Glomstad,

Norwegian University of Science and Technology; L. Sorensen, SINTEF Materials and Chemistry; M. Ramzan, B.M. Jenssen, Norwegian University of Science and Technology; A. Booth, SINTEF Materials and Chemistry. A wide range of carbon nanotubes (CNTs) with different physical properties (diameter, length) and surface functionalisations are being produced and used in consumer products. The application and use of CNTs is increasing which will inevitably lead to their release into the aquatic environment. Studies have shown that natural organic matter (NOM) might adsorb to the surface of CNTs, thus increasing their stability in water. CNTs have strong sorption capacity towards hydrophobic organic contaminants dissolved in aquatic environments, including polycyclic aromatic hydrocarbons (PAHs). CNTs may therefore be an important factor in controlling the fate, bioavailability and toxicity of organic pollutants in waters. In this study, the adsorption of PAHs to suspensions of CNTs in synthetic freshwater containing NOM was investigated. Seven CNTs with different physical and chemical properties were evaluated; one single-walled carbon nanotube (SWCNT) three multi-walled carbon nanotubes (MWCNTs; 1-3) with different physical properties (diameters and lengths) and three MWCNTs with hydroxyl (-OH), carboxyl (-COOH) or amine (-NH<sub>2</sub>) surface functionalisations. Prior to experiments, dry CNTs are characterised by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to confirm their physical properties. The CNTs were dispersed in moderately hard reconstituted water (MHRW) containing environmentally relevant concentrations of NOM. CNT concentrations in the suspensions were determined using ultraviolet-visible (UV-vis) spectroscopy. The suspensions were spiked with phenanthrene or pyrene and allowed to equilibrate. Freely dissolved concentrations (C<sub>free</sub>) of the PAHs were then determined using negligible depletion solid phase microextraction (nd-SPME) followed by GC-MS analysis. Comparison of C<sub>free</sub> from sample solutions with and without CNTs allowed for the quantification of PAHs sorbed to the CNTs. Preliminary results showed differences between MWCNT-1, MWCNT-2 and MWCNT-3 in their sorption of PAHs, indicating that physical properties affect adsorption of organic pollutants to CNTs. The adsorption of PAHs to the SWCNTs and the surface functionalised MWCNTs will be determined. This will give information regarding the influence of different CNT properties (number of walls, diameter and length and surface functionalisation) on the adsorption of organic pollutants, which is important in order to evaluate the effect of CNT contamination in natural waters.

**WE044 Investigating fundamental characteristic of metal toxicity in zebrafish (*Danio rerio*) embryos** L. Sonnack, Fraunhofer IME; E. Muth Koehne, S. Kampe, K. Schlich, K. Hund-Rinke, Fraunhofer Institute for Molecular Biology and Applied Ecology IME; F. Hischen, W. Baumgartner, Institute of Cellular Neurobiology (Biology II), RWTH Aachen; H. Hollert, Institute of Environmental Research (Biology V), RWTH Aachen; M. Fenske, Fraunhofer Institute for Molecular Biology and Applied Ecology IME. Metals as environmental pollutants are a serious global problem. They are released during extraction (mining), processing and manufacturing (industry) of metals, and through use (e.g. in agriculture, medicine) and disposal of metal-containing products. Once metals reach the environment, they pose a continuous hazard, as they are neither chemically nor biologically degradable. In particular aquatic organisms are affected by metal exposure through industrial or waste water treatment discharges and land surface run-offs. Fish are exposed to metals in bio-available form, either ionic or elemental, at nano- to micro-scale size and toxicity occurs often already at very low concentrations. Metal exposure can lead to severe damages and impairs fish development through toxic mechanisms, which are still not clarified in detail. We therefore examine the uptake and the effects of environmentally relevant metals in various forms and modifications in zebrafish (*Danio rerio*) during embryonic development. For adult fish, it is known that the uptake of metals mainly occurs via the gills and the intestines, but for embryos still very little is known about the uptake,

distribution and subsequent effects. In experiments with silver nanoparticles (AgNP) as an example, we could demonstrate that the AgNP are taken up by the embryos but that the toxicity is primarily mediated by the silver ions. In a next step, we are now studying the toxic impact of other metals, in ionic and elemental form, with a special emphasis on the absorption and distribution of the metals in the fish embryos. An overview of the development and implementation of the test and analytical methods will be presented as well as study results on cadmium, demonstrating the effects on apoptosis in 48 and 96hpf zebrafish embryos. Silver nitrate (AgNO<sub>3</sub>) and silver nanoparticles will give an examples for the specific effects of metals in ionic and elemental particulate form related to neurotoxicity, lateral line development and behaviour.

**WE045 Mechanisms of Toxicity of Ionic Cu, CuO nano- and microparticles in epithelial cells** A.T. Thit Jensen, H. Selck, H. Bjerregaard, Roskilde University. The use of CuO nanoparticles (NPs) is increasing and studies examining the toxicity of these particles are therefore needed. The metal, Cu, is essential to animals and higher plants, as it serves as a co-factor for a variety of enzymes (Zhou and Gitschier 1997). However, Cu is highly toxic in excess due to its ability to bind and inhibit proteins, and generate Reactive Oxygen Species (ROS, H<sub>2</sub>O<sub>2</sub> and OH<sup>•</sup>) that can adversely modify proteins, lipids, DNA etc. CuO NPs may cause adverse effects and potentially be toxic, as a result of their small size (1-100nm) and high surface to volume ratio. Engineered metal NPs have been shown to cause organelle damage, DNA-damage, oxidative stress, apoptosis, up/down regulation of proteins etc. However, the sequence of these events and to what extent the toxic effects are contributable to release of metal ions is still fairly unknown. Here we examine the effects of Cu form and particle size on toxicity by conducting continuous exposures of epithelial cells from the proximal tubule of *Xenopus laevis* (A6) with different Cu treatments. The Cu treatments used were ionic Cu and CuO particles of three different sizes: CuO NPs (6nm), Polydispersed CuO NPs (Poly-CuO, 100nm) and CuO micro particles (5µm) at 200µM. The molecular mechanisms explaining the effects of the different Cu forms were tested by examination of morphological changes, cell death, ROS generation and DNA-damage. Our results revealed that toxicity depends on Cu form (ionic and particulate) and particle size and that Cu ions were least toxic and Poly-CuO NPs most toxic to A6 cells. Poly-CuO also caused increased ROS-generation and ROS-dependent DNA damage. Our findings strongly suggest that the cytotoxicity and DNA damage caused by Poly-CuO NPs exposures was caused by ROS-generation.

**WE046 Multibiomarker assessment of TiO<sub>2</sub> nanoparticles effects on *Dreissena polymorpha* and *Gammarus roeseli*: influence of shape and crystal structure** M. Garaud, Laboratory LIEBE CNRS UMR Université de L.; A. Bennasroune, Université de Lorraine; C. Chaneac, LCMCP - UPMC (Paris VI); C. Cossu-Leguille, Laboratory LIEBE - CNRS UMR 7146 - Université de Lorraine; N. Couleau, S. Devin, V. Felten, B. Marchal, K. Mehennaoui, C. Pagnout, Université de Lorraine; S. Pain-Devin, Université de Lorraine UdL / LIEBE CNRS UMR; F. Rodius, Z. Cyrielle, Université de Lorraine; L. Giamberini, Université de Lorraine CNRS UMR. Titanium dioxide nanoparticles (nTiO<sub>2</sub>) are among the most widely used nanoparticles, and the PECs are predicted to be as high as 16,3 µg/L in WWTPs effluents. Those PECs were confirmed by field studies which also measured concentrations up to 3000 µg/L in raw sewage water. Unfortunately, very few studies deal with the risk associated to aquatic environmental exposure to nTiO<sub>2</sub> at those concentrations. Moreover, production processes control has allowed industrials to design and to manufacture tightly calibrated nTiO<sub>2</sub> of various size, shape, coating and crystal structure in order to improve some desired properties (photocatalytic activity, UV shielding properties, etc). However, that multiplicity complicate ecotoxicity assessment as it appears that those properties profoundly influence nTiO<sub>2</sub> toxicity. In particular, studies on murine cells showed that the anatase form of nTiO<sub>2</sub> was more toxic than its rutile form and that rod-shaped nTiO<sub>2</sub> induced more damages than its sphere-shaped counterpart. Therefore, it is of primary importance to take into account



those properties to assess properly nanoparticules ecotoxicity. We decided to address this challenge by exposing two freshwater organisms, the bivalve *Dreissena polymorpha*, a filter-feeding organism with highly developed phagocytosis and endocytosis digestive capacities making it a unique target for nanoparticle toxicity, and the amphipod *Gammarus roeseli*, implied in important ecological processes such leaf-litter breakdown, to realistic concentrations of nTiO<sub>2</sub> (20 and 1000 µg/L). The effects of crystal structure and shape using two forms of anatase (cubic and rod-shaped) and one form of rutile (as little rod) of roughly the same primary size were tested using a wide range of biomarkers (mRNAs induction, immunocapacity, antioxidant and antitoxic defences, cellular damages, lysosomal morphology, energetic reserves, filtration, locomotion and ventilation). The results suggest that nTiO<sub>2</sub> exert a very low toxicity on *Gammarus roeseli* and *Dreissena polymorpha*. However, it seems that the AB form could physically impact gills in relation to its fiber shape, in a asbestos like-manner, triggering inflammatory response and inducing gill mRNAs in *Dreissena polymorpha* and several biomarker changes in *Gammarus roeseli*.

**WE047 Short-term algal testing – a new approach for disclosing silver nanoparticle toxicity** S.N. Sorensen, DTU Environment / Environmental Engineering; S. Laurelle, A. Baun, C. Engelbrekt, Technical University of Denmark. Silver is the most common nanomaterial in commercial products, mainly used for its antimicrobial properties. Increasing use of silver nanoparticles (AgNPs) is expected to cause release into the environment. Several studies explain the toxicity of AgNPs to aquatic organisms by released ionic silver, while other studies cannot relate all of the observed toxicity to this fraction. Overall, still little is known about AgNP toxicity and the underlying mechanisms. The aim of this study was: 1) to determine whether AgNPs display ionic behaviour as measured by algal toxicity under various conditions and 2) to investigate the effect of a shortened exposure period on AgNP and AgNO<sub>3</sub> toxicity. A series of tests were conducted with citrate capped silver nanoparticles (AgNP-Citrate), reference OECD silver nanoparticles (NM-300K) and silver nitrate as a reference for dissolved silver. The algae *Pseudokirchneriella subcapitata* were used as a test organism in both a standard growth inhibition test (ISO 8692:2004) for 48h and a short-term (2h) test, using <sup>14</sup>C-incorporation during photosynthesis as toxic endpoint. Characterization of AgNPs included ICP-OES, DLS, NTA, and TEM. For all three test materials, the two methods were carried out under conditions of pH 7 and 8, and with added cysteine as ionic silver ligand. For AgNO<sub>3</sub>, similar EC values were obtained in the 2h and 48h tests, showing a fast mechanism for algal toxicity of dissolved silver. In all tests, AgNO<sub>3</sub> was significantly more toxic than NM-300K, which again was more toxic than AgNP-citrate. For the nanoparticles, 2h exposure at pH 8 resulted in higher toxicity than after 48h for NM-300K, while AgNP-citrate was less toxic in the 2h test. This difference may be related to the different endpoints of the two tests, as well as the size and composition of the nanoparticles. The toxicity of the three test materials all decreased from pH 8 to 7 in the 48h tests, and similarly for AgNO<sub>3</sub> in the 2h tests. As expected, the toxicity was further reduced by the addition of cysteine. Overall, the observed changes in toxicity arising from varied exposure conditions correspond well with the anticipated outcome for ionic compounds. The 2h algal test setup provides a measure for photosynthesis inhibition and allows for high throughput screening of nanoparticle toxicity while minimizing potential confounding factors experienced in standard algal tests due to e.g. media composition and test duration.

**WE048 Antioxidant enzyme activity after exposure of 28days inhalation of talc** I. SHIM, Department of Environmental Health; H. Kim, G. Seo, M. Lee, K. Choi, P. Kim, J. Kwon, National Institute of Environmental Research. Talc is currently widely used in cosmetic products, paints and rubber manufacturing. The biological effects of talc have been studied extensively, however inhalation study focused on oxidative stress is not accomplished in depth until now. We conducted repeated 28 days inhalation toxicity study of Talc using Sprague-Dawley rats with whole body inhalation exposure system according to the

OECD Test Guideline 412. Male and female groups of Sprague-Dawley rats were exposed to Talc by inhalation of concentrations of 0, 5, 50, 100 mg/m<sup>3</sup>, for 6 h/day, 5 days/week for 4 weeks. The objective of this study is to identify the subacute inhalation toxicity of talc and to investigate antioxidant activity after exposure to talc. As a result, the body weight gain of male rats was statistically significantly suppressed in the 50 mg/m<sup>3</sup> group and 100mg/m<sup>3</sup> group when compared with the control group. Clinical examination results showed that there were no treatment-related toxic symptoms or mortality in any of the animals treated with talc during the study period. The result of cell count of BAL fluid showed that significant increase (p< 0.001) of activated alveolar macrophage dose-dependently. In subacute inhalation toxicity test of talc, infiltration of macrophage was observed on the alveolar walls and spaces near the terminal and respiratory bronchioles in the middle and high dose groups. In addition, the high dose male and female groups, the expression of a typical biological indicator of oxidative damage, SOD-2 (superoxide Dismutase-2) were statistically significantly increased (p< 0.05). These results suggest that inhalation of talc in rats can induce histopathologic alternation and oxidative damage. Key words : Inhalation toxicity, Talc, SOD-2, BAL fluid

**WE049 Aquatic and terrestrial ecotoxicity of nano and bulk forms of nickel** T. Sovova, Institute of Chemical Technology / Department of Environmental Chemistry; I. Konickova, H. Motejllova, Institute of Chemical Technology in Prague / Department of Environmental Chemistry; V. Bartunek, Institute of Chemical Technology in Prague / Institute of Inorganic Chemistry; V. Koci, Institute of Chemical Technology in Prague / Department of Environmental Chemistry. The aim of this work was to evaluate ecotoxicity of nano and bulk forms of nickel (mean particle size 10.9 and >100 nm, respectively). The ecotoxicity tests were carried out using both aquatic (freshwater crustaceans *Daphnia magna*, marine bacteria *Vibrio fischeri*, freshwater algae *Desmodesmus subspicatus*) and soil (seeds of lettuce *Lactuca sativa*, enchytraeids *Enchytraeus crypticus*, springtails *Folsomia candida*) organisms. The algal toxicity of the two forms was similar with EC50 of 17.2 and 13.2 mg Ni l<sup>-1</sup> for the bulk and nano form, respectively. For both the crustaceans and the bacteria, it was the nano nickel that was much more toxic (EC50 *D. magna*: 423.3 and 11.7 mg Ni l<sup>-1</sup>; *V. fischeri*: 302.1 and 76.62 mg Ni l<sup>-1</sup> for nano and bulk form, respectively). In the case of *F. candida*, it was on the contrary the bulk form that was more toxic even if there was not a significant difference (EC50 476.6 and 770.9 mg Ni kg<sup>-1</sup> of soil). *E. crypticus* was less sensitive than the springtails where we did not observe a 50% inhibitive effect even at the highest tested concentration of 2000 mg Ni kg<sup>-1</sup> of soil. In the case of the lettuce, the bulk form did not cause a significant inhibitive effect even at 4000 mg Ni kg<sup>-1</sup>. The nano form on the contrary caused a significant stimulative effect. The results showed a very different effects of both forms of nickel in the different environmental media. While in the aquatic environment, the nano form was more toxic, in the soil environment it was the bulk form that was the more toxic form of nickel.

**WE050 Avoidance of Ag Nanoparticles by earthworms, Eisenia fetida** J. Mariyadas, Aarhus University / bioscience; M.J. Amorim, Universidade de Aveiro / Department of Biology and CESAM; J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience – Terrestrial Ecology. Earthworms are key sentinel organisms playing an important role in improving the soil structure. Here we tested the avoidance behaviour of earthworms, *Eisenia fetida* to silver nanoparticles (Ag NPs). Silver nanoparticles are widely used in a range of consumer products mainly as antibacterial agents and thus causes potential risk to the environment once these particles are released into the environment [1]. In our tests, we were able to show that the earthworms avoided commercially fabricated silver nanoparticles in a dose and time dependent manner. The earthworms were exposed to 3 nanoparticles: NM300K, Ag (PVP Coated), Ag (Non Coated) and a soluble salt AgNO<sub>3</sub>; the avoidance behaviour was noted for different time intervals. Immediate avoidance at 24 hours was observed for the highest concentrations for all the test substances. And, periodical avoidance was

observed for other concentrations. The avoidance behaviour could not be explained by the release of silver ions in the soil-solution. Although, Ag-ions release (if any) may still have had an influence on behaviour. The present results suggests that the earthworms perceive the presence of actual nanoparticles in the soil. Our results suggest that, earthworms avoid the soil spiked with nanoparticles as the concentration increases also with respect to time. **REFERENCE** 1.Heckmann L-H, Hovgaard M.B., Sutherland D.S., Autrup H., Besenbacher F., Scott-Fordsmand J.J. (2011) Limit-test screening of selected inorganic nanoparticles to the earthworms *Eisenia fetida*. *Ecotoxicology* 20, 226-233.

**WE051 CeO<sub>2</sub> NPs ecotoxicity and their behavior in terrestrial and aquatic ecosystem** V. Minatta, C. Porredón, Unit of Experimental Toxicology and Ecotoxicology (UTOX) Barcelona Science Park; V. Celestini, L. De Marzi, Dept. of Basic and Applied Biology. University of L'Aquila Faculty of Biotechnologies; E. Teixidó, N. Brull, J. de Lapuente, M. Borrás, Unit of Experimental Toxicology and Ecotoxicology (UTOX) Barcelona Science Park. The exponential growth in the development of manufactured nanomaterials and their subsequent release into air, water and soil is increasing the exposure chances to humans and ecosystems. Consequently, the potential impact of nanoparticles on aquatic and terrestrial ecosystems is of special interest. Nanoparticles find a great deal of applications because of their unique properties, such as their light absorbing potential or magnetic characteristic usually due to their high specific surface area to volume ratio, which determine an increase of activity and their ability to penetrate and accumulate within organisms and cells. Furthermore, their effects on the environment depend on their composition, synthesis method, size and on the physico-chemical evolution in the environment. All these variables cause uncertainty about their fate and behavior in the environment. CeO<sub>2</sub> nanoparticles have wide applications such as automotive industry, solar cells, gas sensors, pharmaceutical, and agricultural products. Consequently, their impact on terrestrial and aquatic ecosystem must be assessed. There are few studies about environmental effects of CeO<sub>2</sub> nanoparticles and besides some of them have contradictory results. Such differences regarding its toxicity could be attributed both to their intrinsic characteristics (chemical composition, morphology, particle size, surface reactivity, synthesis method...) and to their coating characteristics. Regarding the effects on aquatic ecosystem, acute and chronic toxicity of CeO<sub>2</sub> nanoparticles were studied on algae, crustaceans and fish but few studies had been performed taking into account bioconcentration, biomagnification and bioaccumulation in the aquatic food chain. Different concentrations of CeO<sub>2</sub> nanoparticles were tested on earthworm for 48h and in 3 varieties of seeds (tomato, lettuce and cucumber) for 72 h to study ecotoxicity and fitotoxicity, respectively. Germination rate and root elongation of seeds were measured in the fitotoxicity test and the mortality rate of the earthworm was taken into consideration for the ecotoxicity test. No significant effect of CeO<sub>2</sub> nanoparticles was shown on elongation and germination seed in contrast to their respective controls, while a significant increase of earthworm's mortality was observed at the higher concentration tested (LC50 = 4403mg/L). Genotoxicity was also assessed by Comet assay both in seeds and earthworms, to know the potential DNA damages of CeO<sub>2</sub> nanoparticles. The analysis of Comet assay showed that genotoxicity was directly proportional to their concentration. Finally, the behavior of CeO<sub>2</sub> nanoparticles in the aquatic ecosystem was studied. Different concentrations of CeO<sub>2</sub> nanoparticles were incubated with *Chlorella vulgaris* and Fish *Danio rerio* for 72h and 96h, respectively. CeO<sub>2</sub> nanoparticles resulted toxic for Algae while no behavioral changes or mortality was shown on fish. Analysis with ICP-MS was conducted to study the potential accumulation of CeO<sub>2</sub> nanoparticles in the aquatic organisms. Further experiments are currently in process at different temperatures to simulate the effect due to metabolic stress.

#### WE052 Comparing the toxicity between differently synthesized

S. Gonçalves, Universidade de Aveiro; R.S. Lopes, University of Aveiro / Dept. Biology & CESAM; P. Kouvaris, N. Michailidis, Aristotle

University of Thessaloniki / Physical Metallurgy Laboratory, Mechanical Engineering Department; S. Loureiro, Universidade de Aveiro / Biology. Silver nanoparticles are one of the fastest growing nanoparticles produced and used worldwide. They can be found in all kinds of products, such as domestic disinfectants, cleaning products and even in pharmaceuticals due to their bactericidal properties, which will lead to an increase in concentration in the environment. Silver nanoparticles can be synthesized through different physical and chemical methods or more recently through leaf extraction, a biological and eco-friendly green synthesis. These methodologies are translated in particles with different characteristics. Therefore, this study aimed at comparing the toxicity of silver nanoparticles differently synthesized and with different characteristics on the survival and reproduction of the cladoceran *Daphnia magna* after exposure in laboratory conditions. To evaluate acute toxicity 48h exposure tests will be used and also 21 days exposure tests will be carried out to evaluate chronic toxicity and assess the effect on the reproductive output. Response patterns for the number of juveniles were different and dependent on the synthesis and characteristics. However, both endpoints measured were affected by the silver nanoparticles, despite the synthesis method.

#### WE053 Does the exposure pathway to ZnO nanoparticles in OECD soil influence on the ecotoxicological behaviour?

M. Miglietta, G. Rametta, S. Manzo, A. Salluzzo, J. Rimauro, G. Di Francia, ENEA. Currently, the attention to the potential harmful impact of engineered nanoparticles on the environment is increasing due to the large employment of nanomaterials. A fair number of literature data are already available about the toxicological impact of nanoparticles on terrestrial ecosystem but still the goal is to be reached. The exposure pathway of any particular ecosystem towards nanoparticles is one of the main variables to be considered when addressing the behaviour and toxicity of nanomaterials. This is even more so for terrestrial ecosystem where several ways of introduction can be envisaged (atmospheric fallout, disposal of waste product containing nanomaterials, direct disposal of nanoparticles, etc.). Once the nanomaterials are introduced into soil systems they can undergo different physicochemical transformations that depend on the input pathways and that in turn, may affect their toxic behaviour. The aim of this study was to investigate how the results of standard testing of nanoparticle toxicity towards soil organisms are affected by different exposure procedures. To this purpose, three soil spiking procedures were analysed for the differences in the physicochemical properties of the testing nanomaterials and of their ecotoxicological behaviour. A standard OECD soil was spiked with ZnO nanoparticles through three different approaches: as dry powder and as aqueous suspensions in water and in an aqueous soil extract. Bulk ZnO and ZnCl<sub>2</sub> were also studied for comparison. Soil samples were characterized by Scanning Electron Microscopy and Inductively Coupled Plasma Mass Spectrometry. Water extracts of the soil samples were analysed for particle size distribution by Dynamic Light Scattering and zinc concentration (ICP-MS). Toxicity contact tests, selected by previous investigations, were performed with *L. sativum* and *H. incongruens*. Results show that the spiking procedures give homogeneous distribution of ZnO nanoparticles in soil but the main properties of the testing species differ according to the spiking procedure. The same trend is observed for the ecotoxicological tests. The results show the urgent need of the definition of agreed methods concerning the nanoparticle spiking procedures.

#### WE054 Growth comparison of freshwater diatom *Nitzschia palea* in presence of double and multi-walled carbon nanotubes with or without natural organic matter

L. Verneuil, Ecolab (Laboratoire d'écologie fonctionnelle et environnement); J. Silvestre, F. Mouchet, Université de Toulouse; UPS, INP / Ecolab (Laboratoire d'écologie fonctionnelle et environnement); E. Flahaut, Université de Toulouse; UPS, INP / Institut Carnot CIRIMAT; C. Gancet, ARKEMA / R & D; J. Boutonnet, ARKEMA France; L. Gauthier, Université de Toulouse; UPS, INP / Ecolab (Laboratoire d'écologie fonctionnelle et environnement); E. Pinelli, Université de Toulouse; UPS, INP / EcoLab (Laboratoire d'écologie fonctionnelle et environnement).

Despite increasing production and use of carbon nanotubes (CNTs), only few studies have reported their effects on primary producers at the base of the trophic chain. Diatoms are ubiquitous unicellular algae, considered as main primary producers in many aquatic food chains. They play a key role in the life cycle of many organisms. Studies dealing with the impact of emerging pollutants such as CNTs on diatoms are therefore of great importance for future evaluation of the consequences of their presence in ecosystems. In the present work, the effects of two types of CNTs, double-walled carbon nanotubes (DWNTs) and multi-walled carbon nanotubes (MWNTs), are investigated on a monoculture of benthic diatoms *Nitzschia palea* (Kützing) W. Smith. For this purpose, an experimental device was established to discriminate the CNTs toxicity and the shading effect that they cause. Moreover, this device allows direct observations of CNTs agglomeration to the diatoms biofilm. For a better assessment of ecotoxicological hazards due to CNTs, this study was also conducted in the presence or absence of natural organic matter (at a realistic concentration), used as CNTs dispersing agent. Indeed, in absence of natural organic matter, the EC<sub>50</sub> values are respectively 119 mg L<sup>-1</sup> for MWNTs and 44 mg L<sup>-1</sup> for DWNTs. In these conditions, cell growth is not significantly affected by shading. In the presence of natural organic matter, the EC<sub>50</sub> values were about 5 times lower for the DWNT and 50 times lower for MWNT. Thus, and despite the positive effect of natural organic matter on cell growth, dispersion highly increased CNTs toxicity. Dispersion also strongly increased the shading effect caused by CNTs on algal growth. Moreover, our results show an important interaction between diatom biofilm and CNTs. Furthermore, electron microscopy observations revealed high affinity of CNTs for extracellular-polymeric substances produced by diatoms. This work would highlight an increased risk of CNTs toxicity on aquatic primary producers such as diatoms in the presence of natural organic matter, as well as possible consequences on freshwater food chains.

**WE055 Effect of dissolved and nanoparticulate copper exposure on cardiac development and function in zebrafish embryos.** S. Bakir, Plymouth University / Biological Sci. One of the urgent problems throughout the world today is environmental pollution, which includes the release of free ions of toxic metals from many domestic and industrial applications. Among the metal NPs of concern are Cu-NPs and copper ion. Copper is an essential micronutrient in fish, but elevated concentrations can initiate many toxic effects. Previous studies of Cu toxicity in fish have reported that exposure during sensitive periods of embryonic development, especially the gastrula and segmentation periods resulted in a significant increase in embryonic mortality, decrease hatching, and disturbances in cardiac function. Our objective is to determine the toxicity of copper ions during embryonic development of zebrafish. The results have shown increased mortality, disturbances in heart function, decreased hatching and morphological malformation of larvae. Future research will investigate the expression of genes involved in the embryonic zebrafish cardiac formation (Nkx) and cardiac function (CX) to evaluate the influence of copper exposure on these processes.

**WE056 Effect of zinc oxide nanoparticles in *Daphnia magna*: size dependent effects and counterparts** S. Lopes, Department of Biology; F. Ribeiro, University of Aveiro CESAM / department of Biology & CESAM; K. Jurkschat, A. Crossley, University of Oxford / Department of Materials; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; S. Loureiro, Universidade de Aveiro / Biology. As the production of zinc oxide nanoparticles (ZnO-NPs) and other metal oxides is exponentially increasing, it is important to investigate potential environmental and health impacts of such nanomaterials. Particle size is an important parameter to be considered when analysing the toxicity of nanoparticles (NPs), since it is known that at the nanosize range the properties of materials differ substantially from the respective bulk counterpart. Therefore information on the behaviour of nanoparticles in the exposure media used for toxicity testing is crucial to derive conclusions and related toxicity to particle characteristics. In this study the aquatic model organism, *Daphnia magna*, was used to investigate

the effect of ZnO-NPs with two different particle size (30 nm and 80-100 nm) and then compare them with ZnO-micro-sized and ionic counterparts (ZnCl<sub>2</sub>) on immobilization, feeding inhibition and reproduction endpoints. These tests were carried out in ASTM hard water and the characterization of nanoparticles was also studied in this media, by TEM and EDX, and particles dissolution assessed. The 48h-LC<sub>50</sub> values for immobilization ranged between 0.76 mg Zn.L<sup>-1</sup> for ZnCl<sub>2</sub> and 1.10 mg Zn<sup>2+</sup>.L<sup>-1</sup> for ZnO-NPs 80-100 nm. For the chronic exposures (reproduction and feeding inhibition tests), *D. magna* showed a reduction in the offspring production and feeding rate activities when exposed to high concentrations of all zinc compounds. These results were discussed in terms of particle size and dissolution rate.

**WE057 Effects of Silver Nanoparticles on lung cells: in vitro approach** F. Rosário, Universidade de Aveiro CESAM / Department of Biology; H. Oliveira, Universidade de Aveiro; A.J. Nogueira, University of Aveiro / Department of Biology & CESAM; C. Santos, Universidade de Aveiro. Nanoparticles (NP), considering the building blocks of nanotechnology, have at least one dimension between 1 and 100nm, providing a high surface/volume ratio, leading to high reactivity or intrinsic toxicity of the surface. Their unique properties can be useful in a range of fields. AgNP are the most widely used nanoparticles in commercial products, viz wound dressings, catheters, cosmetics, textiles and food production, due to their antimicrobial activity. However, their unpredicted interactions with biological systems and environmental fate and their unique physicochemical properties increases the potential to cross cell membranes and emphasize the need for proper assessment of the putative toxic effects. In this study, we evaluated the toxic effects of coated AgNP on the viability of lung cells (A549). For this, in vitro cultured cells were exposed to different increasing concentrations of AgNPs up to 100µg/mL for 24h and 48h. Cell growth and morphology was daily observed using an inverted microscope and cell viability was measured by MTT reduction assay. Cell cycle progression was evaluated by staining with propidium iodide (PI) and analysis by flow cytometry. Also, AgNP's induced apoptosis was analysed by flow cytometry using the Annexin V-FITC/PI kit. For both exposure periods, independent MTT assays showed consistent results, with a significant decrease in cell viability at the 50µg/mL concentration. Also, an accumulation of cells at G2/M and a concurrent decrease in the percentage of cells at G0/G1, along with the increase on apoptotic cells was observed. From our data, we concluded that AgNPs decrease cell viability induces apoptosis and also modulates cell cycle distribution in A549 cells.

**WE058 Effects of Waterborne Exposure to Dissolved Copper or Copper Nanoparticles on the Spleen of Juvenile Rainbow Trout (*Oncorhynchus mykiss*)** G. Al-Bairuty, University; R. Handy, University of Plymouth / School of Biomedical and Biological Sciences. Information about the immuno-toxicity of waterborne exposure to copper nanoparticles (Cu-NPs) compared to ordinary form of dissolved copper (Cu) is scarce. Therefore the current study aimed to investigate the toxicity of dissolved Cu (as CuSO<sub>4</sub>) compared to Cu-NPs on the spleen of rainbow trout as well as the immunological response by using spleen prints. Stock solutions of dispersed Cu-NPs were prepared freshly by stirring without using solvents. A semi-static test system was used to expose juvenile rainbow trout to control (no added Cu), 20 or 100 µg l<sup>-1</sup> of either dissolved Cu (as CuSO<sub>4</sub>.5H<sub>2</sub>O) or copper nanoparticles for 0, 4 and 10 days. Spleens of fish were sampled at day 0, 4 and 10 for wax histology, whereas spleen prints were collected at day 4 and 10. All Cu treatments caused injuries and alteration in the proportion of haematopoietic contents in the spleen, but dissolved Cu as CuSO<sub>4</sub> caused slightly more severe injuries than Cu-NPs. Spleen injuries included occasional necrosis of the cells, depletion of lymphoid tissues and increases in the number of melanomacrophage deposits in the spleen. Additionally, all Cu treatments caused a decrease in the proportion of red pulp and an increase in the proportion of white pulp compared to the control group (all statistically significant, ANOVA, *P* < 0.05). The proportion of sinusoid space showed slight decreases with 100 µg l<sup>-1</sup> of Cu-NPs treatment compared to control (ANOVA,

**WE059 Embriotoxic and spermiotoxic Effects of nanosized ZnO for Mediterranean sea urchin *Paracentrotus lividus*.** manzo, M.

Miglietta, G. Rametta, ENEA; S. Buono, Università degli Studi di Napoli Federico II; G. Di Francia, ENEA. Nanoparticles (NPs) of ZnO, having adsorbing properties in the UV wavelength range, are commonly added to sunscreens and cosmetics. During the life cycle of these commercial products, NPs may be released into the environment and become a threat to ecosystems. The aim of this research is to investigate the effects of nanoZnO (nZnO) upon early development, fertilization and offspring quality of the Mediterranean sea urchin *Paracentrotus lividus*. Sea urchins are among the main marine organisms expected to be exposed to these new contaminants. Sea urchin gametes and embryos are often utilized to assess the toxicity of chemical compounds in the marine ecosystem due to their sensitivity and availability. The nZnO toxicity findings are also compared to the toxic effects of Zn ions (ZnCl<sub>2</sub>), to investigate how the Zn<sup>2+</sup> contributes to nZnO toxicity and with ZnO bulk (bZnO) to evaluate the toxicity linked to the primary nanosize. Particles and test solution were thoroughly characterized. Aggregation and sedimentation processes were monitored in order to have an overview of the dynamics of the ZnO particle size distribution in a seawater matrix. The sedimentation rate shows that aggregates formed by nZnO remained in the water column for a considerable long time while the same concentration of bZnO aggregates settling much longer time. The effect of nZnO upon the fertilization and early development of embryos of *P. lividus* is reported herein for the first time. Zn ion (ZnCl<sub>2</sub>) and bZnO toxicity were assessed for comparison. Among the tested compounds nZnO was the most embryotoxic with a 100% effect at 1 μM [Zn]. The EC50s (as [Zn]) were 2.02 [1.97-2.09] μM and 0.98 [0.88-1.19] μM for ZnCl<sub>2</sub> and bZnO, respectively. Furthermore, the frequency of different developmental defects produced were very dissimilar and a specific larval skeletal abnormality trend could be observed for nZnO. Tested chemicals did not affect the sperm fertilization while the effects were dramatic on the offspring quality of sperm exposed to ZnO with an early block of the regular larval development. Our data show that together with the toxic action of ionic zinc deriving from the ZnO dissolution, it is necessary to consider also other factors that may affect the toxicity likely related to the presence of aggregates in the suspensions, and also to the surface interactions of particle/aggregates with target organisms and/or with the media.

**WE060 Food chain transfer of quantum dot in an aquatic ecosystem**

W. Lee, Konkuk University; Y. An, Konkuk University / Department of Environmental Sciences. Food chain is an important exposure pathway for xenobiotics because organisms are connected as dietary exposure. In this study, we performed food chain transfer test of quantum dot in water environment. Protozoa (*Euglena*), cladoceran (*Moina macrocopa*), and zebra fish (*Danio rerio*) were selected as aquatic food chain models. We measured the food chain transfer of quantum dot using a bio-image technique and bioaccumulation. Quantum dot was transferred from *Euglena*, through *M. macrocopa* to *Danio rerio* based on the intravital multi-photon confocal laser scanning microscope (IMP-CLSM). Bioaccumulation of quantum dot was measured by cadmium concentration in model organisms by ICP-MS, and we found that the quantum dot was transferred and accumulated in higher trophic levels via dietary exposure. *This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011-0015985). The authors thank the Korean Basic Science Institute for IMP-CLSM and ICP-MS analyses.*

**WE061 A Quantitative Weight of Evidence Approach for Risk Assessment of Engineered Nanomaterials**

D. Hristozov, S. Gottardo, Ca Foscari University of Venice; A. Critto, University Ca Foscari of Venice; A. Zabeo, Venice Research Consortium; P. Isigonis; A. Marcomini, University of Venice / Department of Environmental Sciences. It has been recognized that substantial limitations and uncertainties make the conventional Risk Assessment (RA) unfeasible to apply to engineered nanomaterials (ENMs), which leaves regulators

with little support in the near term. In the last decade, most scientific activities were focused on the production of new experimental data, relevant for the hazard and exposure assessment of ENMs and minor attention was paid by the research community to developing new methodological approaches that could complement the available toolset and facilitate near-term RA. The deficit of quantitative data has led to uncertain and ambiguous, largely qualitative, risk estimations based on expert judgments, which may fail to inform adequate risk management actions (Hristozov et al., 2012). In this context, a novel approach for RA and prioritization of ENMs, including probabilistic uncertainty evaluation, was developed. Its main goal is to quantitatively assess the risks from ENMs in occupational settings in order to inform regulatory decision making. Based on the conventional RA paradigm, the approach uses exposure and effects data that refer to a panel of commercially available ENM to rank and prioritize them for further testing (in a lower tier) and quantitatively estimate their occupational risks (in a higher tier). These data are further analyzed in order to calculate endpoint-specific margin-of-exposure (MoE) values, which are directly used in a Weight of Evidence (WoE) approach for low-tier relative prioritization of nanomaterials and exposure scenarios. The MoE is a well-established method used for risk ranking in regulatory contexts. It is currently used by the U.S. Environmental Protection Agency (EPA) for non-carcinogens and is proposed for carcinogens with non-linear dose-response characteristics. Risky materials/scenarios were further analyzed and their absolute health risks were estimated using the Derived No Effect Level (DNEL) approach, suggested in the REACH Guidelines for regulatory Chemical Safety Assessment. All uncertainties related to the input data, use of models and the application of the WoE-based aggregation procedures were thoroughly characterized using the Monte Carlo approach. This platform presentation reports the conceptual and mathematical structure of the proposed approach for RA of ENM as well as the results of its application to selected case studies.

*Acknowledgement* - The authors thank the FP7-funded ENPRA project.

**WE062 Hindered Firefly Luciferase Activity upon Silver Nanoparticle Exposure**

A. Kakinen, Laboratory of Environmental Toxicology; F. Ding, Clemson University / Department of Physics and Astronomy; P. Chen, Clemson University / Nano-Biophysics and Soft Matter Laboratory; M. Mortimer, National Institute of Chemical Physics and Biophysics / Laboratory of Molecular Genetics; P. Ke, Clemson University / Nano-Biophysics and Soft Matter Laboratory; A. Kahru, National Institute of Chemical Physics and Biophysics / Laboratory of Environmental Toxicology. The production of synthetic nanoparticles (NPs) rapidly increases and therefore the safety aspects of nanotechnologies have become an essential field of research. The adverse effects of NPs may occur on several levels of biological organization. Enzymes regulate life's processes in all types of cells. When engineered nanoparticles become internalized by the cell, they may interact with enzymes resulting in adverse effects on life processes. To evaluate these potential adverse effects we used silver nanoparticles (AgNPs; citrate coated, 20 nm) and a QuantiLum Recombinant luciferase, a 62 kDa enzyme involved in the light emitting reaction of fireflies. The luciferase-NP interactions were studied by combining various biophysical and chemical methods. We showed that upon exposure to luciferase the hydrodynamic size of the AgNPs in the test medium rapidly increased (> 1 μm; DLS analysis) and their zeta potential was changed from -22 mV for free AgNPs to ~6 mV for AgNP-luciferase complexes, depending on the luciferase concentration. The effective binding of luciferase to AgNP was confirmed by a red-shift of the UV-Vis extinction peak for AgNPs. Our TEM imaging further confirmed the formation of a luciferase corona on AgNPs, with an average size of 60 nm for the corona and a protein layer thickness around 20 nm. The formation of AgNP-luciferase complexes remarkably reduced the solubility of AgNPs whereas this effect depended on the amount of luciferase as well as time. The effect of AgNPs on the enzymatic activity was investigated using a Luciferase assay. The preliminary results showed that binding of the luciferase to AgNPs inhibited the enzymatic reaction. A comparison of the inhibitory effects of AgNPs and Ag-ions allowed us to conclude that Ag ions inhibited the

enzymatic activity via allosteric interactions with the SH- groups in the luciferase. Correspondingly, our circular dichroism analysis revealed minor changes of luciferase secondary structure upon their exposure to AgNPs.

#### **WE063 Impact of nanomaterials on soil functionality and biodiversity**

C. Fajardo; M. Sacca, M. Nande, University Complutense of Madrid; C. Lobo, Instituto Madrileño de Investigación y Desarrollo Rural Agrario y Alimentario; M. Martin, Universidad Complutense Madrid. The application of engineered nanostructures, bringing to the release of such materials into the environment (i.e. soil and water system) is nowadays increasing. Soils host a wide variety of species (microbes and fauna) of which our understanding is very limited. However, recent progress in the molecular characterization of soil biodiversity offers the exciting prospect of exploring its complexity and better understanding its functioning. The effect of nanoparticles on soil microorganisms is an area of great concern. Before manifestation of acute toxic effects on the cell or organism level, initial changes appear at a molecular level. For this reason, sets of responsive genes of a cell (*in vitro*) or organism (*in vivo*) can be considered as toxicological endpoints in gene expression profiling. In this work, nanoscale zero-valent iron (NZVI) particles have been applied to heavy metal contaminated soil microcosms. The aim of this approach is to assess biological indicators of exposure and toxicity, including high-throughput molecular methods and to develop new predictive models for environmental biomonitoring. The impact of NZVI on soil bacteria biodiversity was detected by Fluorescence *In Situ* Hybridisation (FISH), and the expression rates of functional- and stress-response genes was assessed by qRT-PCR. Linking molecular changes with relevant ecological responses may greatly improve the predictive powers of tests based on molecular responses and remains a great challenge in ecotoxicology.

#### **WE064 Influence of nanoparticle size in the ecotoxicity to aquatic organisms**

G.H. Silva, University of São Paulo / Escola Superior de Agricultura; J. Lourenco, University of Aveiro; F. Antunes, University of Coimbra; B. Santos, University of Aveiro; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; S. Mendo, University of Aveiro / department of Biology & CESAM; L.Lopes, University of Aveiro / CESAM. With a variety of potential applications, nanotechnology's scientific research is a relatively recent development entailing its application in diverse fields of science. Within this research field, several new products (nanoparticles-NP) with improved performances have been developed. Despite the increasing amount of research on the toxicity of these NP to biota, knowledge on this area is still limited. The present study aimed at assessing the influence of NP size on its sublethal toxicity to aquatic organisms. To attain this goal, species belonging to different taxonomic and functional groups (*Vibrio fischeri* – bacteria, *Pseudokirchneriella subcapitata* – producer, *Daphnia magna* – primary consumer, and *Danio rerio* – secondary consumer) were exposed to NP of silica (SiO<sub>2</sub>), electrostatically stabilized with negative charges and with sodium as counterion, with different sizes: Ludox TM40 (22 nm), Ludox HS30 (12 nm) and Ludox SM30 (7 nm). The test species were exposed to NP concentrations ranging from 0.2 to 328 g/L (TM40) and 0.15 to 246 g/L (HS30 and SM30) and the following endpoints were monitored during exposure: production of bioluminescence (*V. fischeri*), growth rate (*P. subcapitata*), feeding inhibition (*D. magna*), embryonic development and DNA damage (*D. rerio*). In addition, the characterization of NP suspensions was carried out through dynamic light scattering, zeta potential and rheology. The results obtained showed a size dependency on the sublethal effects of the NP, i.e. smaller NP induced higher adverse effects. Regarding genotoxic effects, a dose-effect curve was not observed as intermediate concentrations caused more damage in DNA when compared to the other tested concentrations.

#### **WE065 Membrane protected nd-SPME extraction of organic pollutants from aqueous dispersions of carbon nanotubes**

Sorensen, SINTEF Materials and Chemistry / Marine Environmental Technology; B. Glomstad, Norwegian University of Science and

Technology; M. Shen, J. Liu, Research Center for Eco-Environmental Sciences, Chinese Academy of Science; A. Booth, SINTEF Materials and Chemistry. Negligible depletion solid phase microextraction (nd-SPME) can be used to determine the concentration of a freely dissolved pollutant in an aqueous system. This represents the bioavailable fraction of the pollutant, and is of interest in ecotoxicological studies. The main advantage of nd-SPME is that it extracts a negligible amount of the analyte (In the present study, an nd-SPME method is optimised for the determination of the polyaromatic hydrocarbons (PAHs) phenanthrene and pyrene which adsorb to carbon nanotubes (CNTs) in aqueous systems. However, CNTs with adsorbed PAHs will adhere to the SPME fibres and give erroneous concentration data. Cellulose dialysis tubes (MWCO 300 kDa) of different diameters are investigated as possible barriers to protect the SPME fibres from interaction with the CNTs. The dialysis bags prevent the CNTs from adsorbing to the SPME fibres but permit the target PAHs to pass through without significant adsorption on to the membrane. To stabilise the dispersed CNTs and create environmentally realistic conditions, natural organic matter (NOM) is added to the synthetic freshwater. As NOM might also interact with the PAH compounds of interest, it is necessary to investigate whether the SPME fibres extract only the free PAHs or also those associated with NOM. Extraction from four different aqueous media is investigated to achieve an detailed understanding of the extraction mechanisms and assess the influence of environmental parameters (dissolved salts and NOM) on extraction efficiency. The four media are: (1) pure deionised water, (2) deionised water with NOM (20 mg/L), (3) moderately hard reconstituted water (MHRW), (4) MHRW with NOM (20 mg/L). For the nd-SPME extraction of the PAHs, disposable fused silica fibres (1 cm x 0.17 mm o.d.) coated with polydimethylsiloxane are used. The fibres are placed inside the dialysis bag which is then sealed using small metal clips. A minimum sample volume of 250 mL is used to ensure truly negligible depletion. The extraction is completed in the dark at 25°C. Different extraction times (1-96 hours) are investigated to determine the required equilibrium time for each analyte. The PAHs on the SPME fibres were desorbed in hexane (24 hr) and analysed by GC-MS.

#### **WE066 NanoCuO toxicity to freshwater microbial decomposers depends on nanoparticle size and humic acids**

A. Pradhan, P. Gerales, S. Sahadevan, C. Pascoal, University of Minho; F. Cassio, University of Minho / Centre of Molecular and Environmental Biology (CBMA), Department of Biology. Enhanced use of nanometal oxides increases the probability of their release in freshwaters, posing a potential risk to freshwater biota and associated ecosystem processes. The effects, however, may depend on factors, such as nanoparticle size or natural organic matter present in streams, such as humic acids. In streams, microbial decomposers (fungi and bacteria) play key roles in detritus foodwebs by transferring energy from plant-litter to higher trophic levels. We investigated the effects of nanoparticle size and humic acids on stream-dwelling microbial leaf-litter decomposers by exposing microbially colonized leaves to different size of nanoCuO (12, 50 and 80 nm powder) at concentrations up to 400 mg/L (5 levels) in the absence or presence of humic acids (HA) up to 100 mg/L (3 levels) for 20 d. In the absence of HA, the effects of nanoCuO on leaf mass loss became stronger as the size of nanoparticles decreased and the concentration increased. Bacterial biomass was much more sensitive to nanoCuO than fungal biomass. EC<sub>50</sub> values for microbial biomass increased with the increase of nanoparticle size. Leaf decomposition and microbial biomass were also affected by exposure to HA alone. The negative effects of smaller size nanoCuO were diminished in the presence of HA; but this was not observed for nanoparticles with larger size (80 nm). Fungal reproduction and diversity (as sporulating fungi) were also affected in a similar pattern. SEM revealed that leaves exposed to increased nanoCuO concentrations showed higher adsorption of nanoparticles. FEDER-POFC-COMPETE and FCT supported this study (PTDC/AAC-AMB/121650/2010), AP (SFRH/BD/45614/2008) and PG (SFRH/BD/75516/2010).

#### **WE067 Nanomaterial phytotoxicity to agricultural crops** J.C.

**White, R. De La Torre Roche, J. Hawthorne, C. Musante, Connecticut Agricultural Experiment Station / Department of Analytical Chemistry.** Although nanomaterial use (NM) has increased dramatically, the existing regulatory framework does not require particle size-specific toxicity data and therefore, risks posed by this emerging class of contaminants have not been thoroughly evaluated. NM are incorporated into pesticides and fertilizers but impacts on agricultural crops and potential trophic transfer are unknown. This lack of understanding is disconcerting given that food crop contamination is an uncharacterized pathway of human exposure. Previous work has shown that traditional seed germination and root elongation assays are inappropriate when evaluating NM phytotoxicity. In preliminary hydroponic investigations, the acute toxicity and element accumulation of Ag, Si, and Au NPs was shown to be significantly greater in zucchini than corresponding bulk materials. Consequently, a large screening study has been undertaken where the acute phytotoxicity of 12 NM (single/multi-walled carbon nanotubes/fullerenes, Ag, CuO, Au, Si, ZnO, CeO<sub>2</sub>, TiO<sub>2</sub>, SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>), as well as corresponding bulk and ion controls, is being evaluated under batch hydroponic conditions to 12 common agricultural crops. NM exposure concentrations are 0-500 mg/L and measured end points include biomass, transpiration, particle content, reactive oxygen species formation, lipid peroxidation, and chlorophyll content. Specifically, particle uptake is being evaluated by liquid chromatography inductively coupled plasma mass spectrometry (LC-ICP-MS), atomic force microscopy (AFM), scanning/transmission electron microscopy with energy dispersive spectroscopy (STEM-EDS) and synchrotron X-ray absorption near-edge structure (XANES). Results to date show numerous instances of particle size-specific phytotoxicity and fate, as well as concentration-dependent and species-specific response to NM exposure. For example, Ag nanoparticles (NP) reduced the biomass and transpiration of soybean and wheat significantly more than did ions or bulk particles. This toxicity correlated well with greater Ag content in the tissues of exposed plants. However, Ag had little impact on zucchini and rice; the element content of these plants was not impacted by particle size. Once complete, the screening study will enable identification of sensitive crop-NM systems and guide more detailed mechanistic and molecular investigations. The relevance of these findings to efforts designed to reduce human exposure to NM through food chain contamination will also be evaluated.

#### **WE068 Nanoparticle-protein corona in invertebrate in vitro testing**

**Y. Hayashi, T. Micaela, C. Scavenius, Aarhus University / iNANO Interdisciplinary Nanoscience Center; P. Engelmann, University of Pécs / Department of Immunology and Biotechnology; H. Autrup, Aarhus University / Department of Public Health; J.E. Enghild, D.S. Sutherland, Aarhus University / iNANO Interdisciplinary Nanoscience Center; J.J. Scott-Fordsmand, Aarhus University / Department of Bioscience – Terrestrial Ecology.** We have previously established an in vitro model of the earthworm *Eisenia fetida* and highlighted a considerable involvement of phagocytes in the cellular responses to silver nanoparticles exposure. Our in vitro model was tested in a conventional cell culture environment with serum supplements, and the primary cells were thus exposed to silver nanoparticles with pre-formed corona of serum albumin (a major serum protein). Here we have profiled proteins forming the hard corona around silver nanoparticles (OECD reference materials, 15 nm and 75 nm) using gel electrophoresis techniques to identify proteins that strongly interact with the nanoparticles. This study was accompanied by multi-parametric flow-cytometry analysis of the cellular responses, in particular nanoparticle accumulation and cytotoxicity. The formation of and differential cellular responses to nanoparticle-protein complexes underscore the need for evaluation of the protein corona in invertebrate in vitro setting.

#### **WE069 Nanosilver effects on the extracellular enzymatic activity of stream periphyton**

**C. Gil-Allue, Eawag / Department of Environmental Toxicology; A. Tlili, M.O. Gessner, Institute of Freshwater Ecology and Inland Fisheries; K. Schirmer, Eawag; R. Behra, Eawag / Department of Environmental Toxicology.** Periphyton is a microbial community of auto- and heterotrophic organisms

embedded in a polysaccharide matrix (EPS), and is involved in ecosystem functions such as primary production and nutrient cycling. It has been identified as a sink of nanomaterials in the aquatic environment, but the fate of nanomaterials within the periphyton and their toxic effects are unknown. We are currently investigating the short-term effects of silver nanoparticles and silver ions on periphyton. In this study, we present the findings on the effects of silver on the activity of three extracellular enzymes:  $\beta$ -glucosidase (B-GLU), leucine-aminopeptidase (LAMP) and alkaline phosphatase (PHOS). Extracellular enzymes are involved in nutrient acquisition by breaking down high molecular weight compounds into smaller ones that can be transported across the cell membranes of periphytic organisms. Periphyton was colonized on glass slides in flow-through channels fed with river water. The periphyton was scrapped from slides and suspended in reconstituted freshwater (pH 7.5). Silver treatments were dosed as AgNO<sub>3</sub> (source of Ag<sup>+</sup>) or citrate-coated silver nanoparticles (AgNP) with an average diameter of 27.5±0.7nm. Periphytic suspensions were exposed for 2 hours to different concentrations of AgNO<sub>3</sub> (10, 50 and 100µM) or AgNP (0.1, 0.5 and 1µM). After the exposure, the enzymatic activities were assayed using fluorescent substrates. Assays were carried out in the whole suspension, and also in filtered (0.2µm) cell-free EPS extracts to discern the direct effects to the enzymes. The addition of the silver ligand DMPS (100µM) was used to provide further information about the role of Ag<sup>+</sup> in the observed effects. The activities of LAMP and B-GLU decreased with increasing AgNP or AgNO<sub>3</sub> concentration. In the case of PHOS, the activity was inhibited at the highest AgNP concentration while it was stimulated at the lower silver concentrations. These responses can be caused by a disturbance of enzyme synthesis or direct enzyme inactivation. B-GLU activity in the EPS extract was also observed to decrease with increasing concentrations of silver, suggesting a direct inactivation of the enzyme. PHOS and LAMP activities were negligible in the extract. The addition of DMPS ameliorated the observed effects. This study shows that AgNP and AgNO<sub>3</sub> might disturb the nutrient acquisition by periphytic organisms.<sup>3</sup>

#### **WE070 Quantitative proteomic analysis of vacuolar proteins isolated from *Saccharomyces cerevisiae* exposed to CuO-, TiO<sub>2</sub>- and ZnO-nanoparticles**

**C.M. Gallampois, Linköping University / Department of Clinical and Experimental Medicine; J. Kuruvilla, Linköping University / Department of Clinical and Experimental Medicine; N. Bayat, Stockholm University / Biochemistry and Biophysics; S. Cristobal, Linköping University / Biochemistry and Biophysics.** Nanomaterials and nanoparticles (NPs) possess chemical properties dictated by chemical composition, their unusually small size and very large proportional surface area. Even chemically inert materials can have significant chemical activity on the nanoscale (e.g. surface catalysis). Recent research suggests that NPs can cross important biobarriers (e.g. blood-brain), enter cells and trigger oxidative stress by production of reactive oxygen species. Key factors in NPs toxicity seem to be size, structure, chemical composition and a "corona" of proteins coating the particle which may confer biological functionality. NPs have extensive applications (e.g. biomedical devices, food packaging). Thus, NPs may present an emerging long term environmental threat. In this study, we have selected the yeast *Saccharomyces cerevisiae* as it is one of the most intensively studied unicellular eukaryotic model organisms in molecular and cell biology. On one hand, this species shares essential cellular and biochemical mechanisms with other eukaryotes including humans and on the other hand, yeasts have been found in association with soil and insects in the environment. In our previous work, we observed that *S. cerevisiae* cells exposed to CuO-, TiO<sub>2</sub>-, and ZnO-NPs caused several cellular responses including enlargement of vacuoles. Yeast vacuole is highly dynamic and rapidly changes its shape and size in response to the cell cycle and a multitude of environmental conditions. Therefore, in this study a quantitative proteomic approach has been performed to understand the differential cellular response to investigate the impact of NPs. Briefly, after exposure, yeast cells were enzymatically disrupted by lyticase and purified enriched vacuoles fractions were isolated using a ficoll gradient and ultracentrifugation.

The vacuolar proteins were trypsinated and labelled by Isobaric tag for relative and absolute quantitation (iTRAQ) before LC-MS/MS analysis. Identification and quantification of differentially expressed proteins after exposure to nanoparticles were performed with Scaffold and Proteome discoverer, and NPs has been thoroughly characterized. Our results indicated that the size and the chemistry determine the qualitative and quantitative variations in vacuoles after exposed to NPs.

**WE071 Investigation of oxidative stress and DNA damages in aquatic *Xenopus laevis* tadpoles after short-term exposure to multi walled carbon nanotubes** E. saria, Ecolab / ENSAT; F. MOUCHET, EcoLab/NAUTILE / ENSAT; E. FLAHAUT, CIRIMAT/NAUTILE; C. LAPLANCHE, Ecolab; C. GANCET, ARKEMA/NAUTILE; J. BOUTONNET, ARKEMA; E. PINELLI, EcoLab/NAUTILE; L. GAUTHIER, Ecolab. Carbon nanotubes (CNTs) focus the attention of many scientists because of their huge potential of industrial applications, but there is a lack of information relating to their toxicological properties. Our laboratory gives a particular attention to the study of the impact of CNTs on aquatic organisms especially on *Xenopus laevis*. Preliminary studies with multi walled carbon nanotubes (MWNTs) highlighted acute toxicity (mortality) at very high concentrations (50 mg/L). For lower doses, growth inhibition and genotoxicity were observed in different experimental conditions. Macroscopic observation of CNTs-exposed larvae showed the presence of nanotubes in gills and intestine while RAMAN analysis did not show any presence of MWNTs in cells and tissues. We therefore suspected a possible indirect effect of CNTs in test conditions. To understand the molecular mechanisms involved in the chronic effects observed, *Xenopus* larvae (stage 50) were exposed to two different concentrations of MWNTs (0.1 and 1mg/L) on time ranging from 2 to 24 hours. Content of reactive oxygen species (hydrogen peroxide [ $H_2O_2$ ]) and activities of antioxidant enzymes (Super Oxide Dismutase [SOD], Glutathione Reductase [GR] and Catalase [CAT]) were measured. DNA damages were assessed according to the alkaline comet assay in red blood cells of *Xenopus*. The results obtained showed a variation of reactive oxygen species production and enzymatic activity depending on the concentration of MWNTs and the exposure time. In the presence of 0.1mg/L of MWNTs, GR and CAT activities slightly increased after 8 hours of *Xenopus* larvae exposure and decreased slowly until 24 hours. In the same conditions,  $H_2O_2$  production reached a maximum value at 8 hours and then decreased until 24 hours. For the highest concentration (1 mg/L), the antioxidant enzyme system was early induced; GR activity was maximal after 4 hours of exposure.  $H_2O_2$  production began at 2 hours and decreased after 8 hours until 24 hours. The Comet assay results showed a significant enhancement of the comet parameters (tail DNA, tail length) at both concentrations (0.1 and 1 mg/L), fluctuating in time. These results suggest that oxidative stress and DNA damages may be involved in the mechanisms of toxicity in *Xenopus* larvae after short-term exposure to low concentrations of MWNTs.

**WE072 Sublethal toxicity of nanoCuO to freshwater shredders can be influenced by nanoparticle size and humic acids** A. Pradhan, P. Gerales, S. Sahadevan, C. Pascoal, F. Cassio, University of Minho / Centre of Molecular and Environmental Biology (CBMA), Department of Biology. In freshwaters, natural organic matter, such as humic acids, may interfere with nanoparticle bioavailability to biota. In streams, the invertebrate shredders play a key role in organic matter turnover and energy transfer from plant litter to higher trophic levels. We investigated the sublethal impacts of nanoCuO with different sizes and the influence of humic acids on the feeding behaviour of the shredder *Allogamus ligonifer* (Trichoptera). Animals were exposed to nanoCuO with 12, 50 or 80 nm at different sublethal concentrations (up to  $100\text{ mg L}^{-1}$ , 3 levels) in the absence or presence of humic acid (HA,  $100\text{ mg L}^{-1}$ ) for 5 days. In the absence of nanoCuO and HA, the feeding rate was  $0.416\text{ mg leaf DM mg}^{-1}\text{ animal DM day}^{-1}$ . In the absence of HA, the animal feeding rates decreased with exposure to increasing concentrations of nanoCuO, particularly in the case of lower size nanoparticles. In the absence of nanoCuO, HA inhibited the feeding rate of the shredder ( $0.197\text{ mg leaf DM mg}^{-1}\text{ animal DM day}^{-1}$ ). However, when animals

were co-exposed to nanoparticles and HA, the negative effects of lower size nanoCuO decreased. A post-exposure feeding experiment was carried out to assess the ability of animals to recover from the stress. For that, animals previously exposed to the stressors were allowed to feed on microbially-colonized unexposed leaves for 5 days. The feeding rates of the animals increased, but the recovery was very low. Shredders exposed only to HA or to lowest concentration of the largest size nanoCuO recovered faster. FEDER-POFC-COMPETE and FCT supported this study (PTDC/AAC-AMB/121650/2010), AP (SFRH/BD/45614/2008) and PG (SFRH/BD/75516/2010).

**WE073 The ecotoxic potential of silver nanoparticles: Effects and behaviour in aquatic model systems.** C. Polleichtner, Ecotoxicological Laboratory; A. Huenken, Federal Environment Agency / Ecotoxicological Laboratory; J. Koeser, University Bremen; C. Kussatz, Federal Environment Agency / Ecotoxicological Laboratory. Engineered nanoparticles and materials are increasingly used in a broad variety of consumer products; their economic importance has risen strongly over the last years. Especially silver nanoparticles (Ag

**WE074 The ecotoxicity of engineered nanomaterials in the oligochaetes *Lumbriculus variegatus*** S. Little, Heriot Watt University / Life Sciences; J. Kinross, HeriotWatt University / School of Life Sciences; H. Johnston, Heriot Watt University / Life Sciences; T.F. Fernandes, HeriotWatt University / School of Life Sciences. Rapid growth in the field of nanotechnology is ever increasing the potential release of nanomaterials (NM) into the environment. As the main recipient of industrial and domestic wastewaters, the fate and behaviour of NMs in aquatic systems has come under much scrutiny in recent years. Sediments in particular are anticipated to be the final sink for NMs due to the processes of aggregation, agglomeration and sedimentation, however, little is known in terms of the effects on sediment fauna. In this study, the toxicity of two engineered nanoparticles; silver (NM-300K) and titanium dioxide (NM-104) to the freshwater, sediment ingesting oligochaete *Lumbriculus variegatus* were investigated using the endpoints of; population number, reproduction, dry biomass and body reversal. Following a 28 day exposure, NM-300K (0, 25, 50, 100, 200 mg/kg dry sediment) and NM-104 (0, 100, 250, 500, 750, 1000 mg/kg dry sediment), toxicity proved to be low. Although not significant ( $p>0.05$ ), *L. variegatus*, dry biomass, population and reproduction generally showed a concentration dependent decline for both nanoparticles tested. However, behavioural body reversal responses were significantly ( $p<0.05$ ) poorer for NM-300K exposed *L. variegatus* at the lowest test concentration, indicating a detrimental effect on worm health. Despite a lower number of worms, dry biomass was greatest in worms exposed to the highest concentration of NM-104 (1000 mg/kg dry sediment), suggesting the possibility of nanoparticle bioaccumulation

**WE075 The Ecotoxicology of Copper Nanoparticles in Marine Mussels** H.M. Alnashiri, HeriotWatt University / School of Life Sciences; M. Hartl, HERIOT-WATT UNOVERSITY; T. Fernandes, Heriot-Watt University. Copper oxide nanoparticles (CuO NPs) are one type of NP widely used in various industrial and commercial applications, such as batteries, inks and heat transfer nanofluids. Thus, this wide range use of CuO NPs is likely to lead to a potential increase in their releases to the environment, particularly the marine environment since it is an ultimate sink for many contaminants. However, the knowledge of the toxicity of CuO NPs is still limited compared to other metal oxides nanoparticles, such as ZnO or TiO<sub>2</sub>. Hence, it is essential to investigate CuO NP exposure and effects on key organisms, such as benthic filter feeders since data in these systems and on these species is still lacking. Hitherto, very few studies have determined the effect of CuO NPs on mussels, and these have concentrated solely on oxidative stress and lipid peroxidation, but have not investigated DNA damage or cell viability nor compared the toxic potential in different marine mussel species, living in different habitats and under different environmental conditions. The focus of this study was directed towards determining and comparing the toxicity CuO NPs to two species of marine mussels



(*Mytilus edulis* and *Modiolus modiolus*) by focussing on DNA effects and cell viability using the Comet assay and flow cytometry. The blue mussel, *M. edulis* was selected due to its wide distribution and sensitivity. The horse mussel, *M. modiolus* was selected as a comparison, because of its ecological role in establishing biogenic reefs, which are increasing being classified as special marine feature status and thus require protection. The initial phase of this study presented in this poster investigated the effect of CuO NP in the haemocytes and the gill of *Mytilus edulis* by evaluating DNA effects and cell viability following exposures to CuO NPs, CuO microparticles and copper sulphate, at different concentrations over a 3 day exposure period. This poster will report on the results and will discuss the effects nanoscale copper oxide has on the endpoints studied when compared with larger particulate or salts of the same material. These initial findings indicate that organisms such as marine mussels are potential targets for nanoparticle exposure and these taxa are important in the assessment of any toxicological effects of nanoparticles in the marine environment. Keywords: Copper oxide, nanoparticles, marine systems, marine mussel Session: Aquatic and Terrestrial Ecotoxicology Preference: Poster presentation

**WE076 The influence of titanium dioxide presence in soil on the toxicity of triclocarban towards the earthworm *Eisenia fetida*** R. Conrad, RWTH Aachen University / Dpt of Ecosystem Analysis; A.J. Schneider, RWTH Aachen University / Institute for Environmental Research; H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V); A. Schaeffer, RWTH Aachen University / Chair of Environmental Biology and Chemodynamics; H.M. Maes, RWTH Aachen / Institute for Environmental Research. Titanium dioxide nanomaterials (nano-TiO<sub>2</sub>) and triclocarban (TCC) are both used in personal care products and may therefore have the same entry pathway into the environment. The influence of nano-TiO<sub>2</sub> on the chronic toxicity of TCC towards the earthworm *Eisenia fetida*, and the biochemical responses involved, were investigated. The earthworm reproduction test was carried out according to OECD Guideline 222. In the mixture experiments a TCC concentration series (0 and 42-675 mg/kg) was combined with either 0, 400 or 1000 mg/kg TiO<sub>2</sub> (PC 105, particle size 15-25 nm). Additionally, both substances were tested as single substances. The enzyme activity of glutathione s-transferase (GST) and catalase (CAT) as well as lipid peroxidation (MDA) were measured in 1000 mg/kg PC 105 exposed adults after 28 days. Furthermore, earthworm tissue was examined for apoptotic cells in stained histological cuts. The effect of TCC on the reproduction of *E. fetida* was higher in the mixture test series with the high PC 105 concentration (EC<sub>50</sub> 692 mg TCC/kg) than in the test where only TCC was applied (EC<sub>50</sub> 956 mg/kg) or where TCC was combined with the lower PC 105 concentration (no effect). A difference in the biomass of 40% was observed comparing control and test organisms. The GST activity was significantly lower in worms exposed to the TCC-TiO<sub>2</sub> mixture and to TCC alone compared to control organisms, i.e. up to 32% and 22% lower, respectively. The CAT activity in worms exposed to 168 and 338 mg TCC/kg was significantly lower compared to the control. This was not the case when PC 105 was added. No significant difference in the MDA level was observed. More apoptotic cells were detected in gut epithelium, than in epidermic tissue after TCC exposure, but no difference between the treatments with and without PC 105 were observed. The TiO<sub>2</sub> materials themselves seem to have no effect on the reproduction of *E. fetida*. Low concentrations of PC 105 seem to lead to lower, and high concentrations to higher chronic toxicity of TCC towards *E. fetida*. This might be due to the fact that PC 105 has a stimulating effect on its feeding rate of earthworms leading to higher TCC uptake, and thus, to higher effects. PC 105 seems to reduce the inhibiting effect of TCC on the catalase activity of *E. fetida*. TCC induced cell apoptosis was limited to the inner organs like the gut. From this setup, it can be concluded that TCC causes damage to the worms after being ingested, but not through dermal contact.

**WE077 Titanium Dioxide Nanoparticle Generation of DNA Base Damage Under Controlled Illumination** E.J. Petersen, National Institute of Standards and Technology; V. Reipa, S.A. Rabb, National

Institute of Standards and Technology; B.C. Nelson, National Institute of Standards and Technology / Biochemical Sciences. Titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) are found in numerous commercial and personal care products. Thus, it is necessary to understand and characterize the potential environmental health and safety risks posed by these ubiquitous NPs. It is well known that photoactivated TiO<sub>2</sub> NPs can generate highly reactive hydroxyl radicals (OH) in aerated aqueous solutions and superoxide radical anions (O<sub>2</sub><sup>-</sup>) in non aqueous media. Hydroxyl radicals can interact with and damage various biological molecules, such as DNA, at diffusion limited rates resulting in the formation of oxidatively induced DNA damage. Recent in vitro studies, using the comet assay, have also shown that non-photoactivated TiO<sub>2</sub> NPs (NPs kept in the dark) can also induce significant levels of SSBs. In this work, we utilize stable isotope-dilution gas chromatography/tandem mass spectrometry (GC/MS/MS) to quantitatively characterize the levels and types of oxidatively generated lesions in genomic DNA exposed to TiO<sub>2</sub> NPs (Degussa P25) under controlled illumination conditions. Using calf-thymus DNA and P25 TiO<sub>2</sub> NPs (NIST standard reference material 1898) in phosphate buffered solutions, we demonstrate that TiO<sub>2</sub> NPs incubated with DNA in the dark do not lead to the formation nor accumulation of lesions when tested over 24 h (exposure range = 1 to 100 µg/mL TiO<sub>2</sub>). However, when the same samples are exposed to either visible light from 380 nm to 750 nm (energy dose of ~ 14.5 kJ/m<sup>2</sup> for 30 min) or UVA illumination at 370 nm (energy dose of ~ 10 kJ/m<sup>2</sup> for 30 min), there is a significant (p < 0.0028 for detected DNA bases) formation of lesions at the 100 µg/mL dose for the visible light exposure and a significant (p values < 0.0001 for detected DNA bases) formation of lesions at the 10 µg/mL and 100 µg/mL doses for the UVA light exposure. These findings suggest that commercial P25 TiO<sub>2</sub> NPs do not have an inherent capacity to oxidatively damage DNA bases in the absence of sufficient photoactivation. Electromagnetic radiation within the visible portion of the light spectrum does appear to induce the formation of DNA lesions except at high NP concentrations. Hence it is probable that the source of the DNA strand breaks detected in recent in vitro dark studies that utilize the comet assay is due to an as yet unrecognized cellular strand break promoter or an artefact.

**WE078 TOXIC EFFECTS OF MAGHEMITE NANOPARTICLES COATED WITH DIMERCAPTOSUCCINIC ACID IN ZEBRAFISH AND GUPPY** J. De Souza Filho, University of Brasilia / Departament of Genetics and Morphology; L. R de Sousa, University of Brasilia; W. S Petermele, University Federal of Rondonia / Department of Chemistry; R. Bentes de Azevedo, University of Brasilia / Departament of Genetics and Morphology; C. Grisolia, University of Brasilia / Genetics and Morphology. By 2015, the nanotechnology industry is predicted to be about 1 trillion dollar. Nanotechnology industry development has raised the use of nanomaterials in several fields of science, from medicine up to electronics. Thus, this increased use of nanomaterials have caused concerns due to the potential environmental impacts of nanoparticle residues on aquatic biota. Indeed, nanoparticle pollution is a new type of water pollution. Magnetic nanoparticles, including maghemite, have shown high interest due to their current and future applications in biomedicine. Become important the understanding of its toxicological properties. The aim of this study was to investigate the toxic effects of maghemite (γ-Fe<sub>2</sub>O<sub>3</sub>) nanoparticles coated with meso-2,3-dimercaptosuccinic acid (DMSA-MNP) on zebrafish (*Danio rerio*) and guppy (*Poecilia reticulata*). For genotoxicity assessments, 42 adults of both sexes were exposed for 96 h to MNPT-DMSA at concentrations of 4.7, 9.3, 18.6, 37.2 and 74.4 mg/l. Comet assay (CA), DNA fragmentation evaluated by flow cytometer (F) and Micronucleus test (MN) were performed using peripheral blood samples. Embryotoxicity was investigated through fish embryo toxicity test (FET - OECD, 2006) assay using the same concentrations stated above. For the FET test, healthy fertilized eggs (10 per concentration) to zebrafish were exposed for 5 days using 24 well plates and the number of coagulated eggs, percent of hatching and standard developmental defects were observed daily. Zebrafish and guppy exposed to nanoparticles in concentrations investigated did not present significant difference compared with control when evaluated by CA, FC and MN

assays. At exposure-concentrations of 37.2 and 74.4 mg/l, DNA damages were highest with prevalence of 3 and 4 scores in CA. Upon exposure to DMSA-MNP (74.4 mg/l), embryos showed an increase of 26% in mortality and a decrease of 40% in hatching after 5 days of exposure. Furthermore, microscopic (5x) observation identified abnormalities in the behavior of fish larvae such as lack of balance and no response to external stimuli, indicating a possible neurotoxic effect. The results indicate no genotoxic potential of DMSA-MNP for zebrafish and guppy and possible embryo toxicity at high concentrations. These results are important because the iron nanoparticles are being used in treating diseases like cancer and need to be properly disposed into the environment.

**WE079 Toxicity and subcellular distribution of silver nanoparticles and ionic silver in the terrestrial isopod *Porcellionides pruinosus***

C. Calhoa, CESAM Centro de Estudos do Ambiente e do Mar / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; C. Svendsen, CEH Wallingford / Pollution and Ecotoxicology; S. Loureiro, Universidade de Aveiro / Biology. Silver is a trace element that occurs at low levels in the environment. However, silver contamination has increased due to anthropogenic inputs, such as industrial activities and sewage treatment plants. Silver nanoparticles (Ag NPs) are widely used in nanotechnology industry and consumer products. The usage of these products will ultimately lead to the soil compartment being required studies to predict the potential risks in soils. The woodlouse *Porcellionides pruinosus* (Crustacea) is a suitable model species for the examination of toxic effects following metal assimilation and accumulation because of their extraordinary capacity to accumulate high concentrations of metals from the environment predominantly in their hepatopancreas. In this study, we aim at assessing how the terrestrial isopod *P. pruinosus* accumulates Ag, by comparing its accumulation pattern and compartmentalization upon exposure to AgNO<sub>3</sub> and Ag NPs. For this purpose survival and subcellular distribution were analyzed using the Lufa 2.2 soil as exposure medium for 14 days. The subcellular fractionation was adopted in this study as a tool to test the hypothesis that different forms of Ag (AgNO<sub>3</sub> and Ag NPs) deployed in soil would influence the manner by which this metal is detoxified and stored in cells. Studied fractions were as follows: cellular debris, metal-rich granules (MRG), organelles, heat denatured proteins (HDP) and heat stable proteins (HSP). The organelles, HSP and HDP are considered trophically available fractions (TAM), organelles and HSP are grouped as metal-sensitive fractions (MSF), and HDP and MRG as biologically detoxified metal (BDM). The present study provides valuable information about the mechanisms beyond Ag-NPs and AgNO<sub>3</sub> toxicity and tolerance in the terrestrial isopod *Porcellionides pruinosus* and how it can influence other trophic levels.

**WE080 Toxicity of silver nanoparticles to microalgae: the role of ionic silver**

M.A. Fernandes, CESAM Department of Biology / department of Biology & CESAM; M.F. Jesus, University of Aveiro / CESAM & Department of Biology; S.P. Pereira, University of Aveiro / Depart Biologia Universidade de Aveiro; S.C. Aguiar, University of Aveiro / Department of Biology; A.J. Nogueira, University of Aveiro / Department of Biology & CESAM. Among nanoparticles, silver nanoparticles (AgNPs) are the most widely used in the nanotechnology industry, mainly due to its remarkable antimicrobial capability. Their extensive production allied to their antimicrobial properties has been raising concern about their toxicity to natural ecosystems. The main issue with AgNPs toxicity is centered in their oxidation to the ionic form (Ag<sup>+</sup>). In this work we intended to answer the following questions: - is silver more toxic to algae in the ionic form or as nanoparticles? - can the toxicity of AgNPs to algae be attributed to Ag<sup>+</sup> release? - can L-cysteine reduce Ag<sup>+</sup> and AgNPs toxicity to algae? To assess the comparative toxicity of both AgNPs (uncoated, 91.25±34.93 nm) and Ag<sup>+</sup> as AgNO<sub>3</sub> we used two microalgae species: *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*. Several toxicity endpoints were tested (72h exposure): growth rate, Chl *a* autofluorescence, cell size and complexity (determined by spectrophotometry and through flow

cytometry). Ag<sup>+</sup> release from AgNPs was measured by dialysis; particle size of AgNPs was measured by DLS. Ag<sup>+</sup> was more toxic than AgNPs to *C. vulgaris* and *P. subcapitata* (on a mass basis). Exposure of *C. vulgaris* to high concentrations of Ag<sup>+</sup> (18µg/L) and AgNPs (600µg/L) caused cells agglomeration, i.e., increased cell complexity. Increasing concentrations of Ag<sup>+</sup> or AgNPs caused a peak reduction in Chl *a* autofluorescence of both species. The presence of Ag<sup>+</sup> and AgNPs caused a decrease in cell size of both algae. The decrease in cell size and the increase in cell complexity could lead to self-shading of light and enhance sedimentation rates, which can be translated in deleterious effects on aquatic systems. Oxidation rate of AgNPs was low (0.17%); thus, toxicity of AgNPs to algae cannot be exclusively attributed to Ag<sup>+</sup> release. Addition of L-cysteine at equimolar concentrations eliminated Ag<sup>+</sup> and AgNPs toxic effects on growth rate of both algae.

**WE081 Transcriptomic analysis on silver nanoparticles and fullerene exposed *Chironomus riparius* using RNA-seq; Nanoecotoxicogenomic approach**

J. Choi, School of Environmental Engineering; S. Park, University of Seoul. The manufacture of large quantities of engineered nanomaterials (NMs) may lead to unintended contamination of aquatic ecosystems, however their ecotoxicological impact is still not yet fully understood, much less on sediment dwelling species. As sediment is a final sink for aquatic contamination, to better understand the sediment toxicity of widely used NMs, such as, silver nanoparticles (AgNPs) and fullerene nanoparticles (C60), we conducted global gene expression analysis on the sediment dwelling aquatic midge *Chironomus riparius*. To assess transcriptomic changes, we quantified mRNA expression using high throughput sequencing technologies (RNA-Seq) in *C. riparius* exposed to AgNPs and C60 using Solexa platform. Previously established Expressed Sequence Tags database generated from 454 pyrosequencing were used as reference database. RNA-seq results were covered 90% region of ESTs and the result revealed that expression of 819 transcripts was altered by AgNPs exposure (more than a 2 fold up or down regulation compared to control), whereas 896 transcripts by C60. Further analysis on differential expressed transcripts by the clustering and by gene annotation revealed that 234 and 337 genes were up and down regulated by AgNPs exposure, whereas 158 and 313 genes by C60. These differential expressed genes indicate a broad and complex transcriptomic response to AgNPs and C60 exposure including genes related to oxidative stress, xenobiotic metabolism, energy metabolism, etc. Several DEGs by AgNPs and C60, such as, Catalase, Hemoglobin, Metallothionein, Cytochrome P450, NADH dehydrogenase, Hemoglobin were individually confirmed using PCR. Some analyses are in agreement with RNA-seq (CAT, Hb), whereas some are not (MT). Ecotoxicological relevance of AgNPs-induced gene expression was additionally investigated by correlating their response to higher level effects (i.e. reproduction, development). Overall results suggest that RNA-seq may be successfully applied to non-model and ecologically relevant organisms that do not have an underlying genome sequence assembly to address ecotoxicity of chemicals, including nanomaterials. Additionally, the observed changes in a large set of transcript expression levels are indicative of a complex response to AgNPs and C60, to which an important sediment ecotoxicity model species, *C. riparius* were exposed. This research was supported by Basic Science Research Program through the NRF (2012R1A1A2041679).

**WE082 Trophic interaction between microflora and nematodes in soil after application of activated sewage sludge spiked with silver nanoparticles**

M. Hänsch, C. Emmerling, Universität Trier. Soil microflora reacts sensitive to silver nanoparticles (AgNP) when applied directly to soil in a range of 3.2 – 320µg/kg Ag d.m. (Hänsch and Emmerling 2010). However, the main potential pathway for silver nanoparticles to soil is expected to be contaminated sewage sludge, which may be used as a fertilizer in Germany. From a recent experiment it can be concluded that this will also lead to harmful effects to the soil microflora. In detail, after application of contaminated activated sewage sludge spiked with silver nanoparticles to soil a significant increase in the metabolic quotient (qCO<sub>2</sub>), followed by a significant decrease in

microbial biomass (C) over several months was found. Moreover, PLFA-data suggested that the structure of the microbial community, especially bacteria and fungi, changed. Until now the question remains, how this will contribute to the trophic interaction with representatives of the next higher level, which is for example represented by nematodes feeding on bacteria and fungi. It is hypothesized that AgNP may induce a reliable change in soil food-web. To get further access on this topic we conducted an experiment with five treatments, such as, soil with AgNP (1), soil with activated sewage sludge spiked with AgNP (2), soil with activated sewage sludge spiked with AgNO<sub>3</sub> (3), and two controls, one with activated sludge solely (4) and a total control (5). The amounts of silver concentration in the treatments with AgNP and AgNO<sub>3</sub> was approx. - 55µg/kg d.m.

**WE083 Trophic transfer of differently coated zinc oxide nanoparticles using crustaceans (*Daphnia magna*) and zebrafish (*Danio rerio*)** L. Skjolding, DTU / DTU Environment; M. Winther-Nielsen, DHI; A. Baun, Technical University of Denmark / Department of Environmental Engineering. During the last couple of years the use of nanoparticles (NP) has dramatically increased. Zinc oxide nanoparticles (ZnO NP) have a wide range of applications e.g. in personal care products, paints and semi conductors. A limited number of studies have so far investigated the ecotoxicity of ZnO NP and to our knowledge the bioaccumulation behavior in regards to difference in functionalization of ZnO NP has not been studied previously. In this study, experiments with trophic transfer using *Daphnia magna* as food source for *Danio rerio* was carried out to test if changes in functionalization of ZnO NP would affect the bioaccumulation behavior compared to ZnO NP. *D. magna* was exposed to pristine and functionalized ZnO NP in concentrations considered non-lethal in a 6341 *Daphnia* immobilization test. Bioconcentration studies with *D. magna* included a 24h uptake and 24h depuration phase and it was found that steady state in regards to body burden was reached after 24h uptake. The trophic transfer studies were carried out as 14 d of uptake feeding with pre-exposed *D. magna* and 7 d of depuration feeding with non-exposed *D. magna*. For the trophic transfer studies, 5 d old *D. magna* were exposed to 1 mg/L ZnO NP (ZnO NP and ZnO-C<sub>18</sub>H<sub>17</sub> NP) for 24h before feeding to *D. rerio* at a daily rate corresponding to 8% wet weight of the *D. rerio*. *D. magna* not eaten after 2 h was removed and the resulting exposure loading was corrected for in data treatment. The tested ZnO NP was of same primary size (35 nm) but with different functionalizations (ZnO and ZnO-C<sub>18</sub>H<sub>17</sub>). Characterization included ICP-MS, DLS, BET and TEM. Results show a fast uptake of ZnO NP in *D. rerio* reaching steady state after 5 d of exposure yielding a total body burden (BB) of 887±184 mg Zn/kg dw. A fast depuration ( $k = -0.13 \text{ d}^{-1}$ ) was observed reaching steady state after 3 d of depuration. The calculated BioMagnificationFactor (BMF) was 0.15 with a biological half-life time ( $t_{1/2}$ ) of 5.3 d. In contrast, ZnO-C<sub>18</sub>H<sub>17</sub> showed linear uptake in *D. rerio* during the 14 d of uptake thus not reaching steady state. A total BB of 2169±414 mg Zn/kg dw was observed past 14 d of uptake. However, the depuration rate was faster ( $k = -0.32 \text{ d}^{-1}$ ) compared to ZnO NP. The BMF for ZnO-C<sub>18</sub>H<sub>17</sub> was 0.42 and a  $t_{1/2}$  of 2.2 d. The studies demonstrate the feasibility of conducting bioconcentration and trophic transfer studies with NP and the results indicate that functionalizing of NP may affect the uptake and depuration of NP in aquatic organisms.

**WE084 Uptake of silver nanoparticles by *Danio rerio* eleutheroembryos** S.P. Pereira, University of Aveiro / Depart Biologia Universidade de Aveiro; M.A. Fernandes, CESAM Department of Biology / department of Biology & CESAM; R. Oliveira, Universidade de Aveiro / department of Biology & CESAM; F.T. Jesus, University of Aveiro / department of Biology & CESAM; E.P. Gray, Colorado School of Mines / Environmental Science and Engineering; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; A.J. Nogueira, University of Aveiro / Departament of Biology & CESAM. Silver nanoparticles (AgNPs) are the most commonly commercialized nanoparticles. These particles are mainly designed to release ionic silver (Ag<sup>+</sup>), which is known for its bactericidal potential.

Ag<sup>+</sup> is one of the most toxic metallic forms in the aquatic environment and their uptake by aquatic organisms is widely studied. However, the uptake of Ag by organisms exposed to AgNPs remains fairly unknown. Furthermore, the dissolution rate of AgNPs to Ag<sup>+</sup> can be affected by AgNP's size, capping, among other factors. Given this problematic essential questions arise: - Do AgNPs exert toxicity through Ag<sup>+</sup> only or does the particle itself cause toxicity? - How do different size and particle surface capping agents influence Ag uptake and toxicity in eleutheroembryos? Thus, the aim of this work is to evaluate the effects of size and capping agents of AgNPs on Ag uptake by zebrafish (*Danio rerio*) eleutheroembryos, through quantitative analysis. For comparison purposes, uptake was also evaluated in eleutheroembryos exposed to Ag<sup>+</sup> (as AgNO<sub>3</sub>). Experiments were carried out with an alternative life-stage of zebrafish, eleutheroembryos (72-120h post-fertilization - hpf), and followed the OECD guideline 305 for the bioconcentration assays. The eleutheroembryos were exposed to three types of AgNPs concerning to size and surface capping type, and also AgNO<sub>3</sub>. For each assay about 1000 eleutheroembryos were selected and separated in different tanks: control (Ag-free media), tank with media spiked at the lowest effect concentration and tank with media spiked at the highest effect concentration. Uptake assays had the duration of 48h and sampling was done at 6, 12, 24, 36 and 48h of exposure. Total Ag and AgNPs, present in the exposure media and in the individuals, were quantified and the uptake rate determined. This work is a contribution in the assessment of Ag uptake by fish and highlights the potential toxicity of AgNPs to aquatic organisms.

**WE085 Effects of ionic, micro- and nano-sized iron on *Lepidium sativum* as a reference biological model** G. Libralato, University Ca' Foscari of Venice; A. Costa Devoti, University Ca' Foscari Venice; L. Manodori, I. Micetic, S. Totaro, E. Sabbioni, ECSIN - Veneto Nanotech SCpA; A. Volpi Ghirardini, University Ca' Foscari Venice; F. Groppi, LASA, Università degli Studi di Milano and INFN-Milano; S. Manenti, LASA, Università degli Studi di Milano and INFN-Milano and University of Ferrara. Engineered nanomaterials are at the forefront of ecotoxicologist agendas due to their widespread use in a broad range of industrial and domestic sectors. Actually, they are manufactured in increasing amounts year-by-year. Particularly, nanoscopic zerovalent iron started to be used in various countries around the world for land and groundwater remediation presenting, apparently, encouraging removal rates particularly with organic compounds. Indeed, the use of iron in remediation activities mainly via the Fenton reaction is considered a sort of common practice. Nevertheless, since now few data are available about the potential adverse effects of the release, even if controlled, of iron within the ecosystem. Terrestrial plants may be considered as interesting biological models for assessing its potential impacts. The effect of ionic, micro- and nano-sized iron were compared using *Lepidium sativum* as reference biological model. Germination, elongation and biomass production inhibition were taken into consideration as endpoints. Besides the full physico-chemical characterisation of the selected chemicals, neutron activation offered the possibility to label them in a way that avoids surface modification and permits both localization and quantification within the model organisms. The main results showed the following toxicity trend: nano-sized iron < micro-sized iron < ionic iron.

**WE086 How may we secure experimental data for future use in mechanistic effect modeling of engineered nanoparticles?** A. Palmqvist, Roskilde University / Department of Environmental Social and Spatial Change; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; A. Gergs, Roskilde University / Department of Environmental Social and Spatial Change; B. Nowack, EMPA; H. Selck, Roskilde University; E. Valsami-Jones, University of Birmingham / Division of Environmental Health and Risk Management. Whereas populations, communities and ecosystems are protection goals for Environmental Risk Assessment (ERA) of chemicals, current ERA methods are based on simple individual level effects. The link between individuals and populations is represented by the application of a safety factor, which is considered to guarantee a protective risk assessment. In

recent years it has become apparent that such simplified RA methods may not provide the measures needed for value relevant RA, and mechanistic effect models (MEMs) may provide a more sophisticated and ecologically relevant tool for ERA of chemicals. Therefore, the primary aim of the present work was to provide information on data requirements for parameterizing MEMs that can eventually be used for nanoparticle (NP) ERA as well as to pinpoint current data gaps. A literature review for data on Ag-NP toxicity to parameterize selected MEMs showed a general scarcity in applicable data for modelling NP toxicity as well as in data for estimating the necessary realistic environmental exposure scenarios. Most available Ag-NP effect data represent short-term exposures measuring sub-individual level endpoints and lethality as the main individual level endpoint. A few available studies on long term exposure and sub-lethal effects measured only effects at the end of the exposure. For MEMs, effect data obtained during longer exposure periods, several census times and endpoints such as growth and reproduction in addition to lethality are needed. We advocate that slight changes to experimental testing procedures coupled with more details in data reporting may provide NP toxicity data that can be used directly to produce MEMs. Though there are NP specific challenges in producing such data, e.g., keeping exposure concentrations constant during exposure, changes in NP sizes and properties over time and characterizing NPs in complex media (e.g., sediment), we nevertheless believe it is necessary to enhance testing procedures to help address risk of NPs for ERA protection goals. Even if data availability for engineered NP is not currently at a stage where it is realistic to include MEMs in nanoparticle ERA, experimental tests and data reporting should still be performed such that results can eventually be used for and implemented in e.g., MEMs. This can be regarded as securing the data for future purposes, and even without the data integration in MEMs more relevant information may be obtained from improved testing procedures.

**WE087 Regulatory ecotoxicity testing of engineered nanoparticles: are the results relevant to the natural environment?** S. Park, Environment Department; J. Woodhall, Food and Environment Research Agency; J.G. Veinot, University of Alberta / Dept of Chemistry; M. Cresser, University of York; A. Boxall, University of York / Environment Department. Due to increasing use of ENPs, the release of ENPs into the aquatic environment is inevitable. Concerns have therefore been raised over the potential risks of ENPs in the environment and a wide range of acute and chronic ecotoxicological studies have been performed to better understand. The speciation of NMs in these standardised media may be very different from the speciation that occurs in the natural environment so the results from standardised ecotoxicity experiments could therefore have little relevance to toxicity in the natural environment. This study was therefore performed to explore the behaviour of a set of model gold ENPs with different surface functionality (amphoteric, negative, positive and neutral) in a range of standardised test matrices and to determine whether behaviour in standard tests reflects the likely behaviour of these ENPs in the real environment. The effects of the presence/absence of test organisms and the addition of humic acid (HA) to the standardised media and behaviour in natural waters were also explored. No significant increase in mean size of the positive and neutral charged particles was seen in any of the test media while great aggregation was shown in natural waters. Limited aggregation of amphoteric and negative charged particles were seen for the majority of the natural waters tested whereas, they were found to aggregate in the standardised media. The addition of test organisms significantly reduced the mean size of amphoteric charged particle. The presence of HA significantly reduced the aggregation of the amphoteric charged particles and significantly increased the aggregation of the positive charged particles but had limited impact on the stability of the negative charged particles. This study shows ENPs had very different behaviour in various types of standardized test media depending on the particle types, presence of test organisms and HA, and in natural waters. The mismatch between behaviour of ENPs in standard media and natural systems raises questions around the relevance of standard ecotoxicity experiments for

use in the risk assessment of ENPs. A better understanding of the behaviour of ENPs and the factors affecting behaviour in the environment is therefore needed. In the future, consideration should be given to development of standardised ecotoxicity media which better reflect the behaviour of ENPs in natural system, this would allow the real risks of ENPs to the environment to be determined.

**WE088 Risk assessment of one specific silver nanoparticle in the sewage sludge pathway** K. Schlich, Fraunhofer IME / Ecotoxicology. The rising use of silver nanoparticles (AgNP) in commercial products increases the potential for environmental contamination and adverse effects. AgNP will inevitably reach the environment, especially the terrestrial environment within the sewage sludge pathway, and there is a need to gather detailed information about their fate and behaviour within soils. The main goal of this study was to formulate a risk assessment of the AgNP NM-300K (from the OECD Sponsorship Programme) for the sewage sludge pathway. For this purpose the study was divided in two steps. In step one, the potential effects of pure AgNP on the terrestrial ecosystem (soil microorganism, plants, earthworms) were determined using standardised test guidelines. In step two, sewage sludge was spiked with AgNP via a simulation of a wastewater treatment plant over ten days. The sludge was added to soil in accordance with the German sewage sludge ordinance (5 t dry matter sludge per ha in three years) and the long term effects on soil microorganism over 140 days were observed. The same test guidelines were used from test one. All tests were performed with silver nitrate as a reference. Biological measurements were done after 32, 60, 100, and 140 days. Results from the first step demonstrated that soil microorganisms, especially nitrifying bacteria, were extremely sensitive to 28 days exposure to AgNP (EC<sub>50</sub>: 0.5 mg/kg dry matter soil; NOEC < 0.56 mg/kg dry matter soil). AgNP were less toxic to microbial biomass, earthworms, and plants compared to nitrifying bacteria. The observed effects of AgNP and silver nitrate were similar in soil. Results from step two indicated that most of the AgNP were located in the sewage sludge. Sewage sludge containing AgNP was added to soil and incubated at 20°C for 140 days. This resulted in a degradation of the sludge and sorbed AgNP became bioavailable. The first adverse effects on soil microorganisms occurred after 60 and 100 days. Adverse effects on soil microorganisms that occurred after 140 days were similar to those of step one. Based on this comparison of effects, the risk assessment for the sewage sludge pathway was implemented using the results from step one (tests with pure AgNP). A PNEC of 50 µg/kg dry matter soil for NM-300K was calculated. This resulted in a PNEC for sewage sludge of 30 mg/kg dry matter sludge, calculated on the basis of the German sewage sludge ordinance.

**WE089 A mathematical model and ecotoxicity reflecting the fate of nanoscale zero-valent iron (NZVI) particles in soil complex media** L. Casasus, Universidad Politecnica de Madrid; G. Costa, L.T. Ortiz, M. Rodriguez-Membibre, M. Nande, Universidad Complutense de Madrid; M. Martin, Universidad Complutense Madrid. Anthropogenic contamination of soils by heavy metals, which threatens human life and the environment, occurs from many sources such as mining, atmospheric deposition, application of sludge and mineral fertilizers and pesticides. The use of nanoscale materials (i.e. nanoscale particles) is a new approach to environmental remediation technologies that could provide solutions to some of the most challenging environmental cleanup problems. Knowledge of the physical behavior of the nanoscale particles in soils would improve the use of these nanomaterials. Thus, the development of mathematical models could be useful to explain the fate of nanoscale particles in the complex soil matrix and furthermore, its effects on soil-dwelling organisms. In this work, a mathematical model on behavior in soils matrices is presented. We construct a mathematical formulation of the fate of nanoparticles in a complex medium (soil), taking into account diffusion, advection, degeneration and chemical reactions with heavy metals present in the soil. Particularly, we pay attention to the effects of different fluxes of water pumped into the soil. The consistence of the model has been validated with laboratory experiments. In addition, we have investigated the effects of leachates

collected from columns on the soil-dwelling nematode *Caenorhabditis elegans*, an useful biomodel for soil and groundwater toxicity tests. To perform the experiments, columns containing control or heavy metal contaminated soils with or without NZVI, have been used. The calculated porosity of the soils ranged between 45-95%. Columns were subject to a six hours continuous water flow each day. From the collected leachates, the conductivity, pH and NZVI concentration were measured. We have analyzed the effects of leachates on reproduction of age-synchronized gravid adults. The results show that this toxicity endpoint was seriously affected by leachates containing heavy metals but after 48 h, the presence of NZVI in the soil reversed the effect.

**WE090 An experimentally derived conceptual approach for risk assessment of metal nanoparticles** K. Syberg, G. Banta, Roskilde University / Department of Environmental, Social and Spatial Change; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; F. Khan, Natural History Museum / Department of Zoology; S. Luoma, University of California at Davis / John Muir Institute of the Environment; H. Selck, Roskilde University; E. Valsami-Jones, University of Birmingham / School of Geography, Earth and Environmental Sciences. The production and use of engineered nanoparticles (ENPs) is rapidly growing within several different industrial sectors. One of the distinctive characteristics of ENPs is that they have unique physical/chemical properties compared to their non-nano metal counterparts. Whether these unique properties can lead to different toxic properties is largely unknown at this point, but some studies indicate that it might be the case in some scenarios. The unique properties along with the expansive use have led to concerns that the production and use of ENPs might pose uncontrolled risks to humans and the environment. It is therefore important to establish risk assessment paradigms that can assess the potential risks from ENPs in a scientifically sound manner. This study proposes a conceptual approach for risk assessment of ENPs. The approach is made as modifications to existing metal risk assessment frameworks, based on a series of experimentally derived conclusions concerning toxicity and bioavailability of metal ENPs. The underlying experimental work was conducted over four years by ten different scientific groups under the NanoReTox cluster (7th Framework Programme, Grant Agreement no. CP-FP 214478-2), and covers synthesis, characterization, human toxicity, ecotoxicity and bioavailability in different media of several different metal ENPs. Seven conclusions regarding nano-specific risks were made based on this experimental work. These conclusions subsequently formed the foundation for the conceptual nano risk assessment approach proposed in this study. The conceptual approach deals with nano-specific aspects that should be specifically assessed. It furthermore offers an approach to handle the high amount of uncertainty that is associated with assessing risks for ENPs and other new and emerging technologies.

**WE091 Assessing the risk of artificial iron-(III)-oxide nanoaggregates used for groundwater remediation to microbial and nematode communities** S. Höss, Ecossa; L. Bleick, University of Bielefeld; G. Pilloni, C. Meyer, Helmholtz Center Munich; A. Fritzsche, Friedrich-Schiller-University Jena; T. Lueders, Helmholtz Center Munich; W. Traunsperger, University of Bielefeld. In the joint research project NanoSan a new treatment technique for the remediation of contaminated groundwater is validated. Artificial ironoxide nanoaggregates (FeOx-NA) are used to stimulate the degradation of BTEX by *in-situ* bacteria. As a considerable amount of FeOx-NA have to be transferred into the aquifer for the purpose of remediation, the risk of FeOx-NA for groundwater relevant organisms had to be assessed before the new technique could be applied in the field. To assess the risk of FeOx-NA on nematodes and microorganisms that were used as representatives of groundwater organisms, the toxic effects of various FeOx-NA were tested on single species (*Caenorhabditis elegans*, *Vibrio fischeri*) and at community level in microcosms. Results of single species tests showed that the nematode *C. elegans* was more sensitive to FeOx-NA (minimal EC50 for nematode reproduction: 0.064 mM Fe) than bacteria (minimal EC50 of *V. fischeri*: 1.1 mM Fe), while the

toxicity ranking was different for nematodes and bacteria. The use of hypersensitive knockout-strains of *C. elegans* revealed that oxidative stress could partly explain the inhibitory effects on reproduction. However, FeOx-MA ("bulk iron-oxide") were comparably toxic to *C. elegans* than nanoaggregates, indicating that the effects are more dependent on the type of iron oxide rather than on the size of the aggregates. Natural FeOx-NA also exhibited similar effects on *C. elegans*, suggesting that the organisms might not be additionally harmed if artificial FeOx-NA are introduced into aquifers. Column experiments with sediments from the headwater of the river Ems showed that the *in-situ* microbial community changed in species composition after exposure to increasing concentrations of FeOx-NA, while *C. elegans* was not affected by the sediments even at the highest FeOx-NA concentrations. To compare effects of FeOx-NA and MA on natural microbial and nematode communities, microcosms with Ems sediment were set up in which nematodes and bacteria could be investigated simultaneously over a period of 3 month. First results showed clear effects of both NA and MA on the bacterial biomass (measured as ATP). In the ongoing microcosm experiment, effects on nematode community structure (composition of species and feeding types; NemaSPEAR) and the bacterial community structure (analyzed with a T-RFLP/Pyrotag approach) will be compared and discussed with regard to the risks of FeOx-NA on groundwater organisms.

**WE092 Toxicity of silver nanoparticles to 8 different bacteria** M. Matzke, Centre for Ecology Hydrology NERC / Hails Section; D. Read, NERC, Centre for Ecology and Hydrology / Hails Section; C. Taylor, K. Jurkschat, University of Oxford / Department of Materials; C. Svendsen, CEH Wallingford / Pollution and Ecotoxicology. Nanoparticles (NPs) display special chemical properties because of their size, shape, composition and electronic structure. These properties lend NPs their functionality, but may also lead to toxic effects. Due to their widespread use in consumer products an exposure of the environment to NPs is anticipated and already proven in first analytical surveys. Especially metal/metalloride NPs are widely used, of which silver nanoparticles (AgNPs) have gained considerable attention due to their broad microbicidal properties implying a specific hazard for exposed (environmental) bacteria. In order to identify suitable test species for the hazard assessment of AgNPs, we determined the toxicity of two AgNPs (3-8 nm nominal size, uncoated and 50nm size, PVP coated) for a selection of 8 different bacteria. Silver nitrate (AgNO<sub>3</sub>) was tested as a reference to distinguish between particle and Ag<sup>+</sup> ion related effects. The following bacterial species were selected based on their occurrence in different habitats as well as their belonging to the gram-negative and gram-positive subdivision: *M. luteus*, *E. coli*, *J. lividum*, *B. megaterium*, *B. subtilis*, *P. stutzeri*, *P. fluorescens* and *C. metallidurans*. *C. metallidurans* was added to the test series as it is known to be very resistant to a broad range of heavy metals. All studies were accompanied by chemical analytics: TEM, graphite furnace AAS (total silver and dissolved silver (ultrafiltration), Nanoparticle Tracking analysis (NTA) to determine the particle size distribution (NanoSight system) and differential centrifugation (particle size and size distribution). Different sensitivities towards AgNO<sub>3</sub> and the two different particles were recorded for the different species with no obvious pattern in relation to their subdivision and/or habitat. In general AgNO<sub>3</sub> was one order of magnitude more toxic than the particles with one exception: EC values for *M. luteus* were differing by several orders of magnitude between AgNO<sub>3</sub> (0.01 µg/L) and the 3-8 nm, uncoated AgNPs (1100 µg/L). Surprisingly *C. metallidurans* reacted very sensitive towards the silver exposure with EC values between 10 and 66 µg/L. For most of the species the PVP coated particles produced less pronounced effects than the uncoated ones supporting the thesis that the Ag<sup>+</sup> ions are the toxicity driving factor. Further studies are necessary to identify the causes for the differences in species sensitivity.

**WE093 Surface plasmon resonance for the detection of non-metallic nanoparticles** n. hma salah, University of Plymouth; D. Jenkins, L. Panina, Plymouth University; R. Handy, University of Plymouth / School of Biomedical and Biological Sciences. Surface plasmon

resonance (SPR) is an established technique for label free sensing of bio-molecular species, including time-dependent reaction analysis. The typical Kretschmann configuration used requires a prism coated with a thin Au film of thickness less than 50 nm, and this is highly sensitive to the localised permittivity at or near to the interface. At present, the focus of SPR research appears to be dominated by bio-molecular sensing, with a strong emphasis on real-time monitoring of proteomic interactions. In Plymouth, environmental toxicology research is concerned with the detection and characterisation of nanoparticles (NP) that are environmentally hazardous; such as silver or titanium dioxide NPs. In the work presented here the aim is the detection of non-metallic particles and nanoparticles, i.e. particles with no free charges. There are many different commercial products that use titanium dioxide nanoparticles. Such particles, inevitably, will be transported into the natural environment and here arises the need for detection methods for NPs in complex samples. This is also to support both environmental and human health risk assessments. The authors have previously detected the presence of TiO<sub>2</sub> of 25 nm and 120 nm diameter NPs, using interferometry. Here the spectral-phase interference method with a simple microscope glass substrate as a sensor interface was used to characterise 5% solutions of TiO<sub>2</sub> NPs. Experiments showed that the signal strength was larger for solution with smaller particles, and that the sensor output goes to saturation much faster for smaller NPs. Whilst this work was encouraging, an alternative system is required for portable environmental monitoring. This study encompasses the development of an SPR system utilising a glass prism, coated with a Cr/Au plasmonic layer to which a functionalised layer, such as graphene, carbon nanotubes is added, to enhance the affinity of the surface in the presence of TiO<sub>2</sub> NPs. SPR is sensitive to small changes in the local permittivity, which is the case when TiO<sub>2</sub> is present. This phenomenon is used to explore the potential to create a portable instrument. In this paper modelling of SPR under these conditions will be presented, and based upon this theory the LSPR condition for self-sensing will be investigated theoretically and experimentally.

**WE094 Lethal and sublethal toxicity of six nanoparticles to freshwater primary producers and consumers.** L. Lopes, University of Aveiro / CESAM. Engineered nanoparticles (NP) possess unique properties that enable their use for a wide variety of applications. Consequently, within the last years the use of NP in consumer products increased considerably. This widespread use of NP in the society foresees their inevitable release into the aquatic environment, where they can constitute a risk to aquatic biota. Accordingly, this study aimed at investigating the lethal and sublethal effects of six NP (vesicles of sodium dodecylsulfate-dodecyltrimethylammonium bromide SDS/DDAB, vesicles of monoline/sodium oleate Mo/NaO, gold rods NM-Au, quantum dot Lumidot<sup>TM</sup> CdSe/ZnS 530, TiO<sub>2</sub>, and TiSiO<sub>2</sub>) to the green algae *Pseudokirchneriella subcapitata* (primary producer) and to the rotifer *Brachionus calyciflorus* (primary consumer). These two species constitute important model organism that have long been used in ecotoxicology studies, as they are very sensitive to chemical contamination and are representatives of different trophic and functional levels. To attain the main goal of this work, each test species was exposed to serial dilutions of the six NP plus the corresponding control (media without NP) for 72h (algae), 24h (lethal effects; rotifer) and 48h (sublethal effects; rotifer). At the end of the assays the population growth rate was calculated for both species and percentage of mortality was computed for the rotifers. In order to characterize NP exposure, zeta potential, hydrodynamic diameter and surface charge were determined by light scattering techniques and the NP size was determined by transmission electron microscopy. All NP formed aggregates when suspended in the aqueous media. Except for Mo/NaO, the size of the NP-aggregates was higher in the rotifer media, comparatively with the medium used for algae assays. The NP of TiO<sub>2</sub> and TiSiO<sub>2</sub> did not exert toxic effects to the rotifer or to the green algae, even at high concentrations (49 mg/L), which is in agreement with data already reported in the literature for Ti based NP. For the other four NP, *B. calyciflorus* exhibited a higher sensitivity than *P. subcapitata*, as in general rotifers mortality occurred at concentrations below those that

induced sublethal effects in algae: LC<sub>50</sub> for rotifers were 0.39 mg/L (SDS/DDAB), 17.27 mg/L (Mo/NaO), 50.24 µg/L (NP-Au), and 3.14 mg/L (QD-CdSe/ZnS), and EC<sub>50</sub> for *P. subcapitata* were 4.54 mg/L (SDS/DDAB), 42.54 mg/L (Mo/NaO), 59.56 µg/L (NP-Au), and 7.86 mg/L (QD-CdSe/ZnS). No association between toxicity and NP size was observed for both tested species.

**WE095 Investigation of toxicity of MWCNT of different functional groups and sizes using in vitro and in vivo (C.elegans) tests.** J. Choi, School of Environmental Engineering. Carbon nanotubes (CNTs) have attracted a great deal of attention due to their unique structural, physical and chemical properties that lend their use to a variety of industrial and biomedical applications. However, there are concerns that the properties of the CNTs might lead to adverse health effects and various factors, such as, tube size, state, functional groups and impurities may contribute to varying toxicological effects of CNTs. Therefore, in this study, toxicity of multiwall carbon nanotube (MWCNT) with functional groups and sizes was compared using HepG2 cells cytotoxicity test and the nematode *Caenorhabditis elegans* survival test, subsequently their mechanism of toxicity was investigated using proteomics and functional mutant analyses. To identify the effect of functional group on toxicity of MWCNT, toxicity of pristine, OH- and COOH-MWCNT was compared. To test the effect of sizes on toxicity, MWCNT with small outer diameter (OD) was compared to that with big OD similarly, MWCNT with short tube length was compared to that with long tube. It was found that functionalized MWCNT was more toxic than pristine MWCNT in *C. elegans* survival test and OH-MWCNT was more toxic than COOH-MWCNT in HepG2 cells cytotoxicity test. MWCNT with small OD is more toxic than that with big OD and short MWCNT is more toxic than long tube in both cytotoxicity and survival tests. Toxic intensity of MWCNT in HepG2 cells was found to be correlated with the hydrodynamic diameters of MWCNT in test media measured with DLS. In the next step, to understand the intrinsic toxicity of MWCNT, proteomics followed by pathway analysis was conducted on pristine MWCNT exposed *C. elegans*. Differentially expressed proteins (DEPs) and pathway analyses revealed endocytosis, phagocytosis and ER stress being involved in MWCNT toxicity. To further confirm this, survival test was conducted on *C. elegans* mutants of the genes detected in stress response pathways raised in proteomics-pathway analyses. Mutants were exposed to MWCNTs with different functional groups and sizes. The results suggest small OD with short OH-MWCNT is the most toxic among tested MWCNTs and ER stress and endocytosis being involved in MWCNT toxicity. Overall results suggest that toxicity of MWCNT is dependent on its functional groups and sizes. The study also suggests integrated approach seems to provide comprehensive insight to understand toxicity of chemicals, of which toxicity is poorly understood, such as, MWCNT.

**WE096 Operationalization and application of “early warning signs” to screen nanomaterials for harmful properties.** S.F. Foss Hansen, Technical University of Denmark / DTU Environment. In 2001 the European Environment Agency (EEA) published a report that analyzed 14 cases of technological developments that later on turned out to have negative side-effects and they identified 12 “late lessons” for current and future policy-makers to have in mind when initiating new technological endeavors. This paper explores how the first lesson - “Acknowledgement and respond to ignorance, uncertainty and risk in technology appraisal” could be applied to screen nanomaterials. In cases of ignorance, uncertainty and risk, the EEA recommends paying particular attention to important warning signs such as novelty, persistency, whether materials are readily dispersed in the environment, whether they bioaccumulate or lead to potentially irreversible action. Through an analysis of these criteria using five well-known nanomaterials (titanium dioxide, carbon nanotubes, liposomes, poly(lactic-co-glycolic acid) and nanoscale zero-valent iron, and carbon nanotubes), it was found that only nanoTiO<sub>2</sub> fulfills all the five criteria. Dependent on the length of the nanotubes, carbon nanotubes fulfills 3 or 4 criteria whereas liposomes, poly(lactic-co-glycolic acid), nanoscale zero-valent iron fulfills only one criteria.

Finally, we discuss how these warning signs can be used by different stakeholders such as nanomaterial researchers and developers, companies and regulators to design benign nanomaterials, communicate what is known about nano-risks and decide on whether to implement precautionary regulatory measures. **[n]**

**WE097 Species sensitivity distributions for silver and zinc oxide nanoparticles: using taxonomic and trait-based risk assessment approaches** **S. Loureiro**, Universidade de Aveiro / Biology.

Nanotechnology industry has been developing potential materials and applications for the welfare and human benefit, resulting in the large scale production of nanoparticles (NPs). The proliferation of manufactured NPs and their consequent entrance in the environment has led to concerns about their potential effects on organisms. Silver and zinc oxide nanoparticles (Ag-NPs and ZnO-NPs) are two metal-based NPs forms that are currently in widespread use (e.g. as antimicrobial agent and in personal care products). This work aims to investigate how well and with what modifications species sensitivity distribution (SSD) methodology can be applied to address the ecological risk of silver and zinc oxide NPs towards aquatic and terrestrial organisms and the estimation of protective environmental limits. Toxicity data was gathered from the published literature and from results obtained within the EU-funded NanoFATE project. Applying SSDs the hazardous concentrations at 5% (HC<sub>5</sub>) were estimated through two different approaches, using the geometric mean effect concentrations (EC<sub>50</sub>, EC<sub>10</sub>) for the Ag-NPs and ZnO-NPs for 1) each taxonomic group and also 2) considering the relevant functional role or physiological traits of the test species. The results analysis also took into account how possible patterns resulting from effects of size and charge of the NPs, media properties, amongst others can be taken into account.

**WE098 Reproductive toxicity of progestogens – norethindrone and progesterone inhibit vitellogenesis** **C. Berg**, Dept of Environmental Toxicology; M. Safholm, Uppsala University / Dept of Environmental Toxicology. Aquatic wildlife is exposed to several different progestogens present in surface waters. We recently showed that environmental concentrations of the progestin levonorgestrel disrupt oogenesis by inhibiting vitellogenesis in the *Xenopus tropicalis* model frog. Information on the effects, potency and modes of action of progestogens in the environment is needed in order to understand the risk for mixture effects of these compounds. The objectives of the present study were to 1) determine the effects of environmental concentrations of norethindrone (NET) and progesterone (P4) on the full cycle of oogenesis, 2) elucidate potential modes of action by analyzing estrogen synthesis. Female *Xenopus tropicalis* were exposed to environmental concentrations of NET (0, 1, 10, 100 ng/L), or P4 (10, 100 ng/L) via the ambient water for 28 days, after which the full cycle of oogenesis, aromatase activity in brain and ovaries, and the morphology of reproductive organs and secondary sex characteristics were analyzed. Both test substances caused reduced relative gonadal weight, increased proportions of previtellogenic oocytes and reduced proportions vitellogenic oocytes compared with the controls, indicating inhibited vitellogenesis. The effects were ascertained also at the lowest tested concentrations. None of the test substances caused an effect on aromatase activity or on secondary sex characteristics. The results indicate that inhibitory effects of the progestogens on oogenesis did not involve reduced estrogen synthesis. We conclude that the vitellogenesis is a sensitive target for progestins and that oogenesis is at risk in wild amphibian populations exposed to this type of compounds.

**WE099 Effects of dietary exposure of polycyclic musk HHCB on *Xenopus laevis* metamorphosis** **M.V. Pablos**, Department of Environment; M.A. Jimenez, The Complutense University / Histology Laboratory; L. San Segundo, INIA National Institute for Agricultural and Food Research and Technology / Department of the Environment; **B. Eulalia M.**, INIA / Environmental; F. Martini, C. Fernandez, Spanish National Institute for Agricultural and Food Research and Technology (INIA). HHCB (galaxolide®) is a synthetic musk used extensively as a fragrance in many consumer products (laundry detergents, soaps,

perfumes, air fresheners, etc). Environmental concern regarding the use of these compounds has arisen due to the persistency and frequent isolation of these chemicals in aquatic and marine compartments as a result of wastewater stream contamination, and also due to the high potential for bioconcentration in living tissues such as blood and human milk. Consequently, synthetic musks, together with other personal care products, have been classified, in the last years, as emergent pollutants. The ecotoxicological information available for HHCB contemplates exposure via water, but the physicochemical characteristics of this compound (limited water solubility and strong adsorptive properties) substantiate that this compound can be frequently adsorbed to particulate matter. The goal of this study was to assess the effects of a dietary exposure to several environmental relevant concentrations of HHCB adsorbed to food during metamorphosis of *Xenopus laevis*. The potential effects of this synthetic musk on diverse endpoints such as development stage and time to metamorphosis, wet weight, length, and gonad and thyroid histology are discussed in detail. This work was funded by the Spanish projects CTM2010 19779-C02 and RTA2010-00004-C02-01. Laura San-Segundo was funded by a fellowship from the INIA.

**WE100 Evaluation of the effects of Benzophenone-3, AHTN and BDE47 on *Xenopus laevis* embryos at organism and molecular levels.** **L. San Segundo**, INIA National Institute for Agricultural and Food Research and Technology / Department of the Environment; **B. Eulalia M.**, INIA / Environmental; M. Pablos, INIA. Benzophenone-3 (BP3) is used as an ingredient of many sunscreens to provide protection against both UVA and UVB radiation. AHTN (Tonalide®) is a synthetic fragrance in cosmetics and detergents. 2,2',4,4'-tetrabromodiphenyl ether (BDE47) is used as a flame retardant and in printed circuit boards among others. These three substances are considered emerging environmental contaminants which can produce adverse effects in humans and wildlife. We evaluated the effect of exposure to BP3 (0.1, 1, 10, 100 µg/L), AHTN (4.8, 24, 120, 600 y 3000 µg/L) and BDE47 (10, 100, 1000 µg/L) on *Xenopus laevis* during the embryonic development. The effects were assessed in terms of survival, deformities and mRNA expression levels of genetic markers involved in general stress responses (hsp70, hsp47 and crf), oxidative stress (cat), apoptosis (p53) and differentiation and lipid accumulation (ppar?). Survivor and deformities observations were made at 24, 48, 72 and 96 hours of exposure (hoe). However, the expression levels of selected genes were measured at 24 and 96 hoe by combining reverse transcription (RT) and real-time quantitative polymerase chain reaction (qPCR). No significant differences on survival and deformities rate between controls and BP3 and BDE47 treatments were found. Significant differences were observed on deformity rate at 48 (+17%) and 72 (+34%) hoe and on survival at 96 (0%) hoe between embryos exposed to the highest concentration of AHTN and controls. After 24 hoe and relatively to control values, significant differences in expression levels were found for ppar at the highest concentration tested of BDE47 and AHTN and at 10 µg/L BP3, for crf and p53 at 100 µg/L BP3. After 96 hoe and relatively to control values, significant differences in expression levels were found for crf and p53 at the highest concentration tested of BDE47, for hsp47 at 10 µg/L BDE47 and BP3, for ppar at 120 µg/L AHTN and for hsp70 at 600 µg/L AHTN. In these cases, the fold change values were between 0.5 and 1.8, except for the expression of HSP70 that was up-regulated approximately 4-fold in the embryos exposed to 600 µg/L AHTN in comparison to control group. Heat-shock-induced expression denoted a stress response to AHTN at a concentration lower than the concentration that caused developmental toxicity. This work was supported by the projects CTM2010 19779-C02 and RTA2010-00004-C02-01. LSS was funded by a fellowship from the INIA.

**WE101 Blood cell responses of *Lithobates catesbeianus* to zinc, cadmium and copper during the larval phase** **H. Utsonomiya**, Universidade Federal de São Carlos; T. Pasquoto, Universidade Federal de São Carlos; M.J. Costa, Federal University of São Carlos UFSCar Soroca / Department of Biology; **M.N. Fernandes**, Universidade Federal de São Carlos / Ciências Fisiológicas; C.S. Carvalho, Universidade Federal de São Carlos; J.B. Fernandes, Universidade Federal de São



Carlos / Quimica. The assessment of the biological responses of aquatic vertebrate species is frequently employed to monitor water pollution, as it provides significant information on bioavailability and environmental concentration levels of pollutants. We investigated the changes in periphirc blood variables of *Lithobates catesbeianus* tadpoles after exposure to Cd, Cu and Zn, Cd+Cu, Cd+Zn, Cu+Zn and Zn+Cd+Cu. Control group was not exposed to metals. After 48 h exposure, the hemoglobin levels increased (22%,  $p < 0.05$ ) in tadpoles exposed to Cd and Cd+Zn and a significant increase ( $p < 0.05$ ) in the red blood cells (RBC) were observed in the groups exposed to Cu (74%), Cd (50%), Zn+Cd (55%), Cd+Cu (42%) and Zn+Cd+Cu (50%) compared to the controls. However, the MCH (mean corpuscular hemoglobin) decreased significantly from 20 to 40% in tadpoles exposed Cu, Cd+Zn, Cd+Cu and Zn+Cd+Cu. After 16 days of exposure, the RBC increased 73% in the group exposed to Cd ( $p < 0.05$ ). The MCH decreased (30%) in tadpoles exposed Cd and decrease from 30 to 45% in the animals exposed to Zn+Cu, Zn+Cd and Cu+Cd. These results showed that Cd, Cu and Zn (singly and/or associated) have different effects on the red blood cell variables evidencing different tadpoles sensitivity to them. The increased hemoglobin levels suggested an adjustment to increase the O uptake indicating that larval *L. catesbeianus* population exposed to metal may have their health status compromised in chronic exposure conditions. Financial support: FAPESP

**WE102 Effects of methyl-parathion on the heart function of bullfrog tadpoles** L. Ribeiro, Federal University of Sao Carlos (UFSCar) / Department of Biology; R. Salla, L. Alves, F. Gamero, Federal University of Sao Carlos (UFSCar); F. Abdalla, UFSCar / Biology; E. Silva-Zacarin, UFSCar; M.J. Costa, Federal University of Sao Carlos UFSCar Soroca / Department of Biology. In the last decades, it has been observed an alarming amphibian decline worldwide. Considering that Brazil is in the fourth position in the world ranking of countries that most use agrochemicals, the aim of this work was to evaluate the effect of an acute exposure (48 h) to methyl parathion (MP - 10 ?M) over the heart function of bullfrog tadpoles. During the exposure (static system/assayed in triplicate), tadpoles' activity level (AL - %) was determined. After the exposure, the heart rate ( $f$  - bpm), and the relative ventricular mass (RVM - %) were determined. Furthermore, ventricle strips were mounted for isometric contraction recordings in order to determinate the twitch force ( $F_c$  - mN.mm<sup>-2</sup>), the times to peak tension (TPT - ms) and to half relaxation (THR - ms). The analysis of the results indicated a reduction of the AL on the exposed group ( $7 \pm 2$  % of active animals) compared to the control group ( $29 \pm 4$  % of active animals). In contrast, the  $f$  of the MP group ( $79.5 \pm 0.6$ ) was higher than that of the CT group ( $66.8 \pm 2.8$ ). The reduction of the AL in the exposed animals seems to be correlated with the fact of MP inhibits the activity of acetylcholinesterase enzyme, responsible for acetylcholine hydrolysis, causing the subsequent reduction of the NA in the animal. However, such effect could not be observed in the heart, considering that at 25 Gosner stage, the animals do not have cardiac cholinergic innervation. Moreover, the *in vitro* experiments showed that the positive chronotropism observed in response to the MP exposure seems to be related with an acceleration in the cardiac dynamics, as can be evidenced by the reduction of TPT and THR duration of ventricle strips of MP group animals towards CT group. Additionally, the  $F_c$  developed by ventricle strips of MP group was lower than that observed to its control, what can be correlated to the reduction ( $P < 0.05$ ) in RVM in MP-group, decreasing systolic volume. These findings suggest that MP has an effect on the mechanisms responsible for calcium transportation through sarcolemma. The tachycardia seems to correspond to a homeostatic adjustment to avoid abrupt decrease in cardiac output due to the negative inotropic effect of MP-exposure. As a consequence, the xenobiotic detoxification and/or other physiologic adjustments needed to permit the development of metabolic increments to confront such adverse conditions would not be impaired.

**WE103 Histological and immunohistochemical study of several organs, in natural populations of the Perez's frog, *Pelophylax perezii* (Seoane, 1885)** G. Lopes, University of Porto / ICBAS-Instituto de

Ciências Biomédicas de Abel Salazar; B. Santos, University of Aveiro; A. Faustino, University of Porto / ICBAS-Instituto de Ciências Biomédicas de Abel Salazar; N.F. Santos, University of Aveiro / Biology; F.H. Caetano, B.F. Pereira, Universidade Estadual Paulista Júlio Mesquita Filho / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; R. Ribeiro, Universidade de Coimbra / IMAR-CMA, Dept. of Life Sciences; I. Lopes, University of Aveiro / CESAM. This work aimed to assess histological alterations and Caspase-3 activity (an apoptotic marker) in several organs obtained from adult animals of *Pelophylax perezii* originated from natural populations subjected to long-term exposure to chemical contamination. For this, four populations were sampled: two at chemically contaminated sites and two at reference sites. Sixteen adult organisms (8 females; 8 males) were captured at each site and transported chilled to the laboratory, where animals were euthanized with MS222. After obtaining several morphometric parameters the organisms were dissected to collect gonads, kidney, liver, spleen, lungs and skin that were immediately fixed in formalin or Bouin and further processed for paraffin wax embedding using routine protocols. All organs were subjected to hematoxylin-eosin staining. Gonads, kidney, liver, spleen and lungs where further processed for immunostaining with specific antibody for Caspase-3. Semi-quantitative assessments were carried out using an image analyzer. Histology of spleen, liver, lungs and kidney did not reveal specific histological lesions in the impacted populations. Liver histology revealed the presence of melanomacrophages in animals from all populations. Histological analysis of testis revealed testicular oocytes (TO) in the impacted populations (25% and 12.5%) while no TO were detected in reference populations. Differences in the mean seminiferous lobule diameter were observed between reference and contaminated populations, and as well between the two contaminated populations. However no differences in testicular Caspase-3 activity were found among population. With regard to ovaries, histological analysis showed no differences in the distribution of the different developmental stages of oocytes among populations. Analysis of Caspase-3 activity in histological sections of spleen, liver, lungs, kidney and ovaries are underway.

**WE104 Effects of long-term exposure to contamination on genetic variability in natural populations of *Pelophylax perezii*** B. Santos, University of Aveiro; R. Silva, A. Amorim, University of Porto / IPATIMUP, Faculty of Sciences; I. Lopes, University of Aveiro / CESAM. Natural populations of amphibians have been exposed for a long time to contaminated waters and soils. Some of these scenarios may contribute to decrease or alter the genetic diversity of populations in order to cope with less favourable conditions. Therefore, it is important to know how different environmental conditions can change the genetic landscape of these populations. Thus, this work aimed to assess genetic diversity of four different populations of *Pelophylax perezii* exposed to different environmental conditions. To achieve this goal, four natural populations of the Perez's frog *Pelophylax perezii*, with different history of chemical exposure, were studied. Sixteen adult organisms (8 females; 8 males) were captured at each site and sacrificed with MS222. The muscle tissue was removed from the animal and deep-frozen in liquid nitrogen for DNA extraction. The analysis was performed based on two mitochondrial markers: 12S (ribosomal) and ND2 (protein-coding). PCR amplifications of a fragment of each gene were performed followed by a sequencing analysis. The data were screened to assess genetic diversity within and between the four populations. Several single nucleotide polymorphisms (SNPs) were found, including both synonymous and missense mutations, and some unique alterations in each population. Aligned sequences were used to build UPGMA trees and the obtained data suggest that, in general, samples from the less impacted sites tend to group together and the same is observed for samples from the most impacted sites. Additionally, one of the less impacted sites has an increased allelic diversity, which may indicate loss of genotypes in the other populations. To further address this hypothesis, nuclear and neutral DNA markers are also being studied.

**WE105 Contaminant-driven genetic erosion: fitness costs and consequences on the viability of wild populations of amphibians.**

Lopes, University of Aveiro / CESAM; R. Ribeiro, Universidade de Coimbra / IMAR-CMA, Dept. of Life Sciences; M. Beneitez, University of Castilla La Mancha / CSIC; I. Domingues, University of Aveiro / Biology & CESAM; R. Francisco, I. Martinez-Solano, University of Castilla La Mancha / CSIC; P. Morais, University of Coimbra; E. Munoz, B. Santos, University of Aveiro / Biology & CESAM; R. Silva, S. Silva, University of Porto / IPATIMUP, Faculty of Sciences; O. Sobral, University of Coimbra / Life Science; R. Soria, University of Castilla La Mancha / CSIC; C. Venancio, Universidade de Aveiro / Biology & CESAM; C. Bishop, University of British Columbia; A.M. Soares, University of Aveiro / Biology & CESAM; M. Ortiz-Santaliestra, University of Castilla La Mancha / CSIC. Increasing emphasis has been assigned to evaluate potential impacts of chemical contamination on the genetic diversity of natural populations. Within this process is essential to understand long-term ecological effects that may follow a loss of genetic diversity. At present, limited empirical evidence exists supporting the general view that genetic erosion leads to negative evolutionary side effects, and many knowledge gaps still exist. As populations are regarded as the minimum units for species conservation and environmental protection, then accurate understanding of how contaminants may influence their genetic diversity, and the associated costs and consequences on their evolutionary potential is mandatory for a better conservation of biodiversity. Currently, most of the published studies addressing this issue fails some of the permits to establish causality between population genetic effects and contamination; some only monitor neutral markers that may fail to detect directional selective pressures, and only few address the impacts that loss of genetic diversity can have on the viability of populations. Also, most work has been focused on invertebrates, but the study of these processes is also extremely important in vertebrate species, as usually they are constituted by populations with a much smaller number of individuals, and, thus, the effects of contaminants in population genetics may be more exacerbated. Accordingly, this project aims at assessing the occurrence of contaminant-driven genetic erosion in natural populations of amphibians, possible fitness costs and consequences in the viability of these populations under scenarios of future environmental changes. To achieve this goal, three specific objectives were delineated, to evaluate if: (i) metal contamination led to genetic erosion in exposed populations; (ii) an increased resistance to metals is associated with fitness costs; (iii) resistance to metals is associated with an increased susceptibility to other stressors. To tackle these objectives, two study sites, with a similar history of metal contamination, were selected: Mina de São Domingos (SE Portugal) and Valle de Alcudia (SW Spain). At both mines, metal contamination is reported to have been occurring for more than a century, and it is expected that amphibian populations inhabiting these sites have had multi-generational exposure to chemical contamination, and thus the occurrence of genetic erosion due to directional selection could have occurred.

**WE106 Paradise Lost? The Effects of Anthropogenic Contaminants on Wetland Species in Bermuda**

J.P. Bacon, M.E. Outerbridge, Bermuda Zoological Society; G.M. Fent, M. Mathis, C.E. Fort, H.M. Fort, D.J. Fort, Fort Environmental Laboratories. Contaminants, particularly diesel range organics, polycyclic aromatic hydrocarbons (PAHs), and metals, are causing a host of effects in Bermuda's wetland species including developmental malformations, endocrine disruption, and immunological stress. To further assess the effects of contaminants on wildlife in Bermuda, a total of 126 adult cane toads (*Rhinella marina*) were collected from 8 sites and a total of 118 adult red-eared sliders (*Trachemys scripta elegans*) were collected from 8 sites, 5 sites differing from the toad collection sites. Additionally, 10 adult red-eared sliders were collected from a reference site in Stillwater, Oklahoma for comparison. Necropsies were performed on all specimens. Contaminant residues were measured in composited toad carcass, liver and ovary tissue samples, toad stomach contents, and in composited slider liver, ovary and testes tissue samples. Liver and gonad abnormalities were

observed in toads at 7 sites and in sliders at 8 sites. Overall 26.2% of toads and 46.6% of sliders had liver abnormalities with lesions and discoloration being the most common abnormalities observed in both groups. Further, 41.5% of male toads and 48.6% of male sliders exhibited testes abnormalities and 32.8% of female toads and 31.8% of female sliders exhibited ovary abnormalities. Segmented testes was the most common abnormality seen in male gonads of both groups while necrotic or underdeveloped ovaries were the most common abnormalities seen in female gonads of both groups. Tissue residue analyses for Bermuda specimens revealed significant levels of PAHs, TPH-DRO, As, Cd, Cr, Cu, Hg, Pb and Zn in all toad and terrapin tissues sampled with the highest residues generally occurring in liver samples. The toads' stomach contents were also highly contaminated. In contrast, no abnormalities were observed in the reference site sliders; hydrocarbons, As, Cd, Hg and Pb were not detected in reference slider tissue samples; and Cr, Cu and Zn residues were markedly lower in the reference site sliders than in the Bermuda specimens. Contaminants (metals and PAHs) thus appear to play a significant role in the induction of the responses we observed in the Bermuda specimens; however, more work is needed to establish direct and in-direct causality.

**WE108 HerPesti: Characterizing the risk of pesticide use on amphibian and reptile populations based on multiple, ecologically relevant responses**

M.E. Ortiz Santaliestra, Instituto de Investigación en Recursos Cinegéticos / Forest and Wildlife Ecology; C. Bruhl, University of Koblenz-Landau / Institute for Environmental Sciences. Environmental pollution is recognized as one of the major causes involved in amphibian and reptile declines, in spite of which these animals have not been traditionally considered in risk assessment of some pollutants like pesticides, being supposedly protected through data retrieved from other vertebrate taxa. The development of the Regulation (EC) 1107/2009 concerning the placing of Plant Protection Products on the market is introducing some changes in this context, like consideration of the published information regarding toxicity on amphibians or reptiles for pesticide risk assessment. However, large information gaps in this context still exist. For example, the permeable nature of amphibian skin or the strong links of reptiles with soils (especially during egg laying) where pesticides are applied make dermal assimilation a very relevant route of exposure of these animals. In this context, a recent FERA report published by the EFSA concluded that information to estimate dermal exposure in amphibians is lacking. Overall, both amphibians and reptiles exhibit particular mechanisms that can make them especially vulnerable to the impact of pesticides. HerPesti project aims at testing the degree of protection that EU legislation on pesticides confers to amphibians and reptiles and, if necessary, developing possible remediation measures. With this purpose, we will identify, on the one hand, the impact of pesticides on amphibian and reptile populations using novel, ecologically relevant endpoints indicative of population status and viability, such as reproductive and social behaviours, immune response and pathogen resistance. On the other hand, we will test effectiveness of risk assessment protocols by comparing effects on native amphibians and reptiles based these ecologically relevant endpoints with effects on surrogate taxa. A field monitoring in areas of pesticide use will serve to characterize the exposure in realistic scenarios. The project strongly pursues the establishment of a fluent communication with industry and government sectors, providing the former with protocols to efficiently evaluate pesticide risks on amphibians and reptiles, and the latter with decision-making tools. Financed by the EC's Research Executive Agency through MC-IEF actions of the 7<sup>th</sup> Framework Program

**WE109 Biospectroscopy as a novel tool to monitor amphibian**

**health: a pilot study comparing UK ponds** R. Strong, Lancaster University / Centre for Biophotonics; C.J. Halsall, K.C. Jones, Lancaster University / Lancaster Environment Centre; R.F. Shore, Lancaster Centre for Ecology and Hydrology / Lancaster Environment Centre; F.L. Martin, Lancaster University / Centre for Biophotonics. Amphibians are regarded as sensitive bioindicators of environmental pollution due to their highly-permeable skin and complex lifecycle, comprising both

aquatic and terrestrial phases. After habitat destruction, environmental pollution is cited as a major reason for perceived amphibian population declines. As such, it is critical to develop biomarkers capable of identifying adverse effects of environmental pollution, ideally focusing on early changes that could potentially have population-level effects. One such approach is to combine attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy, a technique used to derive a chemical signature in the form of an IR spectrum, with subsequent computational analysis potentially allowing for the identification of wavenumber-related biomarkers of effect [1]. Employing this, we investigated if ATR-FTIR spectroscopy could detect differences attributable to environmental contamination in spawn and tadpoles of the common frog, *Rana temporaria*. The sites studied were in North-West England and differed in their sources of contamination: wastewater input, landfill contamination or agricultural pollution. Preliminary results indicated clear differences in mean spectra between sites, an effect that was more pronounced in tadpoles. Principal component analysis-linear discriminant analysis (PCA-LDA) readily distinguished tadpoles, and to a lesser extent spawn, from the sites studied. This pilot study suggests that ATR-FTIR spectroscopy is a promising novel tool towards environmental biomonitoring. Martin FL et al. (2010) *Nature Protocols* **5**: 1748-1760.

**WE110 Diversity of the cutaneous bacterial community in the Perez frog, *Pelophylax perezi*** S. Costa, University of Aveiro & CESAM; P.V. Morais, G. Paiva, University of Coimbra; R. Ribeiro, Universidade de Coimbra / IMAR-CMA, Dept. of Life Sciences; I. Lopes, University of Aveiro / CESAM. Amphibian skin holds a resident bacterial community that may confer tolerance to environmental stressors. Chemical contamination may reduce its diversity and consequently alter the sensitivity of amphibians to future environmental stressors. Understanding the structure, dynamics and specificity of this microbial community is needed to engage a better and broader protection of amphibians. Accordingly, the present study aimed at investigating the skin-associated bacterial community of the frog *Pelophylax perezi*, looking at among and within population variation. Three adults were sampled at five different ponds. For each population, skin swabs were obtained from the entire body after rinsing the individuals with sterile distilled water to ensure the collection of skin-associated microbes rather than pond-associated transient microbes. Cultivable bacteria were recovered from each swab by using R2A medium incubated at 22°C. The microbial concentration per ml per amphibian varied within animals from the same environment and between animals from different environments. The skin bacterial community diversity of each amphibian was then evaluated based on the colony morphology. In addition, the diversity of the skin microbial community was also assessed based on differences in the 16S rRNA gene sequence through the cultivable-independent method denaturing gradient gel electrophoresis. The obtained results showed that both intra- and inter-population variability exist in amphibian skin microbiome, however the latter seems to be higher than the former.

**WE111 Short-term in situ exposure of *Pelophylax perezi* tadpoles to different uranium mine effluent ponds** S.M. Marques, Department of Biology; S. Chaves, University of Lisbon / Science Faculty; F. Goncalves, University of Aveiro / Department of Biology; R. Pereira, University of Aveiro / CESAM, Center of Environmental and Marine Studies, University of Aveiro. Mining activities invariably produce metal contaminated effluents. Depending on factors such as pH and metal concentration the toxicity of the effluent may vary. To assess the effects of three characteristically different effluent ponds from a deactivated uranium mine, presenting toxicologically relevant data, an *in situ* exposure with *Pelophylax perezi* tadpoles was conducted. Tadpoles were exposed to the three effluent ponds, ranked by increasing order of metals concentrations (REF, M1, M2). Survival, metal accumulation, antioxidant enzymes (catalase, glutathione peroxidase and glutathione reductase) and lipid peroxidation (LPO) were determined in tadpoles. Simultaneously, physical and chemical variables of the effluents were measured. Death percentage in the effluents was 3.17 (REF), 9.84 (M1)

and 42.86% (M2) and was not coincident with metal accumulation, which was highest in tadpoles exposed to M1, while metal contents in M2 tadpoles were quite similar to those recorded in REF tadpoles. However, high mortality in M2 was attributed to the extremely low pH (? 3.77). The antioxidant enzymatic activity was only affected in the case glutathione peroxidase (GPx) with significantly higher activity in M1, being in accordance with the highest accumulation of metals. LPO, usually associated with metal accumulation, had the following pattern: M1 > REF > M2. Overall, effluent toxicity in tadpoles exposed to M2 effluent seems to be primarily an effect of pH while in M1 toxicity is mainly owed to high metal concentrations. The effluent acidity seems to reduce metal accumulation probably due to damage in the integument, affecting ion uptake. The results obtained bring a better understanding of the toxicological processes that local *P. perezi* population is subjected to, mainly in the early life stages. Furthermore this study highlights the influence of pH in the toxicity of metal rich effluents.

**WE112 Amphibians and uranium mine ponds: an unexpected co-existence** S.M. Marques, Department of Biology; F. Goncalves, University of Aveiro / Department of Biology; R. Pereira, University of Aveiro / CESAM, Center of Environmental and Marine Studies, University of Aveiro. Despite the widespread concerns with the persistence of amphibians on earth due to their well known sensitivity to different kinds of environmental stressors, green frogs were found, apparently healthy, living and breeding in a uranium mine complex in Portugal. The various effluent ponds in the complex are globally metal and radionuclides-rich, having however distinct acidity that modulates their toxicity to the early life stages, as confirmed through both laboratory and *in situ* long and short term exposures, during which lethal and sub-lethal parameters were evaluated. Despite this short-coming for the early-life stages, adults seem to benefit from the reduced predation pressure offered, being abundant there. Nevertheless this benefit implies some costs in terms of the health of adult animals, which have shown to be effectively exposed to high metal concentrations due to the high levels of some metals like Al, Fe, Cu, Se and U bioaccumulated in the liver and kidney with concomitant histopathological changes in these organs as well as in lungs. To cope with this exposure several mechanisms seemed to be activated depending on the animals' life stage, namely in adults i) the overproduction of melanomacrophages, resulting in high numbers of large melanomacrophagic centers in the tissues, which is probably related with the antioxidant role of melanin, being able to complex metals, preventing them from exerting their toxic effects; ii) higher expression of genes coding for proteins (haemoglobin, albumin and fibrinogen) that can play a fundamental role in metal detoxification and act as antioxidants, providing high basal protection against metal toxicity; and in tadpoles iii) investment in osmotic regulation mechanisms by up-regulating stomatin and peptidyl-prolyl cis/trans isomerase FKBP2, to compensate for any imbalance derived from high metal content in surrounding media, as well as iv) higher investment in energy acquisition through up-regulating voltage dependent anion channel and trypsin, to provide invaluable energy required for the elevated energetic demand of increased osmoregulation. To provide new insights about the ability of amphibians to cope with a particular kind of contamination, which includes both a chemical and a radiological exposure, this work integrates different studies carried out with Iberian green frogs occupying an improbable habitat.

**WE113 Biochemical and Histopathological Changes in the Brain Tissue of the African Toad, *Bufo regularis* Exposed to Diazinon** I. Tongo, L.I. Ezemonye, University of Benin / Animal and Environmental Biology. The sublethal toxicity of diazinon to the adult African toad, *Bufo regularis* was carried out to assess biochemical and histopathological changes in the brain using an integration of biomarkers. Glutathione S-Transferase (GST), Acetylcholinesterase (AChE), Corticosterone, Total protein, and Glucose levels were investigated. Toads were exposed to 0.01, 0.02, 0.03 and 0.04 mg/l for 28 days. Brain AChE activity reduced by 96% in the highest concentration (0.04 mg/l) compared to the control brain. Similarly, brain

GST levels increased significantly after exposure while Corticosterone and Total Protein levels decreased significantly compared to the control. The pesticide also caused differential increase in glucose levels indicating hyperglycemia. Brain histopathology after 28 days diazinon exposure showed oedema, with an associated inflammatory infiltration of eosinophilic granule cells, necrosis in the cerebrum, dark-stained degenerating Purkinje neurons and vacuolar changes with empty spaces appearing as "moth eaten" areas which were absent in the control toad. More pronounced poisoning symptoms was observed at higher concentrations. The results indicate that exposure of the brain tissue of *B. regularis* to sublethal concentration of diazinon caused biochemical and histopathological alterations occurring in a dose-dependent manner. The study suggest that biochemical and histopathological parameters of the brain of *B. regularis* are subject to the sublethal negative effects of diazinon pesticide and can therefore provide early warning signals on the impacts of diazinon contamination on amphibians.

**WE114 Towards a sustained bee health: Recent Updates on the in-vitro bee larvae test** S. Kimmel, S. Hoeger, Harlan Laboratories Ltd / Environmental Toxicology; A. Liedtke, Harlan Laboratories Ltd / Ecotoxicology. The impact of plant protection products and therefore ecotoxicological testing on pollinators in common and on honeybees in special has become an important focus throughout the recent years. Not only since the new regulation 1107/2009, protection and intensive research on bee health has become an important part of the registration process especially for pesticides and insect growth regulators. Scope of this presentation is a brief update on recent proceeding from OECD workshop and seminars on bee larvae toxicity from the CRO perspective. Further on, adaption and modification of the bee larvae toxicity test protocol introduced by Aupinel et al. (2005, 2007 & 2009) will be discussed: - Test design and setup (from the ringtest period until recent improvements) - A worst case approach focussing on larvae mortality after single dose exposure (OECD guideline publication in progress) - An extended and prolonged approach with multiple/daily dose exposure (further validation necessary, intended to proceed with additional ringtests) The split into these two approaches enables a fast and intense experience of a possible hazard effect on bee health as well as emphasis on different endpoints, e.g. LD and NOEL. Further on, this testing strategy fills the gap of having no laboratory worst case test system for bee larvae toxicity which can be conducted prior to tunnel, semi-field and field testing of whole honeybee colonies (e.g. OECD GD 75, 2007; Oomen et al., 1992). The presented methods also suit the recently increased governmental authority requests for intense bee health research within the risk assessment for plant protection products (Alix & Lewis, 2010; US EPA, 2011, EFSA, 2012).

**WE115 LARVICIDAL ACTIVITY OF THE ESSENTIAL OIL OF EUCALYPTUS STAGERIANA** C.H. Soares, Universidade Federal de Santa Catarina / Bioquímica; Y. Simon, Universidade Federal de Santa Catarina; A. Furigo Junior, Universidade Federal de Santa Catarina / Engenharia Química, CTC. *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) is an important vector of pathogens in many cities of Brazil and other countries. The search for methods to eliminate mosquito larvae using extracts of plant essential oils has been a strategy used by many researchers. Though the primary concern is with the efficiency of the extracts of essential oils on mosquito mortality, it is expected that this does not have toxicity to other organisms or invertebrate important for the development of biota. The objective of this study was to evaluate the larvicidal effect of the essential oil of *Eucalyptus stageriana* in *Aedes aegypti*, as well as the degree of toxicity to organisms such as *Daphnia magna*, *Scenedemus sp.* and *Fusarium oxysporum*. The aqueous extracts were tested at concentrations of 100%, 75%, 50%, 25%, 12% and 6% with exposure assessments made at 0, 24, 48 and 72 h for algae; 24 and 48 h for daphnia and mosquito larvae and 10 days for the fungus. Three replicates were used for each concentration and for the control. The essential oil exhibited larvicidal activity at low concentrations killing larvae in 24 hours and 48 hours as well as preventing changes before occurring instar appearance. On the other hand, the results of toxicity tests to algae, fungi and daphnia, in the

same concentration range that we can observe death of the mosquito larvae, demonstrated that there was not acute toxicity. Keywords: Toxicity; *Aedesaegypti*; Bioinsecticide

**WE116 The potential impact of atrazine on the native Australian fish species (M. fluviatilis)** A.F. Miranda, School of Applied Sciences / School of Applied Sciences; L. Zalizniak, J.M. Rodrigues, Ecotoxicology research group, School of applied sciences, RMIT University / School of applied sciences; V.J. Pettigrove, The University of Melbourne / Zoology; D. Nugegoda, Ecotoxicology research group, School of applied sciences, RMIT University / School of applied sciences. The herbicide atrazine is widely used in agriculture for the production of a number of crops. Due to its physical and chemical proprieties atrazine is found in low concentrations in surface waters, potentially causing unintended impacts on the aquatic biota. The effect of atrazine on aquatic species is variable. Atrazine has been implicated in causing hermaphroditism in frogs, but there were no effects reported in fish. It is clear that further research on the effects of atrazine, especially on fish, is required. This study evaluates the effects of atrazine on adult male Murray River rainbowfish. The activity of the biotransformation enzymes ethoxyresorufin-O-deethylase (EROD), glutathione S transferase (GST), the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) was evaluated in fish liver and muscle. The activity of acetylcholinesterase (AChE) in brain as well as the gill ATPase activity at the end of the exposure was determined. Possible adverse effects on the reproductive fitness of the Murray River rainbowfish were assessed using plasma vitellogenin concentrations. Histopathology of gills, gonads and liver were evaluated at the end of a 21-day exposure to three concentrations of atrazine: 1.3 µg/L, 13 µg/L and 130 µg/L. Changes in AChE activity in brain as well as in the biotransformation enzymes SOD, EROD and of the antioxidant enzyme CAT were observed with the exposure. Vitellogenin was detected in male fish exposed to atrazine confirming that atrazine may act as an endocrine disruptor with estrogenic effects. Histopathological sections revealed lesions in the gills including hyperplasia and lamellar fusion at the two highest concentrations tested. The results show that atrazine behaves as an enzyme inhibitor as well a sexual endocrine disruptor

**WE117 Experimental assessment of the environmental fate and effects of triazoles and benzotriazoles** M. Kos Durjava, B. Kolar, L. Arnus, Public Health Institute Maribor; E. Papa, QSAR Res. Unit Environ. Chem/Dep. Structural Functional Biology; S. Kovarich, University of Insubria; U. Sahlin, Linnaeus University, School of Natural Sciences; W.J. Peijnenburg, RIVM / Laboratory for Ecological Risk Assessment. The environmental fate and effects of triazoles and benzotriazoles are of concern within chemical regulation. As part of an intelligent testing strategy, experimental tests were performed on endpoints that are relevant for risk assessment for a selected number of substituted triazole and benzotriazole compounds. Experimental tests included the assessment of the ecotoxicity for algae, daphnids and zebrafish embryos and assessment of ready biodegradability. The triazoles and benzotriazole were selected for the testing on the basis of existing toxicity data for vertebrate and invertebrate species, as well as on principal component analysis on molecular descriptors aimed at selecting a minimum number of compounds to be tested in order to maximize the spanning of the chemical domain of both compound classes. The experimental results show that variation in toxicity of triazoles and benzotriazoles across species was relatively minor; in general the largest factor was about 20. The study conducted indicated that triazoles are not readily biodegradable.

**WE118 POTENTIAL OF THE FUNGICIDE PYRIMETHANIL TO TRIGGER AVOIDANCE RESPONSE IN FISH AND TADPOLES** C. Araujo, C. Shinn, University of Coimbra / IMAR - Instituto do Mar; L.B. Mendes, A.L. Sanchez, D. Delello-Schneider, A. Vasconcelos, M.M. Nogueira, University of São Paulo / Hydraulics and Sanitation; E.G. Espindola, University / Hydraulics and Sanitation. The fungicide pyrimethanil is used pre- and post-harvest in agriculture to prevent fungal diseases. Its potential risk to non-target organisms in

aquatic environments should be taken into account. Thus, the present study intends to verify the ability of juveniles of the fish *Danio rerio* and tadpoles of two species of amphibians, *Leptodactylus latrans* and *Lithobates catesbeianus*, to avoid pyrimethanil-contaminated environments. The commercial formulation Mythos, containing pyrimethanil (300 mg L<sup>-1</sup>) as the active ingredient, was applied to mesocosm systems at a concentration of 1 mg/L, simulating a spray-drift situation. One day after application, samples of the reference (uncontaminated) and contaminated mesocosms were taken to be used in the avoidance assays. Avoidance assays were performed under non-forced conditions, formed by seven interconnected compartments, through which the organisms could move and potentially chose the preferred compartment. Different sample concentrations (contaminated mesocosm water at 0, 17, 33, 50, 66, 83 and 100% diluted with reference water) were placed in each compartment and then three fish or five tadpoles were introduced. Assays were performed in triplicate, in darkness, at 25 °C and during 12 h. Although avoidance was recorded for the three species, the response pattern was not identical. The response of *L. catesbeianus* was not concentration-dependent and avoidance response ranged between 13 and 19%. For *L. latrans* the avoidance was very similar in the concentrations of 17 to 83%, ranging from 17 to 27%; however, in the highest concentration that response was more pronounced: 47%. For *D. rerio* the avoidance was recorded after 4 and 12 h. The shorter time was used as the movement of fish in the system accelerates the mixing of the concentrations between compartments. The fish avoidance response was not so clear in the three lowest concentrations containing pyrimethanil (17 to 50%); however, at concentrations of 66, 83 and 100% the avoidance was 27, 54 and 66% after 4 h exposure, and after 12 h the response pattern reduced to 1, 32 and 44%. Based on these results, it is possible to conclude that pyrimethanil can trigger avoidance response in juvenile fish and tadpoles. Therefore, the presence of this fungicide in an aquatic ecosystem, even at non-lethal concentrations, could interfere in the abundance and distribution of the tested organisms due to their ability to detect and avoid pyrimethanil-contaminated environments.

**WE119 Comparative toxicity of two commercially important organophosphates to *Oreochromis mossambicus*: linking biomarker responses and life-cycle effects** M. Jordaan; S. Reinecke, Stellenbosch University / Dept Botany and Zoology; A.J. Reinecke, Stellenbosch University / Department of Botany and Zoology - Stress Ecology Research Group. Organophosphate pesticides are an integral part of commercial farming and implicated as a major source of environmental contamination in South Africa. This is relevant to the Western Cape Province which has a large deciduous fruit production sector which includes high levels of pesticide use. Various non-target aquatic organisms in agricultural areas are at risk due to the presence of these pesticides and the repetitive nature of pesticide application. The extent to which non-target aquatic animals can be affected by exposure to two commercially important organophosphates, azinphos-methyl and chlorpyrifos, was investigated through monitoring selected biomarker responses and life cycle effects under laboratory conditions in the fish *Oreochromis mossambicus*. Juvenile life stages were subjected to standard acute toxicity tests which showed that azinphos-methyl is more toxic than chlorpyrifos to this species. *Oreochromis mossambicus* was also subjected to an intermittent exposure regime in order to assess the effects of repeated pesticide application on various endpoints at different levels of biological organization. Biomarker responses, growth effects and feeding behaviour were assessed and there was evidence to suggest that in the case of an intermittent exposure scenario, exposure interval played a more important role in inducing an effect than exposure concentration. At a shorter exposure interval, the majority of endpoints showed no difference between higher and lower exposure concentrations, while at a longer exposure interval the effects of exposure concentration became evident. Feeding behaviour was affected in a dose-dependent manner. The present study yielded important results that improve the understanding of biological impacts of pesticide pollution on the environment and provides evidence that environmentally relevant pesticide concentrations are posing a realistic

threat to non-target species occurring in these areas. This work is especially relevant when considering the very diverse and highly threatened indigenous freshwater fish fauna of the Western Cape Province and the fact that many of these species occur in areas subjected to large scale deciduous fruit production. The results of this study can aid in optimising pesticide application not only in terms of eradicating the pest organisms, but also in terms of mitigating the environmental effects and thereby ensuring sustained biodiversity in these areas.

#### **WE120 Combination Effects of Pyrethroids and Neonicotinoids on Life-Cycle Traits and Fecundity of *Chironomus riparius*** V. Kunce, F. Johansson, Uppsala University.

While ecotoxicological risk assessments are conducted on individual substances, monitoring of streams in agricultural areas has shown that pesticides are rarely present alone. Brief but intense pulse events such as storm water runoff and spray drift subject freshwater environments to complex mixtures of pesticides at high concentrations. Both neonicotinoid and pyrethroid pesticides are known to have adverse effects on non-target organisms. The neonicotinoids; imidacloprid and thiacloprid, and the pyrethroids; deltamethrin and esfenvalerate have been detected in Swedish surface waters above the established benchmark values. We exposed first instar *Chironomus riparius* larvae to combinations of these four pesticides in 1 hour pulses then raised them in uncontaminated conditions for 40 days of observation. While the individual pesticide exposures affected life-cycle traits and fecundity, the combination affects could not be predicted from those results.

#### **WE121 Aquatic Ecotox and Fate of inorganic PPPs: Sulphur case study** c. casalegno, ChemService Srl; M. Neri, O. Schifanella, ChemService.

Sulphur, a fungicide broadly used in Europe, has been successfully stewarded onto Annex I inclusion in 2010 after its re-evaluation in the context of the fourth stage of the review program of existing active substances of Directive 91/414/EEC. High volumes used in agriculture combined with difficulty in distinguishing the natural background level in the environment, gave rise to requests of non-standard studies and alternative testing and environmental risk assessment approaches (ERA) for overcoming regulatory hurdles. After a brief introduction on the use of sulphur as inorganic PPP (history, uses, formulation types, current EU regulatory status), the present work gives an overview on non-standard aquatic ecotoxicological test (test types, test media preparation, analytical methods for detection of sulphur in water samples) and non-standard fate approach (aquatic fate models and adopted approach) adopted for the inclusion of the active substance sulphur in Annex I, together with the risk assessment implications of these non-standard approaches. The non-standard testing approach involved tests on sediment dwelling organisms (Chironomids, OECD 218, 219) and aquatic invertebrates (Daphnia reproduction tests, OECD 211) to investigate the environmental behaviour of the test item and to find a better figure for the NOEC (in view of the very low water solubility of a.s. sulphur). As regards to the environmental fate, the non-standard approach used to estimate the Predicted Environmental Concentration of sulphur in surface water (PEC<sub>sw</sub>) and sediments (PEC<sub>sed</sub>) is presented and the applicability of aquatic fate models (FOCUS model) to inorganic PPPs is discussed. As a conclusion, the present work describes the implications of this non-standard aquatic testing and fate approach on risk assessment and highlights the need to build more realistic scenarios through the adoption of standard and non-standard approaches (learning by doing process).

#### **WE122 Degradation pathways of three benzonitrile herbicides – ioxynil, bromoxynil and dichlobenil – in pure bacterial cultures versus agricultural soil** Z. Frkova, Aarhus University; A. Johansen, Department of Environmental Science, Aarhus University; N. Badawi, N. Schultz-Jensen, Department of Geochemistry, Geological Survey of Denmark and Greenland (GEUS); K. Bester, Department of

Environmental Science, Aarhus University; S. Sørensen, Department of Geochemistry, Geological Survey of Denmark and Greenland (GEUS); U. Karlson, Department of Environmental Science, Aarhus University. Due to structural similarities, bromoxynil and ioxynil may be converted

into persistent metabolites the same way as dichlobenil is converted to BAM due to structural similarities. We tested a bacterial strain, *Aminobacter* sp. MSH1, harbouring the enzymes necessary for conversion of dichlobenil to BAM, 2,6-DCBA and further to CO<sub>2</sub>. This strain indeed converted bromoxynil and ioxynil to the corresponding metabolites, but in contrast to BAM and 2,6-DCBA these were not further degraded, suggesting that they might pose an environmental problem. A 2,6-DCBA-induced enzyme study revealed a decrease of the reaction rate for ioxynil and bromoxynil indicating competitive inhibition. We then tested mineralization capabilities of *Aminobacter* MSH1 towards all three benzonitriles over a range of initial cell concentrations (10<sup>5</sup> – 10<sup>9</sup> cells/ml), and observed a strong influence of pH on the activity of enzymes converting dichlobenil to CO<sub>2</sub>, whereas no mineralization by this organism was proven for ioxynil and bromoxynil. This might be explained by the spatial effect of substituents on the benzene ring showing difficulties for enzymes to cleave the ring with halogens at the meta position. Subsequently, we verified the mineralization process in agricultural soil and found that ioxynil and bromoxynil in contrast to dichlobenil have a very high potential for mineralization, so the theoretical problem of persistent metabolites, as seen in pure culture and raised by the literature, was in fact circumvented by the metabolic capabilities of the indigenous microbiota.

#### **WE123 Assessing the toxic potency of Aroclor 1268 to piscivorous marine mammals using mink as a mammalian model** W. Folland;

S.J. Bursian, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science; S. Fitzgerald, Michigan State University / Pathobiology and Diagnostic Investigation; M.J. Zwiernik, Michigan State University / Animal Science Vet Med. Concentrations of polychlorinated biphenyls (PCBs) derived from the commercial mixture, Aroclor 1268, are elevated in aquatic biota in southeastern Georgia, USA. Total PCB concentrations in blubber samples from bottlenose dolphin (*Tursiops truncatus*) in Georgia estuaries have been reported more than 10 fold those typically observed in adjacent regional estuaries. Presently there is great uncertainty associated with quantifying the toxic potency of exposure to this highly chlorinated PCB mixture. The uncertainty originates from the lack of toxicology data combined with differing characteristics of this mixture as compared to the more studied lesser-chlorinated PCB mixtures. Aroclor 1268 is extremely hydrophobic and essentially devoid of coplanar PCB congeners that typically drive PCB toxicity through AhR mediated toxic responses. The American mink is the optimal model for understanding mammalian PCB toxicity and is considered to be a suitable surrogate species for cetaceans due to similarities in diet and taxonomic class. Furthermore, mink are known to be exquisitely sensitive to PCBs, thus the application of mink toxicology data to other mammals is expected to provide a level of safety. In an effort to determine toxic reference values for Aroclor 1268 that can be applied to marine mammals and assign a relative potency value for Aroclor 1268 with respect to 3,3',4,4',5-pentachlorobiphenyl (PCB 126), a trial was conducted to assess and compare their effects on reproduction of adult female mink and on survival and growth of their offspring. Dams and kits were fed a ranch diet spiked with 0, 1.7, 4.0, 10, 17, or 29 ppm Aroclor 1268 or 0.0005 ppm PCB 126. Reproductive, hematological, immunological, enzyme induction and histological endpoints were used to derive toxic reference values so that responses to Aroclor 1268 may be more clearly defined.

#### **WE124 Pollutant accumulation in nestlings of Bonelli's Eagle.**

**Effects on health and influence of diet composition estimated through fatty acid signatures** M.E. Ortiz Santaliestra, Instituto de Investigación en Recursos Cinegéticos / Forest and Wildlife Ecology; J. Resano, A. Hernandez Matias, J. Real, Universitat de Barcelona / Biología Animal; P. Camarero, R. Mateo, Instituto de Investigación en Recursos Cinegéticos (IREC) CSIC-UCLM-JCCM. The accumulation of persistent pollutants like organochlorine compounds (OC) in raptors has been related to toxic effects on adults, while nestlings have received less attention. Nestlings usually demand high amounts of energy that can be obtained from fat consumption, with the potential for mobilization of

stored OC. The accumulation of OC depends on the diet composition, which can be estimated from fatty acid (FA) signatures in plasma. Bonelli's Eagle (*Aquila fasciata*) is an endangered raptor with high dietary variability, and therefore high inter-territorial differences in potential OC intake. The aims of this study were i) to analyse OC accumulation in nestlings from three regions in Spain, ii) to compare plasma OC profiles with FA signatures as diet indicators, and iii) to assess the effects of OC accumulation on fitness. Nestlings from 57 territories in Catalunya, Andalucía and Castilla y León were bled and measured to calculate body condition. Plasma was used to quantify OC, FA, vitamins, carotenoids and biochemical parameters, while cellular pellet was used to measure oxidative stress biomarkers. PCB congener and FA (after calculation of log ratios referred to 18:0) matrices were classified in three principal components each (63 and 81% of variance, respectively). Nestlings from Catalunya, the most industrialized region, showed the highest values for ?PCB, toxic equivalents (TEQ) (p<0.005) and the PCB component related to the dioxin-like congener 126 (p=0.07). No relationship was observed between OC and FA signatures, suggesting that plasma FA profiles are limited as diet indicators to relate to pollutant accumulation. OC profiles significantly explained the variability shown by fitness-related responses (RDA Monte Carlo test: p=0.04). ?PCB and TEQ were selected by permutation tests as significant explaining variables (marginal variance=0.032). Both variables affected plasma vitamins and carotenoids, being negatively correlated (Spearman) to retinol, lutein and zeaxanthin (p<0.038). TEQ also caused oxidative stress through the increase of total glutathione levels (p=0.01) and the inhibition of superoxide dismutase and lactate dehydrogenase activities (p<0.048). Organochlorine insecticides were homogeneously distributed across regions and had almost no effects. Our results suggest that OC accumulation in Bonelli's Eagle nestlings may cause health problems ultimately compromising individual viability

#### **WE125 POP concentrations in eggs of the leatherback turtle (*Dermochelys coriacea*): fatty-acid interactions and effects on reproduction success** E. Andres, CSIC; B. Gomara, Instituto de Química Orgánica General, CSIC; J.C. Navarro, Instituto de Acuicultura de Torre de la Sal, CSIC; D. Gonzalez-Paredes, J. Ruiz-Martin, J.J. Negro, A. Marco, Estación Biológica de Doñana, CSIC. Most

of marine megavertebrates are large predators that acquire and accumulate different types of pollutants, which somehow affect their health and reproductive output. Their fat reserves can play an important role on the dynamics of lipophilic contaminants and their maternal transfer to the offspring. The leatherback turtle (*Dermochelys coriacea*) is categorized as a critically endangered species, mainly due to its decreasing trend on nesting female populations. Thus, reproduction success is a critical feature for this species and potential effects of persistent organic pollutants (POPs) need to be studied. The aims of this study were three: 1) to provide additional baseline data on POP concentrations in eggs of leatherback turtles; 2) to investigate any specific associations between POP congeners and fatty acids; and 3) to clear up how POPs may affect reproductive success in this species. A number of 18 clutches were evaluated during June and August of 2008 at Reserva Pacuare, Costa Rica. CCL, CCW, clutch size and number of SAGs were determined at the time of oviposition. One to three viable eggs were collected from each nest (47 eggs total). Viability, fertility and hatching rates were calculated for each nest. Total lipids from egg-yolk samples were extracted and fatty acid methyl esters were purified by thin layer chromatography and identified by gas chromatography, using helium as carrier gas and comparing peaks to standards. Extraction of POPs was carried out by matrix solid phase dispersion. Purification was performed using a multilayer column. POP congeners were determined by gas chromatography coupled to an ion trap detector using Helium as the carrier gas. PCB and PBDE levels (5068.9 ± 6615.5 and 533.8 ± 371.6 pg·g<sup>-1</sup> wet mass, respectively) were similar to those reported in French-Guiana populations and slightly lower than those associated to Florida populations, probably due to different contamination levels of foraging areas. Both POPs and fatty acids were related to reproductive parameters. This study provides evidences of PUFA enhancing fertility rate (R=0.75; p= 0.009) and hatchling length

( $R=0.826$ ;  $p=0.022$ ), and of PBDEs negatively affecting hatching success ( $R=-0.70$ ;  $p=0.016$ ). A positive correlation found between POPs and PUFAs ( $R=0.615$ ;  $p=0.007$ ) suggests molecular interaction and it may indicate that real harmful effects of these contaminants on the reproduction of leatherback turtles may be masked.

**WE126 Differences in PCB accumulation between male and female moles (*Talpa europaea*) in an area with heterogeneous soil contamination.** M. De Lange, Wageningen UR / Alterra; H. Zweers, D. Lammertsma, J. Faber, Alterra, Wageningen UR. The Krimpenerwaard is an area in the province of Zuid-Holland, the Netherlands, with peaty soil and a large number of small ditches in pastureland. In this area, more than 6000 ditches were filled with different types of waste in the 20<sup>th</sup> century, to create larger pasture fields. However, filling with waste is now recognized as an environmental problem, with known accumulation of contaminants in vegetation (grass for agriculture) and wildlife (birds, mammals). At present, an active management plan is executed, which consists of capping the suspected ditch fills with a layer of clean soil of local origin, and monitor the effectiveness of this capping in reducing the environmental risks for both agriculture and wildlife. As part of this monitoring scheme, European moles were caught and analysed for PCB content in the liver. Monitoring took place during several campaigns (2005, 2007, 2009 and 2012), both before (=T0) and after (=T1) capping of the ditch fills. Reference sites were sampled as well in each year. In total 103 individual moles were analysed for 23 PCB congeners. The ratio between male and females caught in the area differed between years. In the T0-monitoring (2005 and 2007), predominantly males were caught (35 males, 15 females). In the T1-monitoring predominantly females were caught (39 females, 24 males; not all females were analysed for PCBs). The PCB content in the liver (per gram fat) differed between males and females. The natural variation in PCB content was analysed with GLM using the individuals caught on reference sites. Two factors were significant, sex and fresh body weight. Males had a lower PCB content than females, and weight had a positive effect on PCB content. The effectiveness of capping was analysed with GLM using all individuals caught in 2009. The variation in PCB content depended on type of waste, sex, and body size. Again females had a higher PCB content than males, and weight had a positive effect on PCB content. The higher PCB accumulation in females compared with males is consistent over the different sampling years. This result is in contrast with an expected difference of females having lower organic contaminant content due to transfer through lactation, as shown for e.g. marine mammals. Our study on mammals is the first to our knowledge that reports a consistent higher PCB content for females compared with males. Further explanation for the higher accumulation will be investigated using ecological factors.

**WE127 PCBs in Arctic seabirds: the importance of migration, diet, sex and age** L. Baert, Ghent University / Bioscience Engineering; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; K. Borga, Norwegian Institute for Water Research; F. De Laender, Université de Namur ASBL / Lab of Env.Tox&Appl.Ecol.. Trophic Level and the octanol-water partition coefficient ( $K_{ow}$ ) are considered the most important predictors for the polychlorinated biphenyl (PCB) body concentrations of Arctic seabirds. However, interspecific differences in PCB body concentration between seabird species occupying a similar trophic level indicate the need for additional predictors. Accounting for migration, opportunistic feeding, and intraspecific variability – supposed to be caused mainly by age and sex – in bioaccumulation models has been suggested as a potential way to increase the predictive power but has, to our knowledge, never been examined. Based on 726 body concentration measurements for 6 PCB congeners (PCB 28,105,118,138,153 and 180), a statistical model ( $R^2=0.651$ ) was developed for 3 species of Arctic seabirds from the Barents Sea: Common Eider (*Somateria mollissima*), Black-legged Kittiwake (*Rissa tridactyla*) and Glaucous Gull (*Larus hyperboreus*). This statistical model demonstrated that migration caused a 4.1-fold increase (p<0.001) in the PCB body concentrations of the long-distance

migrating Black-legged Kittiwake. Opportunistic feeding caused a 2.7-fold increase (p<0.001) in the Glaucous Gull. The effect of intraspecific variability was modest compared to interspecific variability: immature birds had a 0.7-fold decrease (p=0.00016) compared to adult birds. No significant difference was found in PCB body concentration between adult males and females. Our exercise demonstrates that migration and opportunistic feeding have an effect on PCB body concentrations equal to about a 2 and 1 trophic level increase, respectively, and should therefore be considered in bioaccumulation modeling.

**WE128 Chlorinated compound profiles from different terrestrial sources converge in aquatic bird eggs** H. Bouwman, I. Viljoen, L. Quinn, North-West University; A. Polder, The Norwegian School of Veterinary Sciences. There are many reports about pollutants in birds that concentrate on a single species, compare related species, or consider a specific compound between different species. However, comprehensive perspectives on multiple chemicals in aquatic and terrestrial bird species remain rare. We conducted such a study in an area in South Africa where DDT and other insecticides is used for malaria control, but there is also legacy and suspected current use of chlorinated pesticides, as well as some releases of industrial chemicals from products imported into the region. DDT is applied at 2g/m<sup>2</sup> indoors to thousands of homesteads. Heron and egret eggs were sampled by scaling trees using rock climbing techniques. Sparrow eggs were collected from the nests in the thatch of the homesteads. Eggs contents were shipped to Norway for analyses. Multivariate analyses of the data employed Non-metric Multidimensional Scaling (NMDS). Disappointingly, only a few breeding colonies of aquatic birds could be located. Even more worrisome is that only one heron breeding colony could be found – Grey Herons (aquatic feeders) at Nandoni. Terrestrial feeding Cattle Egret colonies were more readily found. DDT levels in Grey Herons were very high (230 mg/kg lipid; 13 mg/kg wet mass) – some of the highest levels recently reported. The levels found here is the same as the critical level for reproductive success in Brown Pelicans. DDE at 2.8 mg/kg wet mass have been associated with reproductive impairment of piscivore birds, while 1 mg/kgwm has been linked to reduction of heron survival. The levels in some sparrow eggs were also very high (210 mg/kg lipid). NMDS shows that the vector for p,p'-DDT was strongly associated with House Sparrows. The terrestrially feeding Cattle Egrets were associated with the more volatile components of DDT, as well as with PCBs and chlordanes. It is likely that the heron population is under severe threat. DDT levels in Cattle Egrets, although much lower than in the Grey Heron, were higher than in any other known instance of this species from Africa. The DDT levels in House Sparrow eggs were possibly the highest ever recorded. The pollutant profiles of both sources converged in the same congener profile in Grey Heron eggs, indicating the sensitivity of aquatic birds.

**WE129 Exposure to Pb in urban environment impairs breeding success but favours survival in the European blackbird** C. C. Eritsch, University of FrancheComte CNRS / UMR ChronoEnvironnement; H. Pietruszewska, University of Szczecin / Department of Vertebrate Anatomy and Zoology; R. Scheiffler, University of FrancheComte / Chrono-Environment, UMR 6249 University of Franche-Comté / CNRS; D. Wysocki, University of Szczecin / Department of Vertebrate Anatomy and Zoology. Negative effects of environmental metal pollution on bird survival and reproductive success have been found in highly contaminated sites. Besides, it has been shown that avian survivorship and productivity may be decreased in urban areas. These negative effects may be related to some disturbances characteristic of urban ecosystems (permanent presence of pedestrians, poor-quality food resources, habitat fragmentation, light and noise pollution, etc). However, little is known about the role of chronic low-dose exposure to persistent contaminants typically present in urban environments such as Pb. Given the worldwide increase of the urbanization process, it is critical to investigate whether the exposure to pollutants may affect urban bird populations. Within the framework of studies dealing with the ecology of the European blackbird *Turdus merula* in Szczecin (Poland),



individuals have been surveyed for years. Tail feathers were sampled when birds were captured for ringing, and Cd and Pb concentrations were measured. Survival in the 3 consecutive years following feather sampling was computed ( $n=113$ , 92 and 91 for the 1st, 2nd and 3rd year, respectively). Breeding success (number of fledgling/breeding season) was computed on at least 3 breeding seasons ( $n=50$ ). Metal concentrations ( $0.22\pm0.15$  and  $5.8\pm3.6$   $\mu\text{g/g}$  for Cd and Pb, respectively) were within the range of values reported for this species in urban and lowly/moderately polluted sites. A decrease of breeding success with Pb levels in feathers was found (Poisson GLM,  $p=0.02$ ), with a marginally significant gender-based difference ( $p=0.08$ ), females exhibiting a sharper decrease than males. A positive relationship between survival in the 2nd and 3rd year after feather sampling, and Pb levels was found (binomial GLM,  $p=0.05$  and  $0.08$ , respectively). Concerning Cd, we did not detect any significant relationship with survival, but a marginally significant decrease of breeding success ( $p=0.06$ ). Such opposite responses to Pb exposure in terms of breeding success and survival might mirror a trade-off between reproduction and individual health costs, the most stressed birds investing more on their own survival than on breeding, which matches the “stress hormone hypothesis” proposed by Eeva et al (2006) but without gender-related differences. Altogether, the results strongly suggest that chronic low-dose Pb exposure affects life-history traits in urban blackbird populations.

**WE130 Expression of carotenoid-based trait in blackbirds *Turdus merula* increases with metal pollution level** C.C. Fritsch, University of FrancheComte CNRS / UMR ChronoEnvironnement; L. Bervoets, University of Antwerp / Dept. of Biology, Systemic Physiological and Ecotoxicological Research group; R. Pinxten, University of Antwerp / Dept. of Biology, Ethology research group; J. Van Camp, University of Antwerp; M. Boshoff, University of Antwerpen / Dept. of Biology, Systemic Physiological and Ecotoxicological Research group; R. Mateo, UCLMCSIC / Instituto de Investigacion en Recursos Cinegeticos; M.E. Ortiz Santaliestra, Instituto de Investigacion en Recursos Cinegeticos / Forest and Wildlife Ecology; N. Vallverdu-Coll, Instituto de Investigacion en Recursos Cinegeticos; M. Poisbleau, University of Antwerp / Dept. of Biology, Ethology research group; G. Lepoint, University of Liege / Department of Oceanology; K. Das, University of Liege / Laboratory for Oceanology; R. Scheifler, University of FrancheComte / Chrono-Environment, UMR 6249 University of Franche-Comté / CNRS; M. Eens, University of Antwerp / Dept. of Biology, Ethology research group. Carotenoid-based traits in vertebrates, and notably in birds, are considered as “honest signals” of their bearers’ genotypic and phenotypic quality, and thus ultimately of their survival ability and reproductive value. The expression of carotenoid-based ornaments has been widely studied, and is known to be affected by different environmental stressors. For instance, metal environmental pollution has been shown to fade the yellow plumage of the great tit *Parus major*, and it has been suggested that this is related more to depletion of carotenoid-rich food than to metal-induced oxidative stress. We tested the hypothesis that metal pollution affects the expression of a carotenoid-based trait in the European blackbird *Turdus merula* (a sexually dimorphic species in which males exhibit a yellow-orange beak), and investigated whether this may be related to direct toxic effects impairing health and anti-oxidant status and/or to indirect effects related to diet. Fifty-five free-living males were captured during the breeding season along a pollution gradient in Antwerp (Belgium). They were aged (yearling versus older), beak reflectance spectra were measured, and blood and feather samples were collected. Body condition index, hematology, and plasma biochemistry were studied to assess health status. Preliminary analyses showed an increase of beak coloration with environmental metal pollution: carotenoid chroma increased and hue decreased (more orange beak) with pollution level. Brightness did not vary with pollution level. Some health parameters exhibited relationships (positive except for K) with carotenoid chroma (namely Ca, albumin and uric acid levels, and ALP activity in plasma) or hue (MCH and MCHC of blood, plasma K and Mg levels and ALT and AST activities), but according to age-specific patterns in most of cases. However none of the parameters studied as health indicators was

found to differ according to pollution level, except plasma total protein levels in older birds only. Altogether, the results strongly suggest that the factors explaining the increase in beak coloration with pollution were not yet identified. Ongoing analyses on individual exposure (metals in feathers and blood), oxidative stress (blood antioxidant levels and lipid peroxidation) and diet (stable isotopes of C and N in blood and diet items) might allow revealing the mechanisms underlying such a beak color enhancement with metal environmental pollution.

**WE131 Trace metal elements in the common pipistrelle bat (*Pipistrellus pipistrellus*)** B.V. Hernout, University of York / Environment; A. Boxall, University of York / Environment Department. Bats might be expected to be particularly sensitive to chemical contaminants as they are a long-lived species for their body size, they can accumulate contaminants and they also consume a large amount of prey each night which may have been exposed to contaminated soils and water bodies. For example, trace metal can be transferred through the food chain and can therefore be accumulated in bat tissues, fur and bones. To assess the potential risks of metals to bats in the UK, around 200 bats (Adult males) were obtained from different areas across England. They were representative of the metal concentration range for soils in England and Wales. Bats were dissected to extract kidneys, liver, stomach, fur and bones (humerus, radius and femurs). Concentrations of cadmium, copper, lead and zinc in organs and bones were then determined by ICP-MS. Preliminary results (from 93 individuals) showed that average concentrations ranges for the metals were: 0.06 (liver) – 2.94 (fur) mg/kg for Cd; 4.63 (bones) – 26.06 (fur) for Cu; 1.39 (liver) – 678 (fur) mg/kg for Pb; and 24.7 (liver) – 287.9 (bones) mg/kg for Zn. Comparison of tissue concentrations with toxicity data for mammals indicates that approximately 9% of the bats have Pb concentrations of potential concern for bat health.

**WE132 Metal and metalloid concentrations in the livers of Eurasian otter (*Lutra lutra*) from England and Wales** L. Walker, Centre for Ecology Hydrology; A. Lawlor, Centre for Ecology and Hydrology; E.A. Chadwick, Cardiff University / Cardiff School of Biosciences; E. Potter, Centre for Ecology and Hydrology; M.G. Pereira, Centre for Ecology Hydrology; R.F. Shore, CEH Lancaster. The Eurasian otter (*Lutra lutra*) is a top predator in freshwater habitats, feeding predominantly on fish. As a top predator, the otter is exposed, through its diet, to contaminants that bioaccumulate and, in particular, bioconcentrate through food chains. Otter populations have declined markedly over the past 50 years throughout much of the species’ range, particularly in Western Europe. These declines have been attributed, at least in part, to environmental contaminants including Dieldrin and PCBs, although concerns relating to the effects of mercury (Hg), particularly methyl mercury, have been reflected in the majority of studies on metals in otters including work on Hg. Exposure to metals in wildlife may vary geographically due either to natural variation, resulting from weathering of under-lying metal rich rocks, or pollution resulting from local activities such as a metal ore smelting or from the global transfer of metals, for example the release of Hg from coal burning power stations. There is surprisingly little information on metals concentrations in top predators such as the Eurasian otter and very few studies have been conducted in the UK. Those studies in the UK that have reported metals in otters are from limited spatial distributions or do not report the timeframe over which the samples were taken. This study reports spatial variation in the hepatic residues of 17 inorganic elements, including toxic, trace and essential metals, in Eurasian otters sampled between 2007 and 2009. These residues will be assessed in comparison to other studies in Europe and related to possible adverse effects, including correlations with essential and trace metal concentrations and body condition.

**WE133 Is ALAD polymorphism characteristic for animal populations as it is for human?** M. Mikowska, R. Swiergosz-Kowalewska, Jagiellonian University / Institute of Environmental Sciences. Delta aminolevulinic acid dehydratase gene polymorphism found in lead exposed human populations show importance of genomic

differences in body lead management. ALAD polymorphism in human results in existence of two alleles - ALAD1 and ALAD2. The difference between them applies to one nucleotide exchange (one SNP), which causes amino acid substitution (lysine for asparagine). The three possible isosymes (ALAD 1-1, ALAD 1-2, ALAD 2-2) differ in electric charges. It is thought that all ALAD2 carriers have more electronegative form of ALAD enzyme, what increases its affinity to lead ions. Obtained results show a still existing need for complex studies based on biomarkers for better explanation the mechanisms contributing to the differences between ALAD1 and ALAD2 carriers. Our research tried to answer question about possible occurrence of these two alleles in animal populations, by studying ALAD polymorphism in bank vole (*Myodes=Clethrionomys glareolus*) from clean and polluted sites. For purpose of this study we used animals from six sites: three clean forests (Mikolajki, Niepolomice, T. Oszwara) and three from areas located near lead/zinc smelters in Southern Poland (Katowice, Olkusz, M. Sl

**WE134 Monitoring the Exposure of Red Kites to Chemicals** M. Coeurdassier, J. Montaz, M. Jacquot, N. Crini, C. Druart, C. Amiot, University of Franche-Comte / Department Chrono-Environnement - UMR 6249 UFC/CNRS; I. Fourel, Vetagro-sup, campus vétérinaire / Toxicology; P. Berny, VETAGROSUP / Toxicology; R. Scheiffler, University of FrancheComte / Chrono-Environnement, UMR 6249 University of Franche-Comté / CNRS; C.C. Fritsch, University of FrancheComte CNRS / UMR ChronoEnvironnement; L. Caillet, C. Morin, LPO Franche-Comté; R. Riols, LPO Auvergne. The red kite *Milvus milvus* has experienced a population decline in Europe and is consequently classed as "Near Threatened" by the International Union for Conservation of Nature. Poisoning by pesticides, notably anticholinesterase chemicals and anticoagulant rodenticides (AVKs), is though as one of the main threats to this raptor. Moreover, some studies have suggested that it can be exposed to other chemicals such as metals and/or (non-pesticides) persistent organic pollutants (POPs) but this remains little documented. Within the framework of the national conservation plan, some breeding populations of red kite are monitored in France since 2006, several hundreds of nestlings being counted and handled for biometric measurements, ringing, and wing tagging each year. This offers an opportunity for tissue and more particularly blood sampling in order to assess exposure to chemicals. During the spring 2012, a small quantity of blood (~ 1.5 ml) was sampled from 40 nestling kites in the breeding population of the Franche-Comté region (Eastern France). Blood residues of metals/metalloids (As, Cd, Pb, Hg, Zn, Ni, Cr...) are currently under analysis. Specific methodological assays are in progress to check whether blood is usable for AVK exposure assessment. Moreover, 12 corpses of adult Red kites were collected following a dramatic poisoning event with the AVK bromadiolone in the region Auvergne (Center of France) in 2011. We expect to monitor their exposure to metals/metalloids (As, Cd, Pb, Hg, Zn, Ni, Cr...) and POPs, including organochlorines and PBDE. Results will be available for the congress and we expect that they will allow us to: - define reference values of chemical concentrations in the blood of nestling red kites and determine abnormal exposure in kite sub-populations, - link exposure to chemicals with the breeding success between and within the sub-populations monitored. - determine the main chemicals that can be found in adult Red kites. This study is partly funded by the Agence Nationale de la Recherche (RODENT programme, convention 2009CESA00801) and the French ministry for Ecology and Sustainable Development in the framework of the national plan for the conservation of Red Kite.

**WE135 Brominated Flame Retardants: Spatial and Temporal Patterns and Trends in Seabird eggs from the Pacific Coast of Canada** J.E. Elliott, Environment Canada; K. Elliott, M.F. Guigueno, University of Manitoba; L.K. Wilson, S. Lee-Ortiz, A. Idriszez, Environment Canada. Brominated flame retardants (BFRs) have been widely used to reduce fire hazards. One class, the polybrominated diphenyl ethers (PBDEs), are particularly persistent bioaccumulative and toxic chemicals, now classified as POPs under the Stockholm Convention. Marine ecosystems are the ultimate sink for POPs, and thus

there is a continuing need to monitor such contamination. Eggs of marine birds have proven to be an efficient and effective means of measuring and tracking xenobiotic compounds which are transferred from the female bird to the egg via yolk lipids or proteins. Here we report and discuss data from long term monitoring of BFRs in seabird eggs from the northeast Pacific. For this program, the marine system was divided, and representative species selected. The nearshore subsurface is monitored using two cormorant, *Phalacrocorax*, species, *auritus* and *pelagicus*, both feed on a variety of benthic and pelagic fish. The offshore subsurface is monitored using the rhinoceros auklet, *Cerorhinca monocerata*, a feeder mainly on small pelagic fishes, with the offshore surface species, the Leach's storm-petrel, *Oceanodroma leucorhoa*, which feeds mainly on surface plankton and larval fishes. At three breeding colonies each along the Pacific coast of Canada and at four year intervals 15 eggs are collected and archived. Data from a recent retrospective study, using archived samples collected from 1990 to 2011, shows, as reported for more polluted environments, that PBDEs increased in continental shelf ranging auklet eggs until the early 2000s and have declined since then, in response to restrictions on usage. In contrast, another BFR compound, HBCD (hexabromocyclododecane), increased steadily in eggs of both near and offshore species. The possible role of dietary variation, potentially related to marine regime shifts, will be examined by use of stable isotopes in variation in contaminant levels in these monitored seabirds.

**WE136 The influence of POP and metal contamination in Flemish water bodies (Belgium) on ecological water quality and biota populations** E. Van Ael, University of Antwerp / Department of Biology; C. Belpaire, J. Breine, C. Geeraerts, G. Van Thuyne, Research Institute for Nature and Forest (INBO); I. Eulaers, University of Antwerp / SPHERE; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology; L. Bervoets, University of Antwerp / Dept. of Biology, Systemic Physiological and Ecotoxicological Research group. Worldwide industrial development, intensive agriculture and high population densities have led to the presence of numerous pollutants in the aquatic environment. These pollutants such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and metals, threaten the ecological quality of rivers, canals and lakes, and can cause adverse effects on local invertebrate and fish communities. However, it is hard to determine what pollution level will lead to detrimental effects on population level, because low level effects are not always translated into higher level effects and often they will manifest on a long term base. Indices, such as the Belgian Biotic Index (BBI) and Ecological Quality Ratio (EQR), try to describe the ecological quality of a water body by studying its macroinvertebrate or fish communities respectively. The indices are based on several parameters, including species composition and their tolerance for disturbance. Since a well-balanced and adaptive community of organisms can only be maintained by a healthy ecosystem, the indices reflect the ability of the ecosystem to do so. In this study, pollution concentration levels of PCBs, pesticides and metals in sediment and European eel (*Anguilla anguilla*) of Flemish fresh waters, are linked with the Belgian Biotic Index (BBI) and Ecological Quality Ratio (EQR), as indicators of the ecological water quality. The main objectives of this study were 1) to investigate if ecological water quality, as indicated by BBI and EQR, was correlated with the pollution levels, 2) to determine which parameters (PCBs, metals, O<sub>2</sub>, water depth,...) influence the ecological status of Flemish water bodies the most 3) to formulate concentration thresholds from which a community effect occurs.

**WE137 Effect assessment of fipronil treated seeds in red-legged partridges** A. LópezIREC / Departament of Ecotoxicology; M.E. Ortiz Santaliestra, Instituto de Investigación en Recursos Cinegeticos / Forest and Wildlife Ecology; F. Mougeot, EEZA; R. Mateo, UCLMCSIC / Instituto de Investigación en Recursos Cinegeticos. Seed coating is a widespread practice in agriculture that reduces environmental risks by minimizing diffuse application of pesticides. However, for granivorous farmland birds like the red-legged partridge

(*Alectoris rufa*), treated seeds provide large amounts of food in a short time, thus posing a risk of poisoning. Fipronil is a phenyl pyrazole insecticide used for maize seed coating whose toxicological effects on avian species have hardly been studied. The aim of this study was to determine the responses of partridges when fed with a mixture of commercial fipronil-coated and untreated maize seeds through the analysis of i) the degree of rejection of the treated seeds, and ii) the effects their ingestion on bird survival, physiology, fitness and reproduction. Thirteen breeding pairs were fed with a 20:80 mixture of treated and untreated seeds during ten days while other 13 pairs were fed with untreated maize only. Exposition lasted for 10 days in April coinciding with the maize sowing season. Partridges did not discriminate between treated and untreated seed, being the percentage of treated seeds in the diet (17%) not statistically different from what was initially provided. However, exposed partridges reduced their overall amount of consumed food with respect to controls ( $10.13 \pm 1.06$  vs  $33.47 \pm 10.95$  g / individual / day;  $p < 0.001$ ). We found three casualties in the fipronil treatment versus none in the controls ( $p = 0.083$ ). Exposure to fipronil treated seeds also tended to reduce body condition in a 19.6% compared to controls ( $p = 0.069$ ), decrease the eye ring pigmentation (10.7% lower than in controls;  $p < 0.001$ ) and compromise the cellular immune response (27.53% lower than in controls;  $p = 0.059$ ). With regards to reproduction, we found an increase in the mass of fertilized eggs laid by the treated partridges (3.4% heavier than controls;  $p = 0.043$ ) but a reduced fecundation rate (69.05 vs 84.05%;  $p = 0.062$ ). The use of maize treated seeds is a widespread practice and although at the time of maize sowing partridges may have alternative food sources, the ingestion of a small amount of these seeds causes significant sublethal effects that may ultimately contribute to population decline. Upcoming studies must include an assessment of the environmental exposure of partridges to maize coated seeds. Financed by FEDENCA and Oficina Nacional de la Caza with the partnership of Fundación Biodiversidad.

**WE138 Monitoring Plant Protection Product and Rodenticide Exposure in Scottish Wildlife** J. Hughes, E. Sharp, L. Melton, SASA, Scottish Government / Pesticides and Wildlife; M. Taylor, Scottish Agricultural Science Agency. Scottish wildlife is exposed to a range of environmental contaminants, including secondary exposure to plant protection products and biocides such as rodenticides. The Wildlife Incident Investigation Scheme (WIIS) Scotland at Science and Advice for Scottish Agriculture (SASA) operates a monitoring programme of rodenticide exposure levels in Scottish wildlife. Rodenticide residues have been found in a range of non-target animals including iconic Scottish species such as golden eagles, otters, pine martens and Scottish wildcats. The WIIS has also employed an extended analytical method screening for plant protection products in non-target species since 2010. The results of both monitoring programmes are summarised in this poster and exposure data are compared with Scottish rodenticide and PPP use patterns collected by the pesticide survey unit (PSU) at SASA.

**WE139 Biomarker investigation on the contaminant exposure of European Eel (*Anguilla anguilla*) in German rivers** M. Brinkmann, RWTH Aachen University Institute for Environmenta / Department of Ecosystem Analysis; S. Stoffels, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis; M. Freese, J. Pohlmann, U. Kammann, Thünen Institute of Fisheries Ecology; R. Hanel, Thünen Institute / Institute of Fisheries Ecology; H. Hollert, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis. The panmictic stock of the European Eel (*Anguilla anguilla*) continuously declines since the early 1980s and reached a new minimum in 2011. According to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), it is classified as a species deserving special protection according to Appendix 2. The IUCN red list classifies the European Eel as „critically endangered“. There are several hypothesized causes for this population decline of the European Eel. Among others, habitat destruction, migration obstacles, overfishing, parasitism and diseases, but also climate change are under discussion as potential

causes. In addition, the effects of environmental pollution on physiology and fitness of spawners are unknown - especially in light of the migration of eels to their spawning grounds in the Sargasso Sea, which are located several thousand kilometers away from the continental growth (or feeding) habitats. Pollutants like polychlorinated biphenyls (PCBs), polychlorinated dioxins/furans (PCDD/Fs) and polycyclic aromatic hydrocarbons (PAHs) are still present in fresh water sediments at high concentrations, some of which highly accumulate in fatty tissues and are known to have diverse negative effects on aquatic organisms. In context of the European Data Collection Framework (DCF), a large number of eels were derived from commercial fishermen for gathering biological information for stock assessment, covering several German river catchments. In this context, liver tissue was sampled for analyses of several biomarkers. Here, we report on the measurement of EROD (ethoxyresorufin O-deethylase) and GST (glutathione S-transferase) activities in liver homogenates that are, among others, indicative of exposure with PCBs, PCDD/Fs and PAHs. EROD activity differed significantly between the different catchments. Specimens from the Elbe catchment showed the highest values. In the lower Elbe River, a gradient was observed, where the activities increased following the course of the river. Furthermore, the maturation stage of the eels had an influence on EROD activities, where silver eels (the non-feeding migratory stage) had significantly lower activities than yellow eels (the feeding pre-migratory stage). In conclusion, the different biomarker responses indicate differential contamination of the river catchments. In combination with other studies, our results could help identify suitable regions for stocking measures.

**WE140 Effects of sertraline on feeding behavior and success of planktivorous perch (*Perca fluviatilis*)** M.L. Hedgespeth, Lund University / Dept of Biology; J. Jonsson, Lund University / Department of Chemistry; O. Berglund, Lund University / Dept of Biology. This study examines the behavioral effects of pharmaceutical exposure on interactions between aquatic predators and prey, i.e. the European perch (*Perca fluviatilis*) and the small crustacean *Daphnia magna*. The effects of the selective serotonin reuptake inhibitor (SSRI) sertraline were quantified on predator feeding rates at both low and high prey densities to examine changes in the predators' functional response. Sertraline exposure resulted in decreased feeding rates of fish at low and high prey densities in comparison to controls, indicating a decrease in both predator search rate and handling time. The application of ecological models to this work allows for predictions in terms of the effects of pharmaceuticals on population dynamics of aquatic organisms, and potentially community structure in aquatic environments.

**WE141 Evaluating the minimum effective dose of rotenone for the eradication of alien smallmouth bass (*Micropterus dolomieu*) from a South African river** M. Jordaan; O. Weyl, South African Institute for Aquatic Biodiversity. The Rondegat River, situated in the Cape Floristic Region, was the first river in South Africa where the piscicide rotenone was evaluated as a potential biodiversity restoration tool. The presence of alien invasive smallmouth bass (*Micropterus dolomieu*) has caused a local extirpation of the highly threatened endemic fish community. The removal of this species will potentially allow recolonization of the native species. In preparation for this treatment, the sensitivity of smallmouth bass to various concentrations of a rotenone formulation (5% active rotenone) was evaluated using standard toxicity tests to determine the minimum effective dose (MED) that would result in 100% mortality after an exposure of four hours. The MED was 0.0125 mg/L active rotenone, resulting in complete mortality of smallmouth bass within four hours of exposure. Adverse effects, which included erratic swimming, loss of equilibrium, and death, occurred in a dose-dependent manner with smaller fish responding faster than larger fish. Standard operating procedures for the use of rotenone in streams recommend treatment at twice the calculated MED but due to the uncertainty associated with rotenone losses under field conditions (e.g. through hydrolysis and photolysis) and the possible occurrence of smallmouth bass larger than those tested, a concentration of twice the recommended treatment dose (0.050 mg/L rotenone) was finally used to

treat the Rondegat River for a duration of six hours. This resulted in the reduction of smallmouth bass to undetectable levels, providing strong evidence for successful eradication.

**WE142 An assessment of contaminants and their biological effects in Scottish flatfish** C.D. Robinson, K. MacNeish, L. Webster, Marine Scotland Science; J. Barsiene, L. Butrimaviciene, Natural Research Centre; J. Bignell, I. Katsiadaki, M. Sebire, Cefas; M. Gubbins, Marine Scotland Science. The UK is committed to undertaking monitoring and status assessments of the marine environment under the Joint Assessment and Monitoring Plan of the OSPAR Convention on Protection of the NE Atlantic; Marine Scotland Science are responsible for undertaking this monitoring in Scottish waters. The recent EU Marine Strategy Framework Directive also requires regular assessments of environmental status. Here we present findings of a recent research project designed to demonstrate an integrated status assessment of contaminants and their effects in Scottish flatfish. Dab (coastal/offshore) or flounder (estuarine/inshore) were collected in winter 2010/11 from 10 sites around the Scottish coastline, including the industrialised estuaries of the Forth and Clyde and analysed for contaminants and biological effects. PCBs, PBDEs, Pb and Cd were determined in fish liver, Hg in fish flesh and PAH metabolites (1-hydroxypyrene) in fish bile. The biological effects studied included hepatic CYP1A activity (EROD), hepatic lysosomal membrane stability, hepatic and gonadal histopathology, erythrocyte micronucleus frequency and plasma vitellogenin concentrations. Analytical results were compared with internationally recognised Assessment Criteria (ACs) to inform on environmental and health status. Contaminant concentrations were generally lower in fish from the offshore sites and higher in fish from the estuarine sites. At most offshore sites, contaminant concentrations and biological effects responses in dab were close-to-background. Exceptions included Hg, which exceeded the WFD EQS at all sites, and Cd and liver neoplasms at the east coast offshore site, which also had elevated micronucleus frequency. Inshore, liver PCB concentrations were above the upper Assessment Criteria in the Firth of Clyde, and biological effects responses were often above background. Despite close-to-background concentrations of the determined contaminants at most sites, elevated levels of biological effects, including liver tumours, were observed at some sites. These could result from other, unmeasured, contaminants, and/or exposure to contaminant mixtures. In most cases, contaminant concentrations and biological responses were greatest in the industrialised estuaries, although some coastal and offshore sites (e.g. St Andrews Bay, Solway Firth, Montrose Bank) also showed above background responses.

**WE143 Vg mRNA induction in an endangered fish species (*Anguilla anguilla*) from the Loire Estuary (France)** J. Blanchet Letrouve, Université de Nantes, LUNAM université, Université de Nantes, MMS, 1 rue G. Veil, 44035 Nantes; A. Lafont, Muséum National d'Histoire Naturelle; L. Poirier, Université de Nantes / UFR Sciences Pharmaceutiques; S. Baloché, Muséum National d'Histoire Naturelle; A. Zalouk Vergnoux, Université de Nantes; S. Dufour, Muséum National d'Histoire Naturelle / UMR BOREA; C. Mouneyrac, Université Catholique de l'Ouest / MMS EA 2160. Estuarine zones are extremely fragile due to increasing stress from anthropogenic activities. The Loire estuary (France) is potentially exposed to various contaminants including Endocrine Disruptors Compounds (EDCs) able to impact the reproduction physiology of fish. Even if the European eel (*Anguilla anguilla*) is not recognized as the most suitable sentinel species among Teleosts, this study aimed to investigate whether it may still be the target of estrogenic disruption, which could contribute to the decline of this species. Vitellogenin (Vg) appears as a valuable biomarker of EDCs, as well as for exposition and effects. Quantitative real-time Reverse Transcription Polymerase Chain Reaction (qRT-PCR) was used in this study to amplify responses of hepatic Vg transcripts. European eels (*A. anguilla*) were sampled in May 2009 (N=57) and November 2010 (during the downstream migration, N=10) in two sites of the Loire estuary (upstream: Varades; downstream: Nantes) potentially differing by their level of contamination.

Reproductive (gender, sexual maturity stage) and biometric parameters of collected eels were determined. A laboratory exposure of silver male to steroid hormones (Testosterone (T), 11-KetoTestosterone (11-KT), Estradiol (E2)) was conducted in parallel to validate the qRT-PCR approach on hepatic Vg mRNA. Results enabled to show the responsiveness of exposed silver male eels, since hepatic mRNA Vg induction was observed in E2-treated males compared to control specimens. In the field, while only female silver eels should express hepatic Vg mRNA, abnormal levels were also detected in a large proportion (38%) of the other individuals: undifferentiated, yellow females, yellow and silver males. This field study provides the first evidence of xenoestrogenic exposure in the European eel from the Loire estuary.

**WE144 *Campylobacter*, *Salmonella*, vancomycin resistant *Enterococcus*, antibiotic resistant Gram-negative bacteria from crows in urban/agricultural settings** M.C. Roberts, University of Washington; J. DeLap, University of Washington / School Environmental & Forest Sciences; D. No, University of Washington / Environmental & Occupational Health Sciences; J. Marzluff, University of Washington / School Environmental & Forest Sciences. Isolation of *Campylobacter*, *Salmonella*, vancomycin resistant *Enterococcus*, antibiotic resistant Gram-negative bacteria from crows in urban and agricultural settings Marilyn C. Roberts, Jack DeLap, David No, and John M. Marzluff Department of Environmental & Occupational Health Sciences and School of Environmental & Forest Sciences, University of Washington, Seattle, WA 98195 USA; E-mail contact: marilyn@u.washington.edu The general role of birds as reservoirs and vectors for the spread of infectious disease, while acknowledged, is not fully understood in the context of coupled agricultural and urban human systems. We have hypothesized that close coupling of human and natural systems allows pathogenic bacteria and antibiotic resistant bacteria to be shared between the environment, wild birds and people. Crow and cow feces were collected from three dairy farms surrounding agricultural landscapes of Seattle and from crow feces collected three times from a sewage treatment plant along with primary and secondary wastewater in urban Seattle during 2012. Two more collection time points are planned in 2013. We collected cow feces directly from barns and collected primary and secondary wastewater samples. Standard protocols were used to isolate *Campylobacter*, *Salmonella*, vancomycin resistant enterococci (VRE), ampicillin, chloramphenicol and/or tetracycline resistant Gram-negative bacteria. Thirteen [42%] of the crows carried one or more of the three pathogens, with five (16%) of the crow samples positive for *Campylobacter*, *Salmonella*, and vancomycin resistant enterococci [VRE], 26 (84%) of the crows carried antibiotic resistant Gram-negative bacteria [ARGN]. Three (75%) of manure samples were positive for *Campylobacter* and/or *Salmonella*, and/or VRE and/or carried ARGN. All manure samples tested directly carried specific macrolide and/or tetracycline resistance genes, with 1-3 different genes/sample. Five (71%) samples had culturable *Salmonella*, four (57%) samples had culturable *Campylobacter*, or VRE. Findings from this pilot study indicates that crows may effectively spread these microbes and play an important role in the epidemiological dynamics of microbial carriage and disease transmission in nature as well as contribute to the evolutionary dynamics and spread of antimicrobial resistant bacteria and genes between wildlife, the environment and man.

**WE145 Epidemiology and pathology of chronic industrial fluoride toxicosis in Australian marsupials** C.E. Death, J. Hufschmidt, The University of Melbourne / Faculty of Veterinary Science; G.M. Coulson, The University of Melbourne / Department of Zoology; I. Beveridge, The University of Melbourne / Faculty of Veterinary Science. Particulate and gaseous fluoride emissions contaminate vegetation surrounding fluoride-emitting industry, with potential for skeletal and dental disease following long-term consumption by herbivorous wildlife. Eastern grey kangaroos (*Macropus giganteus*) resident near an aluminium smelter in southeastern Australia have been affected by chronic fluoride toxicosis. In the current study we extend the investigation to other species of marsupials. We discuss dental and

skeletal pathology in relation to species, age, bone fluoride concentration and location relative to the emission source. Necropsy examinations of red-necked wallabies (*Macropus rufogriseus*), swamp wallabies (*Wallabia bicolor*), koalas (*Phascolarctos cinereus*), brushtail possums (*Trichosurus vulpecula*) and ringtail possums (*Pseudocheirus peregrinus*) from the smelter site have revealed varying degrees of skeletal and dental lesions consistent with fluorosis. Through better understanding of how dietary fluoride exposure leads to clinical fluorosis in marsupials, including further description of the associated epidemiology and pathology, we aim to produce management options that may prevent disease in marsupials resulting from industrial fluoride emissions.

**WE146 Spatial models to assess the risk of trace metal exposure to avian populations through food chain transfer.** K. Arnold, University of York / Environment Dept; B.V. Hernout, University of York / Environment; A. Boxall, University of York / Environment Department. Trace metal concentrations in the environment have increased above background levels over the past two centuries largely due to human industry. There is growing concern over the effects of these metals on wildlife. Wild bird populations around smelting works have been shown to be sensitive to metal residues, with health, behaviour and reproductive effects being seen. Such local studies, however, do not give an indication of the risk to birds across their range. This project aimed to use a model incorporating spatial distribution to determine the risk of trace metal exposure to wild bird populations across England and Wales: First, we estimated the level of metal uptake from soil in different regions to invertebrate prey. Second, we calculated the exposure of avian species across their geographic range to metals through their diet. Finally, the risk of toxic effects due to exposure was characterised. Incorporation of GIS allowed risk maps to be produced, identifying regions of highest risk based on soil contamination and species' distribution. The regions of highest risk were found to be the former mining areas, particularly the uplands of England and South Wales. The risk posed by each metal was ranked in the order: Pb > Cd > Zn > Cu. The species most at risk of metal poisoning were shown to be the blackbird *Turdus merula*, song thrush *T. philomelos* and starling *Sturnus vulgaris*. The main risk factor for metal exposure was the inclusion of earthworms in the diet. Nestling birds were found to have significantly greater exposure to trace metals due to a higher proportion of invertebrates in their diet than adults. This model provides a low cost and time efficient method of assessing risk of environmental contaminants to species across their range. The model now needs validating with field studies of toxicological and population effects, become it can be used in the development of avian conservation plans and the derivation of soil guideline values for trace metals.

**WE147 Effects of Environmental Stochasticity on Individual Variability** A. McLeod, Great Lakes Institute for Environmental Research; D. Haffner, University of Windsor; K. Drouillard, University of Windsor / Great Lakes Institute for Environmental Research. Understanding the individual variation of contaminants is critical for quantifying hazards of contaminants to aquatic organisms and for the establishment of fish consumption guidelines. Until recently, individual variability was largely neglected, or treated as statistical noise as most hazard assessment models were based on steady state assumptions. For instance, models of contaminant accumulation simulate the chemical body burden of the average fish, failing to incorporate the inherent inter-individual variation present in natural populations. Polychlorinated Biphenyls (PCBs) have been recognized as persistent, toxic chemicals since the 1970s and decades of research have gone into understanding and quantifying their accumulation in aquatic ecosystems. Previous research pinpointed water exposure, sediment exposure, and dietary exposure as drivers of variability, and their contribution varied with changes in log( $K_{ow}$ ). Here I further test the strength of these possible drivers by comparing results from a caged fish experiment looking at temporal variation between fish held at different depths in the water column. Caging fish ensures that all fish within the same cage will experience the same environment, allowing us to resolve the relative

importance of environmental drivers and physiological drivers of contaminant variability. Finally, it allows us to test the relative importance of water exposure and sediment exposure to variability in low and high log( $K_{ow}$ ) congeners.

**WE148 Determination of PT in wildlife risk assessments - a new option** D. Nickisch, Efate & Modelling; J. Ludwigs, Rifcon GmbH; F. Von Blanckenhagen; C. Dietzen, Rifcon GmbH. The 'proportion of time in treated area' (hereafter PT) is a refinement factor for higher tier risk assessments and modeling of dietary exposure. Based on the theoretical background (EFSA Wildlife guidance 2009) PT means the time where an individual is potentially at risk from intake of contaminated food. For the determination of the PT an individual is radio-tracked during a whole day. Based on these data a minimum convex polygon (hereafter MCP) is derived which will be overlaid with land use maps. We compared the MCP method with two recently developed methods for determining animal movements based on GPS data: a) Brownian bridge movement model (BBMM) and b) dynamic Brownian bridge movement model (dBBMM). The models are continuous-time stochastic models of movement in which the probability of being in an area during the time of observation is conditioned on starting and ending locations. Both methods identify the 'habitat use' of an individual based on its movement path rather than individual points. The difference between the two methods is that BBMM assumes homogenous movement behavior whereas the dBBMM allows heterogeneous changes using likelihood statistics. We are further interested in how many observation points are necessary to derive a plausible polygon with the BBMM methods because the MCP method efforts high temporal resolution data for active species. Thus, it is essential that an individual is not lost for a longer time period during a day. The more observations could be taken the greater is the likelihood that the MCP polygon reflects the home range of an individual. Based on different telemetry data sets of birds we demonstrate and discuss the results of these different PT determinations. Since the dBBMM has recently been published the evaluation of PT is still in process, but first results showed that MCP differs from PT values derived by the other two methods.

**WE149 Application of enzyme immunoassay for measuring toxicity of chlorinated/brominated dibenzo-p-dioxins and dibenzofurans in industrial source samples** E. Samara, American University of Sharjah / Biology, Chemistry and Environmental Sciences; B.K. Gullett, U.S. Environmental Protection Agency / Office of Research and Development, National Risk Management Research Laboratory. Enzyme immunoassay (EIA) measures cross-reactivities (the ability of an analyte molecule to bind to the anti-dioxin antibody in comparison to a standard molecule) and sample based toxic equivalents (TEQs) by responding to the toxic polychlorinated dibenzo-p-dioxin/furan (PCDD/F) congeners in proportion to their toxic equivalency factors (TEFs). Traditionally, toxicity levels of chlorinated "dioxin-like" compounds have been determined by gas chromatography/high resolution mass spectrometry (HRGC/HRMS) analysis for congener concentrations which are weighted by congener-specific toxic equivalency factors (TEFs). Limited information is available on the applicability of PCDD/F toxicity assays to their brominated counterparts: polybrominated dibenzo-p-dioxins/furans (PBDDs/Fs). In this study, EIA-based TEQs, showed overestimation for PCDDs/Fs (0-4 orders of magnitudes higher) and underestimation for PBDDs/Fs (0-1 orders of magnitude lower) when compared to HRGC/HRMS-based TEQ calculation (using WHO TEFs) in samples from an industrial source line. No correlation was found between the EIA and the HRGC/HRMS data, which could be attributed to differences in homologue-specific cross-reactivity responses, sample matrix type, and presence of other compounds competing for antibody binding in the immunoassay.

**WE150 Use of zebrafish cell lines to assess estrogenic compounds in environmental samples** M. Sonavane, N. Creusot, E. Maillot-Marechal, F. Brion, S. Ait-Aissa, INERIS. Aquatic endocrine disrupting

chemicals (EDCs) encompass a great diversity of chemicals that affect exposed organisms through different molecular mechanisms, including their ability to bind to estrogen receptors (ERs) and to modulate the transcription of target genes. Till date, most of *in vitro* assays used to assess xeno-estrogens have been established using human ER in either yeast or mammalian cell lines. However, cross-species differences due to cellular context (metabolic capacities, transcription) or different receptor sequences may lead to significant differences between-assay in estrogenic potency of chemicals and eventually influence the response of complex environmental mixtures. We recently established an *in vitro* luciferase assay based on stable expression of zebrafish ER subtypes (zER?, zER?1, zER?2) in the ZFL zebrafish liver cell line (Cosnefroy et al 2012). Luciferase induction by 17?-estradiol (E2) in zER? and zER?2 cell lines results in EC<sub>50</sub>s of 0.2 and 0.03 nM, respectively. In the present study, we have implemented this assay to screen and characterize the individual effect of different chemical contaminants, including bisphenols, zearalenone, alkylphenols, parabens and phthalates. Comparison with the response in the human cell based MELN assay revealed differences in both sensitivity (i.e. EC<sub>50</sub>) and receptor binding potency (i.e. full versus partial agonists). In addition, the ability of the zebrafish *in vitro* assays to detect active compounds in complex mixtures will be tested by screening organic extracts of river sediment, effluents and surface waters. These results will be compared with those obtained with the MELN assay which will provide new information on fish specific effects of environmental xenoestrogens present in aquatic ecosystems.

**WE151 Zebrafish embryos as an integrative model to evaluate mechanism-specific toxicity of surface waters in Effect-Directed analysis** C. Di Paolo, RWTH Aachen University / Ecosystem Analysis ESA; M.M. Lam, RWTH University Aachen; T. Seiler, RWTH Aachen University / Institute for Environmental Research (Biology V); I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy, Physiology and Cell Biology; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis; H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V). The EDA-EMERGE Initial Training Network is developing a new generation of Effect-directed analysis approaches, integrating innovative biological and chemical tools for the improvement of monitoring of surface waters in Europe and identification of non-target substances. As part of this strategy, a simplified protocol consisting of the different methods involved in EDA is demonstrated in a joint European demonstration program, to evaluate surface water samples from selected contaminated and reference European sites. Our battery of tests is presented in another poster at this conference (see M.Lam *et al.*). In parallel, innovative biological and chemical methods are being developed, and as part of that initiative we propose the zebrafish embryo as an integrative model with strong value to evaluate mechanism-specific toxicity at the organism level. As a starting point, we extensively reviewed the literature to identify already established mechanism-specific methodologies using zebrafish embryos and non-feeding larvae. Methods evaluate a broad list of mechanism-specific toxicity, such as genotoxicity, dioxin-like activity, endocrine-disruption, neurotoxicity; using different strategies, that include adaptation of previously established mechanism-specific methods to the zebrafish embryos, -omics techniques as transcriptomics, reporter gene assays, and behavioral assays. Also, methods for the application of qPCR to zebrafish embryos were considered, as well as genes that might have relevance to assess emerging contaminants in surface waters. Finally, the applicability of such mechanism-specific methods with zebrafish embryos to EDA is evaluated, considering its specific methodological requirements and possible solutions to potential limitations, as small volumes of samples / fractions and high number of samples to be tested. The methods that are considered to be the most relevant for our application are being selected, and their further adaptation and improvement is planned. The ultimate aim is to adapt, improve and apply the selected mechanism-specific methods as part of the tiered testing in EDA approach to evaluate surface waters samples and fractions.

**WE152 Mixture aSSessment of Endocrine Disrupting Compounds (EDC) with emphasis on thyroidogenicity - using cats as model for human indoor exposure** J. Weiss; J. Norrgran, Stockholm University / Materials and Environmental Chemistry; L. Norrgren, Swedish University of Agricultural Sciences / Biomedical Sciences and Veterinary Public Health; G. Carlsson, B. Jones, Swedish University of Agricultural Sciences; P. Andersson, Umea University; J. Legler, VU University; A. Bergman, Stockholm University. In 2012 a research project funded by the Swedish research council started, abbreviated MiSSE (Mixture aSSessment of EDCs). The project consists of 4 partner Universities from Sweden and The Netherlands and will be active for 5 years. The research questions in MiSSE are devoted to today's most intensely discussed subjects regarding risks of exposure to anthropogenic endocrine disrupting compounds (EDCs); 1) the most critical chemicals for exposure indoors, including not yet known or poorly studied chemicals e.g. transformation products, 2) exposure to mixtures of chemicals, 3) thyroidogenic disruption as an endocrine endpoint of growing concern for human health and the environment, and 4) improved knowledge on exposure situation during early childhood. The project is aiming to assess the exposure situation to anthropogenic thyroid hormone disrupting compounds (TDCs) in homes; and accordingly the mixture effects of these compounds. Key emerging TDCs are searched for by applying emission modeling from indoor consumer goods, applying similarity models comparing known TDCs, and using the effect-directed analysis (EDA) approach to indoor pet cats as a model for the internal exposure to these chemicals and to dust as a route of external exposure. A battery of TDC *in vitro* screening tests and *in vivo* frog and fish models will be applied for detailed studies of individual compounds and realistic mixture signatures. The results are to be translated to improve the understanding of human and child exposures to indoor related chemicals and their thyroidogenic effects in mixtures, which is the central Hub of MiSSE. The project is aimed to feed in scientific results for improved management of EDCs. More information about the project is available on the webpage at [www.mmk.su.se/misse/](http://www.mmk.su.se/misse/)

**WE153 Effect-Based Water Quality Trigger Values Accounting for Mixture Effects of Organic Micropollutants in Water** J.Y. Tang, The University of Queensland / ENTOX; M. Dutt, E. Glenn, S. McCarty, The University of Queensland / National Research Centre for Environmental Toxicology (Entox); P.A. Neale, The University of Queensland / National Research Centre for Environmental Toxicology (EnTox); C. van Daele, The University of Queensland / National Research Centre for Environmental Toxicology (Entox); M.S. Warne, Centre for Envir Cont Research / Ecotoxicology Unit; B.I. Escher, The University of Queensland / National Research Centre for Environmental Toxicology (Entox); R. Altenburger, UFC Centre for Environmental Research / Department of Bioanalytical Ecotoxicology. Organic micropollutants occur in abundance and typically at very low concentrations in the environment. Complex mixtures of unknown composition are formed by biotic and abiotic transformation processes. Regulations and guideline values for mixtures are limited, in particular there are no effect-based water quality trigger values that relate to bioanalytical assessments. The mixture toxicity concept of concentration addition (CA) gives robust predictions based on information of the components for multicomponent mixtures of chemicals acting according to the same mode of toxic action. An extensive number of studies were undertaken with the Microtox and algal toxicity assays, however, no mixture toxicity concepts have been tested on adaptive stress response pathways. Here, we propose a systematic approach to derive effect-based water quality trigger values for three different types of toxic action: a) bioluminescence inhibition of *Vibrio fischeri* (Microtox), b) induction of an adaptive stress response pathway in the Nrf-2 mediated oxidative stress response, c) inhibition of photosystem-II herbicides in the combined algae test. In Microtox we showed that chemicals present at very low concentrations below the analytical detection limits can contribute to the mixture effects and that mixture experiments of chemicals in equipotent concentration ratios and in ratios of the

guideline values were in fairly good agreement with CA. In the combined algae test the mixture effects could be rationalised by a two stage model using CA for 12 photosystem II herbicides and CA of the baseline toxicity of all remaining chemicals, and combining these two subsets via a binary mixture model invoking independent action (IA). In the oxidative stress response bioassay all active inducers acted according to CA in equipotent mixtures and guideline mixtures, but mixtures of inducers and non-inducing chemicals sometimes gave effects higher than expected by CA for only the active compounds. Lastly, we proposed an algorithm to derive effect-based trigger values based on regulated chemicals in the guidelines for each mode of toxic action to provide the option to integrate mixtures into water quality assessment. If the trigger value exceeds the set values for a particular type of water then further investigations are prompted. The trigger value should not be used alone but in combination with other endpoints.

**WE154 NORMAN MassBank – bridging communities from metabolomics to environmental chemists** T. Schulze, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis; E. Schymanski, Eawag Swiss Federal Institute of Aquatic Science; S. Neumann, Leibniz Institute of Plant Biochemistry - IPB; J. Slobodnik, Environmental Institute; M. Krauss, Helmholtz Centre for Environmental Research / Effect-Directed Analysis; Y. Nihei, Nara Institute of Science and Technology - NAIST; G. Schramm, Helmholtz centre for environmental research - UFZ; T. Nishioka, Nara Institute of Science and Technology - NAIST; J. Hollender, Eawag / Dept Environmental Chemistry; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis. The web-based MassBank mass spectral database [1] (<http://www.massbank.jp> or <http://massbank.eu/MassBank>) allows the storage and searching (e.g. spectra, substructure and peak search) of any kind of high and low resolution mass spectra including EI-MS, ESI-QToF-MSMS and ESI-FT-MS. MassBank was developed within the metabolomics community of Japan and is now the official mass spectral database of Mass Spectrometry Society of Japan. In 2011 NORMAN Association started to build up an own MassBank server to support its purposes. The main aims of NORMAN are the exchange of information regarding environmental unknowns and emerging pollutants as well as the improvement of the prioritisation process of hazardous compounds. A prerequisite to achieve the goals of NORMAN is the availability of a mass spectral database for the identification and structure elucidation of unknown compounds in environmental samples such as polar compounds and transformation products. However, these compounds are often not included in commercial databases and thus a community-driven database can help to fill this gap. In 2012 NORMAN joined the International MassBank consortium and the NORMAN MassBank server was upgraded to act as the European mirror server of the whole MassBank in order to eliminate the problems with big latency times between query and response due to intercontinental internet connections. The closeness of the MassBank consortium with the NORMAN association is a vital success story of interdisciplinary cooperation. [1] H. Horai et al., Journal of Mass Spectrometry 45 (2010) 703.

**WE155 A novel onsite large volume solid phase extraction device for the effect-based monitoring of water resources** T. Schulze, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis; A. Bahlmann, Helmholtz Zentrum für Umweltforschung UFZ; M. Krauss, Helmholtz Centre for Environmental Research / Effect-Directed Analysis; C. Hug, Helmholtz Centre for Environmental Research / Effect-Directed Analysis; K. Walz, MAXX Mess- und Probenahmetechnik GmbH; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis. The traditional monitoring based on lists of few priority pollutants or of river basin specific compounds is more and more supplemented by scientific studies using multi- and nontarget analysis as well as bioanalytical assessment. These approaches aim to unravel adverse effects potentials of so far unknown or neglected compounds such as transformation products. The cost effective and targeted implementation of novel scientific concepts demands for the availability of an automated large

volume solid phase extraction device (LVSPE) for the onsite and challenging collection of water volumes of up to 1000 litres. Recently, we developed a device to obtain such big water samples, which are required for extensive fractionation, biotesting and chemical analysis in effect-based analysis. The LVSPE consists of a stainless steel chamber to take the sample and pressurise it for subsequent filtration through a glass fibre filter cartridge (0.63 µm) and extraction using a multi-sorbent extraction cascade. The extraction cascade contains different sorbents, i.e. a hydrophobic polystyrene-divinylbenzene sorbent and weak ionic exchangers as well as active carbon. We present the concepts and results from laboratory as well as first field experiments of the novel LVSPE approach. Furthermore, it will be applied in a bioassay intercalibration study of NORMAN Association (<http://www.norman-network.eu>) and in the 3<sup>rd</sup> Joint Danube Survey in 2013.

**WE156 Analytical method of volatile aromatic compounds in water and meconium by HSSPME/GC/MS: influence of matrix** M. Meyer; F. Lestremieu, INERIS; I. Morel, CHU de Rennes / Laboratoire de Toxicologie Biologique et Médico-légale; K. Tack, INERIS. Meconium is the earliest stools of newborn. It starts forming from the 13<sup>th</sup> week of gestation in intestinal contents and accumulates until birth. Usually, it is expelled by the newborn between 24 and 48 hours after birth. This complex matrix has the advantage to integrate a large period of exposure of the foetus to xenobiotics while urine and blood only represent a short-term exposure. This work presents analytical consideration for the detection and the quantification of volatile aromatic compounds (VOCs): BTEX (benzene, toluene, ethylbenzene and xylene) and chlorinated solvents (trichloroethylene and tetrachloroethylene) in meconium. This biological matrix is very viscous and sticky, very difficult to manipulate and homogenize. To analyze VOCs, a headspace analysis method avoiding laborious sample preparation due to the complexity of meconium was selected. Analyses were then carried out by Headspace Solid Phase MicroExtraction (HSSPME) coupled with Gas Chromatography coupled to Mass Spectrometry (GC/MS). As meconium is a rare and heterogeneous matrix from one infant to the others, matrix calibration is difficult to perform. This work has investigated if calibration in water could be used for quantification of target components. The addition of isotopic labelled internal standard for each of the target compounds has allowed a correction of these differences when ratio of standard on internal standard is applied. External and internal calibrations were compared in these two matrices. It was particularly studied if the correction with isotopic labelled internal standards is adequate to use an internal calibration in water to quantify molecules in meconium (it contains 70% of water). Influence of the matrix was observed on the response obtained with a significant decrease in amount of molecule, adsorbed on the fibre, when comparing sampling using meconium and water. The results have shown that the quantification by external calibration is less reliable than the internal calibration in these matrices. It also appears that the correction with isotopic labelled internal standards is not enough to allow the transposition of internal calibration in water to quantification in meconium for all target compounds. Analyses of meconium were carried out with an internal calibration in meconium for BTEX and chlorinated solvents. The limit of quantification was 80 pg/g for most of the compounds.

**WE157 From molecular formula to structure – comparing software-based strategies for the identification of unknowns from LC-high resolution MS data** M. Hu, The Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis; M. Krauss, Helmholtz Centre for Environmental Research / Effect-Directed Analysis; T. Schulze, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis; N. Ulrich, Helmholtz centre for environmental research - UFZ / Department of Effect-Directed Analysis; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis. **Keywords :** High resolution LC-MS/MS, identification of unknowns, structure elucidation For the identification of unknown compounds in environmental samples in non-target screening or effect-directed analysis (EDA), liquid chromatography-high resolution tandem mass



spectrometry has emerged as a mature technique. However, data evaluation procedures lag behind these analytical developments and their performance has hardly been assessed so far for contaminants in complex environmental samples. In this study we focus on different software-based strategies to derive compound structures from molecular formulas, which can be routinely obtained from the accurate measured mass and isotope patterns with a high degree of confidence. Starting from a search in large compound databases such as Chempider or Pubchem, often a large number of candidate structures is obtained for a particular molecular formula. To select the most probable candidate structure, MS fragmentation prediction and retention prediction can be employed. We will compare the performance of different approaches for candidate selection. To this end, a test set of about 100 known environmental micropollutants (pesticides, pharmaceuticals, industrial chemicals, etc.) was used and the candidate list for their molecular formulas was obtained from Chempider. We compared MS fragmentation prediction by different software tools and models for retention prediction alone and in combinations and assessed the ranking or correct classification of the correct molecular structure from these candidate lists.

**WE158 Characterisation and cartography of foetal exposure to pesticides and theirs metabolites in meconium and maternal hair by UPLC-MS/MS** T. Berton, INERIS; K. Chardon, F. Mayhoub, Peritox, Université de Picardie Jules Verne; J. Caudeville, INERIS; V. Bach, Peritox, Université de Picardie Jules Verne; K. Tack, INERIS. Pesticides use has increased since 1940s and one million tonnes are presently dispersed every year in the world. Human exposure to pesticides through the environment and the food is therefore inevitable and the detection of foetal exposure to pesticides is important because some effects on human health have been reported, particularly in the case of an exposure during the early stages of development like the prenatal period. The aim of this study is to characterize the foetal exposure to pesticides and metabolites through meconium (baby's first faeces) and maternal hair analysis. These matrices are representative of the foetal exposure during a wide window of pregnancy since meconium is an accumulative matrix starting its formation from the third month of gestation and all xenobiotics will be accumulating in meconium over the last two trimesters of pregnancy. For this sample collection, a collaboration with 11 nurseries in Picardie was established in order to include 700 couples mother/child, and an analytical strategy for the quantification of pesticides and metabolites based on liquid chromatography-tandem mass spectrometry system was developed. This method targets the main pesticide families used in Picardie (carbamates, organophosphates, pyrethroids, phenylureas and phenoxy herbicides) and 21 compounds were selected. This selection is based on the quantity used in Picardie, their toxicity and their physico-chemical properties. These target compounds have been measured with limits of quantification between 0.2 ng.g<sup>-1</sup> and 200 ng.g<sup>-1</sup> according to the molecule. These results have demonstrated a significant exposure of the foetus to organophosphate pesticides, dithiocarbamates and pyrethroids that are also used as domestic pesticides. Indeed, the highest detection rate was observed for the metabolites of organophosphates and dithiocarbamates (probably mancozeb which is the main pesticide used in Picardie) with respective percentage of detection of 57.9% and 22.8%. The parent pesticides were rarely detected and only in very low concentration unless for cyfluthrin and cypermethrin which have been quantified in high concentrations between 43.8-479.8 ng.g<sup>-1</sup> in 7.6% of the meconium samples. A correlation between concentration measured in meconium and maternal hair was investigated for each couple mother/child, and these first exposure data for the foetus in France have been mapped with geo-localization software.

**WE159 Development of Normal Phase - High Performance Liquid Chromatography (NP-HPLC) fractionation for the isolation of active compounds in sediment sample** C. Gardia Parege, EPOCLPTC / EPOC-LPTC UMR 5805 CNRS; M. Devier, University of Bordeaux / EPOC-LPTC UMR 5805 CNRS; N. Creusot, S. Ait Aissa, INERIS / Unit ECOT; H. Budzinski, University of Bordeaux / EPOC-

LPTC UMR 5805 CNRS. Aquatic ecosystems are the sink of a wide diversity of compounds, among them endocrine disrupting compounds (EDCs). Many chemicals as hydrophobic compounds are trapped in sediments. Physical disturbance and bioturbation release chemicals into water column allowing them to be bioavailable for aquatic organisms. Investigations were performed on a sediment sample from a small river subjected to agricultural and urban pressure. Previous studies reported the occurrence of multiple endocrine disrupting activities of the crude extract sample. In order to isolate the active chemicals and to identify them, Effect-Directed Analysis (EDA) method was used. This approach aims at progressive isolation of active chemicals detected by bioassays using sequential reduction of complex mixture by fractionation process (solid phase extraction (SPE) and HPLC). The diversity of contaminants (with different physico-chemical properties) present in sediment sample requires multi-step fractionation methods. A preliminary fractionation of the sediment sample by SPE using increasing solvent polarity led to 4 fractions (F1-F4). Each fraction was tested using *in vitro* cell lines based on luciferase gene reporter. The four fractions have been a biological effect. F2 and F3 were hyperfractionated by reverse phase - HPLC (RP-HPLC). But, a part of observed biological activities of F2 was lost with RP-HPLC hyperfractionation. Considering this, normal phase - HPLC (NP-HPLC) hyperfractionation was developed as complementary process of RP-HPLC. This hyperfractionation was performed using NH<sub>2</sub> column. The gradient used was from 100% pentane to 100% dichloromethane, and then to 100% acetonitrile at a flow rate of 1 mL/min. This fractionation procedure has been calibrated using a mixture of 17 chemicals with different physico-chemical properties and biological effects. The environmental application is under progress using sediment fractions (non polar fraction F1 and F2). Biological activities of their hyperfractions will be presented as well as first identifications of toxicity-associated compounds.

**WE160 Identification of toxic constituents of the dissolved organic fraction of oil sands process affected water using bioassay directed fractionation** S.B. Wiseman, University of Saskatchewan / Toxicology Centre; R. Mankidy, University of Saskatchewan / Toxicology; Y. He, H. Alharbi, University of Saskatchewan / Toxicology Centre; A. dos Santos Pereira, University of Alberta / Division of Analytical and Environmental Toxicology; J.W. Martin, University of Alberta / Laboratory Medicine and Pathology; J.P. Giesy, University of Saskatchewan. Canada has the world's second largest proven reserves of oil, with the majority being present in oil sands deposits in Alberta. In the surface-mining industry a byproduct of the extraction of bitumen is oil sands process-affected water (OSPW) that is pumped to settling basins for storage and recycling. Greater than 1 billion m<sup>3</sup> of OSPW is held in tailings ponds. Fresh OSPW can be toxic to aquatic organisms and it has long been assumed that chemicals responsible for acute toxicity are "naphthenic acids". However, there is no direct evidence to support this hypothesis. Rather, the evidence suggests that the dissolved organic acid fraction, a very complex mixture of possibly greater than 250,000 chemicals, is responsible for most of the acute toxicity, while nothing is known about which chemicals cause chronic and sub-chronic effects. The goal of our study is to use a coordinated application of fractionation, high resolution instrumental analyses, and a battery of *in vitro* and *in vivo* bioassays that have previously been used to assess effects of unfractionated OSPW, to identify classes of compounds (rather than individual isomers) that cause toxicity of OSPW. Fresh OSPW was fractionated using dichloromethane extraction at neutral pH (neutral fraction), then at acidic pH (acid fraction), and finally at basic pH (base fraction). Neutral, acidic, and basic fractions of OSPW were acutely toxic to embryos of fathead minnows. At 144h of the exposure survival of embryos exposed to control conditions was greater than 95%. Embryos did not survive exposure to 5x concentrations of the acidic or neutral fraction but approximately 38% of embryos survived exposure to 5x of the basic fraction. Survival of embryos exposed to 1x and 0.5x concentrations of acidic and basic fractions were greater than 90%. However, survival of embryos exposed to 1x and 0.5x of the neutral fraction was 0% and 60%, respectively. All fractions of OSPW were estrogenic in the MVLN

assay, and the greatest estrogenicity was in the neutral fraction, followed by the acidic fraction and the basic fraction. The rank order of effects of the fractions is: neutral > acidic > basic. Classic naphthenic acids ( $C_{20}H_{32}O_2$ ), which were previously implicated in the acute toxicity of OSPW, were primarily present in the neutral fraction. Fractionation of the neutral fraction is being performed to investigate compounds responsible for effects observed.

#### **WE161 Effect-directed analysis of mutagenic compounds and dioxin-like activity inducers in Yangtze River (China) sediments**

H. Xiao, RWTH Aachen University / Department of Ecosystem Analysis; T. Floehr, Institute for Environmental Research RWTH Aachen; B. Scholz-Starke; M. Ross-Nickoll, RWTH Aachen / Biology V; W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect-Directed Analysis; A. Schaeffer, RWTH Aachen University / Chair of Environmental Biology and Chemodynamics; H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V). Sediment contaminants with toxicity, e.g. carcinogenic, mutagenic, reproductive and endocrine disruptive potencies under low-dose and long term exposure are of great concern. Effect-directed analysis (EDA) - combining bioassay, fractionation and chemical analysis - has shown to be a powerful tool for key toxicant identification in complex environmental samples. Fractionation procedures allow isolating complex components, and identify specific groups of pollutants responsible for the toxic activity in biotests. Positive bioassay responses of the extracts allows the subsequent targeted and specific chemical analysis and - in the best case - unambiguous identification of the responsible contaminants. The Yangtze River (Changjiang) is polluted by huge amounts of industrial wastewater, urban sewage discharge, ship navigation and oil containing wastewater discharge from ships, and was rated amongst the top 10 rivers in the world at risk (Wong et al. 2007). The environmental issue becomes more emerging after the Three Gorges Dam (TGD) operated at full capacity since 2009. To evaluate and assess the sediment quality of the Three Gorges Reservoir (TGR) in the Yangtze River, sediment extracts at four 'hot spots' (first from the mainstream of Yangtze River at Chongqing city; second from the mainstream of Yangtze River downstream of Chongqing city; third from Jialing River, one of Yangtze Rivers tributaries; fourth from Hanfeng Lake in Kai county, also part of Yangtze Rivers tributaries) were investigated. An automated fractionation procedure was performed on three coupled normal phase HPLC-columns separating compounds according to their polarity, planarity and numbers of aromatic carbon. Mutagenicity (AMES assay) and dioxin-like activity induction potency (Ethoxyresorufin-O-deethylase [EROD] assay) were detected according to previous sediment quality triad studies and were further tested in the different fractions with a combination of chemical analyses. The data to be presented will help to identify the key toxicants for mutagenicity and dioxin-like activity in TGR sediments. Wong C, Williams C, Collier U, Schelle P, Pittock J (2007): World's top 10 rivers at risk. Working Papers

#### **WE162 Effect-directed analysis of surface water samples regarding mutagenicity, endocrine disruption and dioxin-like activity**

M.M. Lam, RWTH University Aachen; C. Di Paolo, T. Seiler, Institute for Environmental Research, RWTH Aachen; C. Hug, R. Bloch, W. Brack, UFZ-Helmholtz Centre for Environmental Research; H. Hollert, Institute for Environmental Research, RWTH Aachen. The production and the number of chemicals are growing steadily. In contrast, only a small number of chemicals are considered priority substances and are well monitored in the EU. Non-priority - in partial unknown - substances may also pose a risk for both the environment and human welfare. Effect-directed analysis (EDA), which combines biological and chemical tools, is an integrated approach to identify ecotoxicologically relevant substances in complex matrices such as environmental samples. The EDA strategy has already been applied to surface water samples in previous projects, as in a study conducted in the Saale/Mulde catchment. Another ongoing project is the ITN EDA-EMERGE, which is developing innovative chemical techniques and biotest systems to improve the EDA of surface waters. In the present study, selected bioassays are applied for the toxicological screening of samples from the

Saale/Mulde study and of samples from the EDA-EMERGE project, with focus on endocrine disruption. An interlab comparison of the mentioned assays is expected as an additional outcome of this study. The results from biotests will be combined with chemical fractionation and analysis performed by project partners to identify novel candidate substances which have adverse effects on aquatic systems.

#### **WE163 Effects directed analysis of urban air pollution: influence of composition and particle size**

S.R. Mesquita, Center of Marine and Environmental Research CIIMAR / ; B. Van Drooge, CSIC; M. Casado, Environmental Chemistry; L. Guimaraes, CIIMAR / Laboratory of Environmental Toxicology; C. Barata, CSIC / Environmental Chemistry; B. Pina, IDAEACSIIC / Environmental Chemistry. Air pollution in urban areas is a recognized risk to human health, although the toxic effects of airborne particulate matter (PM) are not yet fully understood. It has been suggested that organic PM constituents, such as polycyclic aromatic hydrocarbons (PAHs), are important contributors to its toxic potential. Some of the adverse effects caused by PAH exposure are mediated by their binding to the aryl hydrocarbon receptor (AhR), thereby eliciting the so-called dioxin-like response. In this work, we used two complementary assays to evaluate toxic activities associated to the organic fraction of airborne PM samples. The AhR-based recombinant yeast assay (AhR-RYA) can detect and quantify the receptor-binding activity of dioxin-like compounds, such as PAHs. The zebrafish embryotoxicity test (ZET) is an *in vivo* method to assess toxic effects of chemical compounds in the early life stages of a vertebrate species. Both activities were tested for a series of air filter samples and the results compared to their organic and inorganic composition. Air samples were collected from October 2008 to December 2009 in the city of Barcelona, Spain, using three different particle aerodynamic diameter cutoffs (i.e. PM<sub>1</sub>, 2.5 and 10). PM extracts with the highest PAH concentrations (November and December in both 2008 and 2009) induced the highest response in both bioassays. Data analysis linked the toxic effects to the abundance of heavier PAHs in the samples. No differences in PAHs concentration, AhR-RYA or ZET results were observed for samples collected at the same day with different PM size cutoffs, indicating that the toxicity driven by the organic content of air samples corresponds to the sub-micron fraction of the PM (PM

#### **WE164 Babies chewing on plastic: Effect-directed identification and characterization of endocrine disrupting compounds in teething rings**

E. Berger, Department Aquatic Ecotoxicology; J. Oehlmann, Goethe Universität Frankfurt am Main / Department Aquatic Ecotoxicology; T. Potouridis, Goethe Universität Frankfurt am Main / Department of Environmental Chemistry; W. Puettmann, Goethe Universität Frankfurt am Main / Department of Environmental Chemistry; C. Wallner, Goethe Universität Frankfurt am Main / Department of Environmental Chemistry; M. Wagner, Goethe Universität Frankfurt am Main / Department Aquatic Ecotoxicology. Due to their potential to interfere with endogenous hormone signalling, concerns have been raised regarding the human and wildlife exposure to endocrine disrupting compounds (EDCs, Vandenberg et al. 2010, Kortenkamp et al. 2012). Interestingly, many of those chemicals are associated with plastic materials, for instance bisphenol A (BPA, a plastic monomer) and phthalates (plasticizers). Despite legislative measures to ban EDCs from plastic toys and bottles, such products may still leach chemicals with endocrine disruptive potential, since not all chemicals used in their manufacture have been tested. In the present study we therefore screened leachates of plastic baby teething rings (toys used for the soothing of teething babies) for estrogenic and anti-androgenic activity using the Yeast Estrogen Screen (YES) and the Yeast Anti-Androgen Screen (YAAS), respectively. In order to identify the chemicals responsible for the observed endocrine activity, active teething rings were extracted with methanol to recover as high analyte concentration as possible. These extracts were then fractionated using reverse-phase HPLC to separate different plastic components. Fractions were again tested in the YES/YAAS and those with a positive response, where analysed *via* GC-qMS. Two out of eleven baby teething rings leached EDCs into ultra-pure water. Methyl- and propylparaben were

identified as responsible for estrogenic and simultaneous anti-androgenic activity. We confirmed the chemicals' identity and biological activity using authentic standards and quantified the amount of chemicals leaching from one gram of plastic. A fraction exhibiting anti-androgenic activity alone contained two isomeric substances that are not listed in GC-MS databases. In conclusion, our study confirms that some plastic products may leach potential EDCs, namely estrogenic and anti-androgenic parabens and an unidentified anti-androgen. Since those compounds migrate to water readily, human exposure via saliva appears realistic. However, the actual health effects for babies chewing on plastic cannot be deduced from our present *in vitro* data. It has also been shown that a combination of biological screening and chemical analysis is a powerful tool to identify possible priority substances for further risk assessment.

**WE165 Use of trees as indicators of site pollution: phytoscreening and dendrochemistry applied on a former oil and solvent recycling company** J. BALOUET, Environnement International; M. CHALOT, Université de Franche-Comté / UMR 6249 Laboratoire Chrono-environnement; B. DUBEARNES, EAUGEO; H. Roussel, ADEME / Urban Brownfield and Polluted Sites. A case study was realized on an orphan industrial site, that used to be an oil and solvent recycling unit as well as a painting and soap manufacture. The aim was to characterize soil and groundwater pollution of the former industrial site using trees as proxy-recorders and integrators of soil and groundwater contamination. Two techniques were used: Phytoscreening to measure the actual contamination and Dendrochemistry to age-date the past contamination. These methods have been already experimented for HVOC around the world with convincing results. However, few studies have been performed for PAH, BTEX and PCB. The phytoscreening is a simple method that aims at delineating and quantitatively mapping the plume. Trees are able to take up pollutants through the roots and to transfer them through the sap. On this site, micro-samples of the outer ring were taken on 31 trees in one day and analyzed for PAH, HVOC, PCB and BTEX contents. A wood core was taken on the oldest tree of the site for Dendrochemistry analyses, using tracers of pollution measured by line scanning methods (EDXRF). Simultaneously, to validate tree results, the site was investigated with classical methods, using 18 piezometers to monitor groundwater contamination, 40 drills to monitor soil contamination and 6 soil gas wells for soil gases. Results showed that trees were relevant indicators of chlorinated solvent contamination. The source zone of groundwater contamination by chlorinated solvents (mainly TCE) was well identified with the trees and the proportion of TCE and Cis1,2 DCE found in the sap was corresponding to the classical measures obtained with the piezometers. These methods were tested on 15 other study sites, as part of a larger research project named PIT (Pollution Investigation by Trees). The project showed that phytoscreening and dendrochemistry methods are non-invasive, cheap, integrative and rapid to implement as compared to classical methods. They can be an interesting complement to classical methods for contaminated site investigation, used as screening technic and historical data.

**WE166 Analysis and removal of siloxanes from sewage sludge biogas produced in WWTPs in Europe and USA** J. Raich, N. de Arespacochaga, CETAQUA; Y. Tsai, DENARD, United Wercs; M. Crest, CIRSEE, Suez Environnement; C. Ribas, F. Broto, Institut Quimic de Sarria; L. Bouchy, J. Cortina, CETAQUA. The necessity to reduce greenhouse gas emissions and the demand for renewable resources indirectly appoint to the use of biogas as a relevant alternative for green energy production. Biogas apart from methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>), also contains some other minor compounds such as hydrogen sulphide, halogenated compounds and siloxanes, which are known to have the most adverse effect on the utilization of biogas. Nowadays, there is not yet reliable analytical methodology for the sampling, analysis and quantification of these compounds in biogas and, therefore, misinterpretations to control and to evaluate the removal efficiency of different systems of treatment for the reduction of the concentration of these compounds in biogas may easily occur.

Under this context, different sampling techniques such as canisters, tedlar bags, impingers and adsorbent tubes were assessed sampling directly from the sewage sludge biogas source and also from a pre-filled and homogenized 200L tedlar bag. To this ending, analysis and quantification of siloxanes in biogas matrices by gas chromatography coupled to mass spectrometry was optimized. Adsorbent tubes were selected among the different sampling techniques under study. Sampling directly from the source or from the 200L pre-filled tedlar bag resulted in no significant differences. Recently, an increasing number of siloxane removal techniques have undergone development. Though granular activated carbon (GAC) is nowadays the most widely used adsorbent media for siloxane removal, non-carbon based media such as silica gel and carbon based media with special features such as narrow range of pore sizes have emerged. In order to evaluate some innovative adsorbent materials, an eight months pilot study was conducted. In this study, five siloxane adsorbents including two types of silica gels, GAC, polymorphous graphite and hydrophobic polymeric were compared. The study showed that both silica gels provided more than twice siloxane removal capacity compared to GAC, and it was also higher than polymorphous graphite or hydrophobic polymer sorbents. Compression/refrigeration not only allowed the required operational conditions but also removed significant amount of siloxanes (from 20 to 40%). Finally, different campaigns in different WWTPs from Europe and USA were carried out. In all of them D5 followed by D4 were the major compounds detected. Higher concentrations of D5 were linked to the anaerobic digestion (AD) temperature.

**WE167 Fate of the fungicide climbazole in soil after the irrigation of treated wastewater** U. Kunkel, A. Wick, T. Ternes, Federal Institute of Hydrology. Irrigation of treated wastewater is a common practice for improving the water balance and nutrient supply of agriculturally used land. However, treated wastewater usually contains organic micropollutants such as pharmaceuticals, personal care products, and biocides. By the irrigation of treated wastewater these micropollutants are introduced to soils where the micropollutants and their transformation products (TPs) may have adverse effects on the ecosystem. Recent studies revealed the ecotoxicological relevance of the anti-dandruff climbazole and first measurements discovered its ubiquitous presence in treated wastewaters in the mid range. The aim of this study was to examine the fate of climbazole in soil after the irrigation of wastewater on agriculturally used fields. To this end, we performed experiments on three different complexity scales: (1) batch experiments under defined redox conditions, (2) laboratory-scale soil column experiments, and (3) lysimeter studies and soil sampling at field-scale. Batch experiments showed that climbazole is aerobically transformed to one single TP (TP 295). The major fractions of climbazole and TP 295 are sorbed to soil and desorption from soil was determined to be very slow. Climbazole was effectively retained in the soil during the column experiments and no breakthrough of the applied climbazole was observed after several pore volumes. Moreover, the formation of TP 295 was also detected in the soil column. Consistent with the results from the batch experiments, the formation was restricted to the oxic zones in the soil column. In field studies, we determined an accumulation of both climbazole and TP 295 in the upper most parts of the soil, while neither climbazole nor TP 295 were observed in the percolate water from the lysimeters. The combined results from the different experimental approaches revealed a very low mobility of climbazole and its aerobically formed TP in soil. Due to a strong retardation in the top soil layer, leaching to groundwater seems very unlikely. Moreover, the strong sorption affinity and the persistence of the sorbed climbazole and TP 295 might lead to an accumulation of both compounds in soils. Therefore, further tests on the bioavailability of both climbazole and TP 295 in soil and their respective ecotoxicity for terrestrial organisms are required.

**WE168 Homogeneity aspects in analysing micro-pollutants in sludge composting degradation** Y. Sadef, T.G. Poulsen, Aalborg University; K. Bester, Aarhus University / Environmental Science. One

disposal route of sewage sludge is composting with following use on land. For this process sludge is mixed with straw, garden wastes etc. to allow appropriate access of air into the material. A major issue in this technology is whether or not organic micro-pollutants are degraded or not in this during composting as it is used as the better alternative to using the sludge directly on land. Currently known challenges are flame retardants, personal care compounds as well as some regulated compounds. This assessment on removal or degradation is challenged by the extreme inhomogeneity of the starting material which can reach uncertainties up to one order of magnitude (Poulsen and Bester, 2010). In this study we assess on which sampling intake, mixing devices and processes are appropriate for such full scale incubation experiments. Results indicate that a combination of large scale sampling and industrial scale homogenization and particle size reduction of the compost material yields the highest measurement accuracy and reproducibility.

**WE169 Occurrence and distribution of organic UV-stabilizers in sediments of the German Bight** H. Wolschke; F. Heydebreck, Helmholtz-Zentrum Geesthacht, Institute of Coastal Research; Z. Xie, Helmholtz-Zentrum Geesthacht; R. Suehring, R. Ebinghaus, Helmholtz-Zentrum Geesthacht, Institute of Coastal Research. Sediments from the coastal area of the German Bight represent a complex ecosystem with various habitats and ecosystem functions. Especially the mudflats of the North Sea, a world natural heritage site, are influenced by organic pollutants, which are discharged into the North Sea over e.g. rivers. Organic UV-stabilizers filters are commonly employed in several personal care products (PCP) as well as in formulations of textiles, varnishes and plastics. The pathways of UV-stabilizers into the aquatic environmental are waste water treatment plants as well as deposition from the atmosphere. Some of these investigated chemicals are known to be hormonally active, functioning as endocrine disruptors. In the last few years, UV-stabilizer have increasingly been discussed as emerging contaminants. In this study, the most commonly used UV-filters were investigated in sediments of the river Elbe and costal area of the German Bight. The analytical method has been validated for the determination of UV-filter using gas chromatography and mass spectrometry (GC-MSMS). The extractions methods pressure liquid extraction (PLE) with acetone/hexane and a solid phase clean up using Oasis HLB-cartridges was validated. This study shows levels of contamination and distribution of UV-stabilizer in the surface sediments along the river Elbe and the German Bight.

**WE170 Removal of Polar UV Stabilizers in Biological Wastewater Treatments** D. Molins Delgado, CSIC / Department of Environmental Chemistry; S. Diaz-Cruz, IDAEACSIC / Environmental Chemistry; D. Barcelo, IQABCSIC. The raising concern about stability and conservation of goods has made that the use of preservation compounds show a heavy progressive increasing during the last decades. Compounds as UV filters are used in many industrial processes and formulas to ensure the stability of personal care products. Benzotriazoles, on the other hand, are a huge family of compounds widely used to preserve packages from degradation. Due to their physicochemical properties, leaching processes from the package to the product may occur and either the package or the product will eventually reach the environment, in particular the aquatic environment. These compounds usually are not efficiently removed at wastewater treatment plants (WWTPs) and ultimately could reach the drinking water supply [1]. Unfortunately, the lack of a complete set of ecotoxicological data on potential chronic effects of these chemicals should caution against the widespread environmental contamination of them. Among these high production chemicals, the compounds considered in this study, i.e. 1H-benzotriazole and 5-Methyl-1H-benzotriazole, are widely used in addition to their great UV light-absorption capacity by their anticorrosive, antifreeze, coolant, vapour phase inhibitor, photographic developer and biocide properties. Both compounds are fairly water-soluble, resistant to biodegradation, and show a limited sorption tendency [2] that makes them potential candidates to pass water treatment processes. In order to assess the removal rates of these two

UV stabilizer we analysed samples from influent and effluent water streams from the most important municipal WWTPs in Catalonia (Spain), by Solid Phase Extraction (SPE) sample treatment [3] and further analysis by High Pressure Liquid Chromatography attached to a Quadrupole-Lineal Ion Trap Mass Spectrometer (HPLC-QqLIT-MS/MS). The performance of the optimized analytical method and the discussion of the outcomes of the study will be presented. Acknowledgements We acknowledge the research funding from the NANOTROJAN project (CTM2011-24051) from the Spanish Ministry of Economy and Competitiveness, and from the Generalitat de Catalunya (Consolidated Research Group: Water and Soil Quality Unit 2009-SGR-965). References S. Weiss, T. Reemtsma. Anal. Chem. 77 (2005) 7415 C. Domínguez, C. Reyes-Contreras, J. M. Bayona. J. Chromatogr. A 1230 (2012) 117 H. Janna, M.D. Scrimshaw, R.J. Williams, J. Churchley, J. P. Sumper. Environ. Sci. Technol. 45 (2011) 3858

**WE171 Occurrence of pharmaceuticals in wastewater treatment plants and in receiving water bodies around the city of Rome (Italy)** n. ademollo, IRSACNR; S. Capri, L. Patrolocco, Water Research Institute - National Research Council. Pharmaceuticals (PPs) have become ubiquitous in the aquatic environment, detected in surface water and wastewater at ng/L to µg/L levels. It is well known that municipal wastewater treatment plants (WWTPs) are the major pathways for PPs into the aquatic environment because they are not specifically designed to remove these emerging pollutants. Their presence in the aquatic environment can have adverse effects on biotic communities with changes in morphological and/or metabolic functions and endocrine disruption. The registration and marketing of PPs are exempted from REACH regulation and, currently, a procedure to establish their possible environmental impact is not required. However, the need to monitor pharmaceuticals in water ecosystems has been recently recognized by the EU which has proposed the two steroid hormones 17-β-estradiol and 17-α-estradiol and the anti-inflammatory diclofenac to be included in the list of the priority substances regulated by the WFD, with a suggested EQS of  $3.5 \times 10^{-5}$ ,  $4 \times 10^{-4}$  and 0.1 µg/L, respectively. The aim of this study was to provide a better understanding of the occurrence of PPs in surface waters by monitoring in different seasons selected PPs in influent-effluent from WWTPs around the city of Rome (Italy) and from contaminated sites along the urban stretch of the Tiber River. The compounds investigated belong to different classes of pharmaceuticals, such as non-steroidal anti-inflammatories, lipid regulators, antiepileptic and steroidal hormones. The extraction-clean-up from aqueous samples was performed by solid-phase extraction (SPE) followed by an analytical determination with HPLC-MS and HPLC UV-fluorescence. The results showed the occurrence of almost all target compounds in the WWTPs samples with concentrations ranging between 0.1 and 10.9 µg/L in the influents and between 0.01 and 2.39 µg/L in the effluents. Mean removal efficiencies varied between 30% and 85%. Along the urban stretch of the Tiber River the PPs concentration levels varied from 50 to 264 ng/L, overcoming, in some cases, the suggested threshold limit. The results confirmed that WWTPs were the main source of river contamination. Although the effluent wastewater input into receiving water should produce a dilution of contamination, the continuous release of PPs into the aquatic environment confers to PPs the characteristic of pseudo-persistence which, in turn, may lead to chronic exposure of organisms at all levels of the food chain.

**WE172 Method development for determination of essential oils in biological matrices** K. Löfstrand Stockholm University / Department of applied environmental science; M. MacLeod, ITM Stockholm University / Department of applied environmental science. Essential oils are extracts from plants and are used in many personal care products as fragrances or as flavorings in food or beverages. Essential oils are complex mixtures of a number of chemicals with different properties, making analysis in biota challenging. Here, we selected seven model compounds to validate an extraction and clean-up method that could be applied to essential oils in preparation of bioaccumulation studies. Homogenate of rainbow trout tissue, used as a model matrix, was spiked

with camphene, cashmeran, musk xylene, cyclohexyl salicylate, acetyl cedrene, 2-*t*-butylcyclohexanol acetate and 8-cyclohexadecen-1-one and was left to equilibrate overnight. The analytes were extracted from the tissue using acetone and *n*-hexane (1:1) and the extract washed with a saline solution (1% KCl). The extract was evaporated to dryness under as gentle stream of nitrogen gas at room temperature. A volatile fraction was separated by heating the sample in a closed system with a continuous gentle stream of nitrogen gas. The volatile analytes were collected on a SPE column (ENV<sup>+</sup>, 10 mg) and eluted from the column with *n*-hexane. The non-volatile fraction was cleaned-up using C18-columns eluted with acetonitrile. The solvents were changed to *n*-hexane prior to analysis. Both fractions were analyzed using GC-EI-MS (SIM). Due to the lack of appropriate surrogate standards, standard addition was used for quantification of the analytes. The method gave sufficient recoveries for all compounds over 70 % recoveries in the volatile fraction (camphene, cashmeran and 2-*t*-butylcyclohexanol acetate) and above 60 % in the non-volatile fraction.

**WE173 Benthic Invertebrate Exposure and Chronic Toxicity Analysis for cVMS Materials – A Combined Probabilistic and Monte-Carlo Approach** K.B. Woodburn, D.E. Powell, Dow Corning Corporation / Health & Environmental Sciences. Lipophilic chemicals, such as cyclic volatile methylsiloxanes octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), adsorb extensively to particles and surfaces in aqueous systems, making sediments the predominate repository when performing risk assessment evaluations. A widely accepted step for estimating the possible risk posed by such chemicals to sediment-dwelling species is to compare the observed sediment concentration with either published ecotoxicity guidelines or to chronic no-observed effect concentrations (NOECs) from toxicity testing with benthic invertebrates. The objective of this work was to compare field exposure sediment levels of D4 and D5 to chronic benthic invertebrate NOEC values, using a probabilistic and Monte-Carlo simulations to estimate the likelihood of exposure/toxicity overlap and to analyze the uncertainties associated with the risk assessment. For both D4 and D5, no overlap of sediment exposure concentrations and chronic NOEC values was noted, with exposure distribution probabilities ranging from 95% to 99.9% and with chronic NOEC extrapolated probabilities ranging from 5% down to 0.1%. PRA successfully described the overlap of sediment exposure and chronic benthic NOEC distribution datasets for both cyclic methylsiloxane materials. The use of Monte-Carlo analysis yielded descriptions of the probability of overlap of exposure and chronic NOEC, i.e., a quantitative description of the risk posed by these materials to benthic invertebrate organisms/populations. Using both sediment OC-based and lipid tissue-based analyses, the cumulative probability of exceedence of chronic NOEC levels for tested benthic invertebrate species is < 0.1% for both D4 and D5. Overall, this risk analysis indicates that existing D4 and D5 sediment residues pose minimal quantifiable risk to benthic invertebrate species.

**WE174 Poorly Soluble Compounds in Cosmetics – an approach for a reliable environmental risk assessment (the ECOSM project)** Stibany, RWTH Aachen / Institute for Environmental Research (Bio 5); S. Damme, H.M. Maes, H. Hollert, RWTH Aachen University; K. Rettinger, J. Steber, German Cosmetic Toiletry, Perfumery and Detergent Association; A. Coors, ECT Oekotoxikologie GmbH; C. Schulte, German Federal Environment Agency; A. Schaeffer, RWTH Aachen University. Personal Care Products (PCP) - often referred to as cosmetics - include a multitude of substances with various physico-chemical properties since they are designed for many different purposes. A lot of these compounds are poorly soluble in water, i.e. their water solubility is below 1 mg/L. Due to the high production volumes of PCP, and their typical 'rinse off' application, substantial amounts of poorly soluble chemicals end up in waste-water treatment plants and may subsequently enter river systems with the effluent. This causes the need for a reliable assessment of their environmental behavior and toxicity to organisms, as requested by the European REACH-Regulation. Standard ecotoxicity tests are not suitable for poorly soluble substances. Due to

their high lipophilicity, extensive adsorption to surfaces like test vessels and organisms is expected. The lack of consistent, reliable results due to the difficulties in maintaining constant test concentrations may lead to improper assessment of possible environmental risks associated with these chemicals. A promising approach addressing this problem is the so called 'poorly solubles approach' making use of an 'ecotoxicological threshold concentration of no concern' (ETNCAqu) for inert substances with a narcotic mode of action. Below this threshold concentration, neither acute nor long-term adverse effects on aquatic organisms are expected to occur. The aim of the project ECOSM (ECotoxicity investigations of COSMetic ingredients) is to develop pragmatic tools to test the ETNCAqu hypothesis in order to allow better environmental risk assessment of poorly soluble substances. Existing aquatic ecotoxicity tests will therefore be adapted including passive dosing techniques to obtain constant exposure conditions. Selected poorly soluble compounds contained in PCP will be tested: The surfactant precursor Dodecylbenzene has been selected as a suitable model compound and already passed the first evaluation steps. A method for chemical analysis using solid phase micro extraction and gas chromatography has been developed. With these techniques, the maximum solubility in distilled water and different standard media for selected test organisms as proposed by the OECD were exactly determined. The results of various ecotoxicity tests with organisms of different trophic levels (e.g. algae, daphnia, fish) will be presented. Furthermore, adaptation of the guidelines for the use of passive dosing techniques will be discussed.

**WE175 Novel Method for the Preservation and Determination of Cyclic Volatile Methyl Siloxanes in Surface Waters and Waste Water Treatment Plant Effluent** S.M. Knoerr, T.H. Schramke, Dow Corning Corporation / Health and Environmental Sciences; J.A. Durham, Dow Corning Corp / Health and Environmental Sciences; D.E. Powell, Dow Corning Corporation / Health & Environmental Sciences; D.A. McNett, Dow Corning Corporation / Health and Environmental Sciences. Cyclic volatile methyl siloxanes (cVMS) are common ingredients in many consumer products, including personal care products such as hair conditioners and anti-perspirants. The wastewater stream represents a major post-use disposal route and subsequently enters the environment through the waste water treatment plant's (WWTP) effluent. In order to understand the role of WWTP emissions of cVMS into the environment, it is important to be able to accurately quantify cVMS in aqueous matrices. The primary objective of the current study was to develop a method to preserve and accurately determine concentrations of cVMS in surface waters and waste water treatment plant effluent in an effort to understand the fate of cVMS in the environment. The studied compounds – octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) - are volatile, have low solubility in water, and very high Henry's law constants, making accurate quantification in WWTP effluent and associated surface waters difficult. Additionally, these compounds have relatively high values of log  $K_{ow}$ , indicating a strong tendency to partition into organic matter. Methods for collection and analysis of aqueous matrices must take into account the dissolved and labile fractions of cVMS that may readily evaporate, as well as those fractions that are associated with the organic content within the water column. Consequently, methods for collection and analysis must incorporate some means to sequester the dissolved and labile fractions, as well as recover the sorbed fraction from the suspended solids. The method reported here for total cVMS utilizes low density polyethylene to inhibit evaporative loss of cVMS during sampling and transport to the laboratory. The sample is extracted into hexanes containing isotopically labeled cVMS as an internal standard and analyzed by gas chromatography mass spectrometry. The benefits of the method are its ease of sampling and simple sample preparation. The limit of detection has been demonstrated to be < 0.2 µg/L. In addition, this method has shown recoveries of >70% during storage of the water samples for up to 14 days at room temperature conditions. Linearity of the method has been established from the limit of quantification up to 300 µg/L. This work was supported in part by the Centre Européen des Silicoes.

**WE176 Comparison of Solvent Extraction and Purge-and-Trap Methodologies for Determination of Cyclic Volatile Methyl Siloxanes (cVMS) in Biological Matrices** S.M. Knoerr, Dow Corning Corporation / Health and Environmental Sciences; J.A. Durham, Dow Corning Corp / Health and Environmental Sciences; D.E. Powell, Dow Corning Corporation / Health & Environmental Sciences; D.A. McNett, Dow Corning Corporation / Health and Environmental Sciences. The purpose of this study was to compare a solvent extraction method and a purge-and-trap method for measuring concentrations of cyclic volatile methyl siloxanes in biological matrices. The cyclics – octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) – are widely used in industrial applications and home and health care products, and are being studied to assess their fate and distribution in the environment. In order to accomplish this, it is imperative that analytical methods can analyze organisms consistently across the food web, from invertebrates to fatty fish, so a compound's bioaccumulation potential can be accurately determined. Various biological matrices were measured using a solvent extraction schema developed at Dow Corning Corporation. Using whole body fish homogenate from fish which were dosed with radiolabeled cVMS, the solvent extraction method has demonstrated extraction efficiencies of 90%. The method is carried out whereby the biological matrix is extracted in duplicate with a 2:1 (solvent:sample) ratio on a multitube vortex mixer. The first extraction is carried out in tetrahydrofuran (THF) containing isotopically labeled cVMS as an internal standard and the second extraction uses neat THF. The combined extracts are then analyzed by gas chromatography/mass spectrometry (GC/MS) for quantification of cVMS concentrations. The results from these samples were then compared with the purge-and-trap analysis. The purge-and-trap method disperses the tissue in water as it is heated and stirred, which causes the cVMS to be volatilized. Volatilization is aided by a nitrogen purge which carries the compounds through a condenser and ENV+ sorbent cartridge where they are retained. The compounds are eluted from the sorbent with hexanes and then analyzed by GC/MS.

**WE177 IFRA Environmental Standards and RIFM program advances: Update for 2013** A. Lapczynski, RIFM / Environmental Specialist; D.T. Salvito, Research Institute for Fragrance Materials Inc / Department of Environmental Science; C. Sachse-Vasquez, Research Institute for Fragrance Materials, Inc; M. Vey, International Fragrance Association. The Research Institute for Fragrance Materials, Inc. (RIFM) in coordination with the International Fragrance Association (IFRA) has been responsible for the assurance of safety of fragrance ingredients in consumer products. The environmental risk and hazard (i.e., PBT) screening of fragrance materials has been incorporated into the RIFM testing program and the results of these assessments have formed the basis of the IFRA Environmental Standards which are part of the fragrance industry's voluntary safety program. Furthermore these assessments and the new data generated have been reported in the peer-reviewed literature and at past SETAC meetings. (RIFM provides an updated report of these studies and the associated revised safety assessments at both the North American and European annual SETAC meetings.) In order to identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental Framework and IFRA Volume of Use Survey as reported for both Europe and North America. The Framework for this evaluation was published in Environmental Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). The focus has been on a 'down-the-drain' emission scenario. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In addition, hazard assessment on these materials was also performed and reviewed. Studies to refine the risk or hazard screening assessments include persistence testing (ready biodegradation tests and die-away studies), bioaccumulation, and acute and chronic aquatic toxicity. In an effort to advance the risk and hazard assessments of fragrance materials (i.e., high volume, lipophilic materials used globally), several projects are underway: the use of higher tier studies for persistence and bioaccumulation assessment,

development, for priority materials, of terrestrial and sediment risk assessments, developing approaches for hazard characterization of fragrance materials derived from natural products, and considering the potential environmental risks in regions with little or no wastewater treatment.

**WE178 Environmental monitoring and risk assessment of Linear Alkylbenzene Sulfonate (LAS) in urban river in China** N. Tagawa, M. Yamane, Y. Honda, N. Nishiyama, Kao Corporation / Safety Science; D. Wu, C. Li, Z. Zhang, S. Hirano, Shanghai Jiaotong University. Environmental issues in developing countries, especially in China, are important and various. The economic growth in China is significant and some environmental concern of chemical pollution is occurred. Recently, some literatures have reported that a cause of water pollution in China has been changed to household wastewater from industrial wastewater, and also the number of literatures regarding to the PPCPs (Pharmaceuticals and Personal Care Products) has increased. However, the report about environmental risk of surfactant in China is very limited. Now, it is one of the urgent issues to understand the environmental behavior and the risk of surfactants in China. In order to assess the environmental risk in China, we have been monitoring a major surfactant, Linear Alkylbenzene Sulfonate (LAS) and common parameters of water quality (BOD, NH<sub>4</sub>-N, MBAS etc.) from 2010 to 2012. River water samples were collected from 3 sites of Suzhou river in Shanghai. The value of water quality parameters were relatively higher than advanced countries. It was assumed that coverage ratio of Waste Water Treatment Plant (WWTP) and high population density of Shanghai caused this low quality of river water. However, water quality parameters of Suzhou river have been improved during the last two decades. Because the number of WWTP will be expected to increase in Shanghai area, it is expected that water quality will be continuously improved in near future, LAS concentrations in river water were in ranging from < 1 µg/L to 183 µg/L with seasonal fluctuation. No difference of LAS concentration in each sites were observed. We used the 95<sup>th</sup> percentile, 106 µg/L as final PEC (Predicted Environmental Concentration) and the reported PNEC (Predicted No Effect Concentration) of (C12) LAS as 270 µg/L for the risk characterization. Consequently, PEC was lower than PNEC, therefore, it was concluded that the environmental risk of LAS in Shanghai was low.

**WE179 Bioaccumulation of pharmaceutical compounds and metabolites in aquatic organisms from Mediterranean rivers** S. Huerta Buitrage, Catalan Institute for Water Research ICRA / Department of Water Quality; V. Osorio, IDAEA-CSIC / Department of Environmental Chemistry; A. Jakimska, Gdansk University of Technology / Department of Analytical Chemistry; S. Rodriguez-Mozaz, Institute for Water Research ICRA; S. Perez, D. Barcelo, IDAEA-CSIC / Department of Environmental Chemistry. Pharmaceuticals have been increasingly detected in water, sediment and other environmental compartments in the aquatic ecosystems in the last years. The constant presence of these pollutants and their transformation products has raised questions about their environmental fate, toxic effects on biota, and ecological risks. Among these issues, uptake of pharmaceutical residues in biological tissues and their subsequent bioaccumulation in aquatic organisms has been considered an important indicator of exposure via water or sediment in the field. In this study, samples (water, sediment, and biota) were taken at five locations along the length of two Mediterranean rivers in Spain, Ebro and Llobregat, to achieve a pollution gradient during the summer of 2010. Partitioning of pharmaceutical compounds to water and sediment, as well as bioconcentration and biomagnification in invertebrates (Tricoptera) and fish (*Cyprinus carpio*, *Barbus haasi*, *Silurus glanis*) were determined the rivers, both heavily impacted by anthropogenic activities. Several pharmaceutical families were repeatedly found in water and sediment, whereas pharmaceutical residues were only detected in 9% of biota tissues. Psychiatric drugs, non-steroidal anti-inflammatory drugs (NSAID) and  $\beta$ -blockers were the most prevalent families. Bioaccumulation was found in the most polluted sites of the rivers, where maximum concentrations were detected for anti-

inflammatory diclofenac ( $15.4 \pm 0.1$  ng/g), psychiatric drugs citalopram ( $1.4 \pm 0.3$  ng/g), and venlafaxine ( $0.6 \pm 0.02$  ng/g), and  $\beta$ -blocker propranolol ( $4.2 \pm 0.2$  ng/g). Findings were consistent with similar studies performed in USA and Canada, which reported concentrations of several compounds in fish in the low ng/g range. This is the first monitoring study reported in Europe related to the occurrence of pharmaceutical compounds in freshwater fish.

**WE180 Application of Environmental Scanning Electron Microscopy and X-ray spectroscopy to determine the fate of silver nanoparticles in wastewater** i. hannah, School of Life Sciences; T.F. Fernandes, Heriot Watt University / School of Life Sciences; M. Hartl, Heriot Watt University / Centre for Marine Biodiversity and Biotechnology, School of Life Sciences; N. Christofi, Edinburgh Napier University / Faculty of Health, Life and Social Sciences. AgNP with citrate, polyethylene glycol and polyvinylpyrrolidone coatings are representative of the most commonly used forms of AgNP in product applications. Post-use, AgNP are likely to enter the wastewater gathering and treatment system, and assessing their toxicological risk depends upon determining their fate, both in terms of their interaction with the components of wastewater and how they may partition in the wastewater stream. AgNP with the specified coatings were specifically prepared for use in this investigation to achieve tight control over characterisation parameters, including particle size, shape, date of origin and storage conditions. When exposed to a range of wastewater components, including free-ions and humic acids, these AgNP exhibit differences in stability, as determined by DLS and UV-vis spectroscopy assessment of the incidence of particle aggregation. Environmental Scanning Electron Microscopy (ESEM) in conjunction with X-ray diffraction spectroscopy allows the aggregated and non-aggregated products of these exposure tests to be analysed. Critically, ESEM allows analysis of samples in their natural, wet state and therefore avoids introduction of aggregation artefacts. The information yielded assists in determining the chemical composition of any AgNP transformation products, the solubility and precipitation characteristics of those transformation products, and therefore facilitates prediction of partitioning behaviour between the different output streams from wastewater treatment – surplus sludge and effluent. **Session :** Environmental and analytical chemistry **Keywords :** Silver nanoparticles environmental fate **Presentation preference :** Poster **Authors :** Presenting – I. Hannah<sup>1</sup> Co-authors - T. Fernandes<sup>1</sup>, M. G. J. Hartl<sup>1</sup>, N. Christofi<sup>2</sup> <sup>1</sup>Centre for Marine Biodiversity and Biotechnology, Heriot-Watt University <sup>2</sup>Faculty of Health, Life and Social Sciences, Edinburgh-Napier University **Correspondence :** Iain Hannah. lah4@hw.ac.uk

**WE181 Antivirals and antibiotics in two Swedish STPs and a river during the 2009 Influenza A(H1N1) pandemic** H. Söderström Department of Chemistry Umeå University / Department of Chemistry; J. Jarhult, Uppsala University / Section of Infectious Diseases, Department of Medical Sciences; A.C. Singer, Centre for Ecology Hydrology; R.H. Lindberg, Umea University / Department of Chemistry; R. Grabic, University of South Bohemia in CB / Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses; G. Ali Khan, Umea University / Department of Chemistry; G. Fedorova, University of South Bohemia in CB / Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses; J. Fick, Umea University / Department of Chemistry; B. Olsen, Uppsala University / Section of Infectious Diseases, Department of Medical Sciences. In 2009, a new influenza caused by influenza A virus subtype H1N1 developed into a pandemic, which was the first pandemic on the highest level of pandemic alert since the Hong Kong influenza pandemic in 1968. Each country developed a strategy to protect its citizens against the pandemic. Many countries used antivirals for treatment and prophylaxis measurements and antibiotics for treatment of secondary bacterial infections. Concerns were raised that the extreme pharmaceutical use during the pandemic could contaminate, via discharged sewage water, the aquatic environments and cause several

effects such as resistance to oseltamivir in the natural reservoir for influenza viruses, dabbling ducks, and selection for antibiotic resistant bacteria. In this study, we present environmental levels of OC during the 2009 Influenza A(H1N1) pandemic together with the levels of 14 antibiotics. The levels of OC and 14 antibiotics in sewage effluents were also studied. Water samples were collected once each week during week w 44 to w50, 2009 and w41, 2010, from the effluent of the sewage treatment plants (STPs) of two major cities in Sweden (Stockholm and Uppsala), and from two sites in the river Fyrisån, Uppsala, Sweden. The environmental levels of OC correlated well with the levels in Uppsala STP effluent and the peak of the pandemic, (highest in w46 at 23 ng/L and 243 ng/L). In Stockholm STP effluent the levels were lower with no specific trends between weeks (average 43 ng/L). Further, the levels of the antibiotic erythromycin were among the highest during and after the pandemic (highest in w46 at 183 ng/L). This is the first detection of environmental OC levels during the 2009 Influenza A(H1N1) pandemic and in Sweden, a country with traditionally restricted use of antivirals and antibiotics. Furthermore, the human health consequences of the pandemic did not become as serious as they were first predicted to be. This indicates that there is a risk that higher environmental levels can occur during a pandemic at which resistance in influenza viruses circulating among wild ducks can be induced and selection for antibiotic resistant bacteria occur. As influenza viruses can cross species barriers, oseltamivir resistance could spread to human-adapted strains with pandemic potential, which disabling oseltamivir. We propose strategies to lower environmental levels of OC including improved sewage treatment and, more importantly, a prudent use of antivirals and antibiotics.

**WE182 Mechanism of action of AROCLOR 1248 in *Vibrio fischeri*** V. Kalciene, Vilnius University / Centre for Ecology and Environmental Studies; A. Cetkauskaitė, Vilnius University / Department of Biochemistry and Molecular Biology. AROCLOR 1248 is an example of a dielectric chemical (now phased out), which is a mixture of PCBs (polychlorinated biphenyls) and is highly bioaccumulative and persistent in the aquatic environment. The effects of PCBs on bioluminescence bacteria were analyzed in few studies (Chu et al., 1997; Salizzato et al., 1998a; Salizzato et al., 1998b). However, bioluminescence was used as the end-point of toxicity only, and target sites of PCBs in bioluminescent bacteria are still unrevealed. Hence, the objective of this work was to evaluate the mechanism of action of the AROCLOR 1248 toxicity to bioluminescent bacteria *Vibrio fischeri*. Results showed that AROCLOR 1248 (5 mg/L) had multiple effects in *V. fischeri*: i) it inhibited cells bioluminescence; ii) generated ROS; and; iii) diminished membrane potential *in vivo*. Moreover, AROCLOR 1248 inhibited oxidation of NADH and bioluminescence caused by NADH:FMN oxidoreductase-luciferase complex *in vitro*. Additionally, the results of comparative inhibitory analysis, using AROCLOR 1248, sulfur, oleic acid, decanal, N-ethylmaleimide, revealed that the principle target site of AROCLOR 1248 is a NADH:FMN oxidoreductase in *V. fischeri* bioluminescence complex. Chu S, He Y, Xu X. 1997. Determination of acute toxicity of polychlorinated biphenyls to *Photobacterium phosphoreum*. *Bull Environ Contam Toxicol* 58:263-267. Salizzato M, Bertato V, Pavoni B, Ghirardini AV, Ghetti PF. 1998a. Sensitivity limits and EC<sub>50</sub> values of the *Vibrio fischeri* test for organic micropollutants in natural and spiked extracts from sediments. *Environ Toxicol Chem* 17:655-661. Salizzato M, Pavoni B, Ghirardini AV, Ghetti PF. 1998b. Sediment toxicity measured using *Vibrio fischeri* related to the concentrations of organic (PCBs, PAHs) and inorganic (metals, sulphur) pollutants. *Chemosphere* 36:2949-2968.

**WE183 The TOMPs Network – continuous data on the UK air quality for 20 years** C. Graf, Centre for Chemicals Management, Lancaster Environment Centre; K.C. Jones, R. Gioia, A. Birgul, J.K. Schuster, A. Katsoyiannis, A.J. Sweetman, Lancaster University / Centre for Chemicals Management, Lancaster Environment Centre. The Toxic Organic Micro Pollutants (TOMPs) Network, which has operated since 1991, currently collects ambient air samples at six sites across England and Scotland, using high-volume active air samplers. Lancaster



University has been operating this UK Department of Environment, Food and Rural Affairs (Defra) funded network from its inception, delivering long-term ambient air trend data for a range of Persistent Organic Pollutants (POPs) at both urban and rural locations. Data from the network provides Defra with valuable information on emission/source controls and on the effectiveness of international chemicals regulation. It is also used to demonstrate UK compliance with its obligations under the 2001 Stockholm Convention on Persistent Organic Pollutants and the 1998 UN/ECE Long-Range Atmospheric Transport Protocol. Moreover, long-term analysis of air pollutants at trace levels allows detailed studies on atmospheric fate and behaviour processes of persistent chemicals and is the inevitable basis of their successful modelling. The target chemicals of TOMP have been polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and, since 2010, polybrominated diphenyl ethers (PBDEs). PCBs, PCDDs, PCDFs, and selected PBDEs are all listed under the Stockholm Convention. The continuous monitoring of these compounds has demonstrated the constant decline in their UK air concentrations over the last decades. The concentrations of all compounds are generally significantly higher at urban compared to semi-rural and rural sites, with estimated clearance rates between 2 and 9 years for PCBs at all sites, 2 to 4 years for PBDEs at urban and semi-rural sites, and 4 to 5 years for PCDDs and PCDFs at urban sites. All these data are in good agreement with emission estimates. Additionally, an archive is maintained, which can be used for analysing emerging chemicals, such as alternative flame retardants, pesticides, and further substances of interest as soon as they have been identified.

**WE184 Environmental factors affecting the concentrations of chlorinated polycyclic aromatic hydrocarbons associated with particulate matters** Y. Kamiya, Meijo University; F. Ikemori, Nagoya City Institute For Environmental science; T. Ohura, Meijo University. Recently, chlorinated polycyclic aromatic hydrocarbons (CIPAHs) with 3- to 5-ring have been detected in the environment, which are possible to be concerned in hazardous pollutants. On the other hand, the sources and behaviors of atmospheric CIPAHs have not been revealed. In this study, we investigated the annual variations of twenty-four species of CIPAHs associated with particulate matters at two sampling sites (residential area and industrial area) in an urban city, Nagoya, Japan. All of twenty-four species of CIPAHs targeted were detected in both sampling site. The mean concentrations of total CIPAHs in residential area and industrial area were 16.0 and 19.5 pg/m<sup>3</sup>, respectively. A seasonal variation of particulate CIPAHs concentrations was observed that the concentrations in winter elevated than those in summer. Of the CIPAHs detected, 6-chlorobenzo[a]pyrene (6-ClBaP) was abundant, followed by 1-chloropyrene (1-ClPy) and dichlorobenzo[a]pyrene (Cl BaP) in the both sampling site. Especially, the concentrations of chlorinated BaP (ClBaP: n = 1 ~ 3) were relatively high contributions (ca. 50% in total CIPAH concentrations) among the CIPAHs in both sites. The composition of total CIPAHs in residential area was somewhat constant during the campaign but not in industrial area. These result suggested that emission sources of CIPAHs associated particles are different in both areas; moreover, several and/or significant sources of CIPAHs could be present in industrial area. Furthermore, the concentrations CIPAHs in particles collected from tunnel were investigated for source analysis. As a result, almost CIPAHs targeted were detected from the tunnel particles whereas its composition was differ from that observed in residential area and industrial area. It suggests that vehicle exhausts are minor contributor for particulate CIPAHs in the air, so that other emission sources as the major contributors are present in the areas.

**WE185 Levels and source regions of polychlorinated biphenyls (PCBs) measured in background air in Leova, Moldova** M. Nordum, University of Oslo / Department of Environmental Chemistry; K. Breivik, Norwegian Inst for Air Research; W. Aas, Norwegian Institute for Air Research; S. Eckhardt, Norwegian Institute for Air Research / Atmosphere and Climate Change Department; M. Schlabach,

Norwegian Institute for Air Research; V. Balan, Hydrometeorologic State Service, Ministry of Ecology and Natural Resources. Polychlorinated biphenyls (PCBs) are persistent, toxic and bio-accumulating industrial chemicals that can undergo long-range atmospheric transport. While production ended in most countries during the 1970s and 1980s, it continued in Eastern Europe until 1993. The Republic of Moldova never produced PCBs, but accumulated large amounts over the years, for the most part as dielectric oil used in electric equipment. Even though efforts have been done in recent years to safely manage and dispose the stockpiles of PCBs in Moldova, data on atmospheric levels of PCBs in this region remain rather limited. However, such data are of vital importance to support monitoring efforts under the European Monitoring and Evaluation Program (EMEP) as well as the Stockholm Convention on POPs. The objectives of this study were (i) to determine ambient concentrations of PCBs and other POPs in air at a background site in Moldova (Leova: 46°29'18"N, 28°17'0"E), (ii) to compare and contrast measured levels of PCBs with data from other countries and regions, as well as (iii) to evaluate possible source regions using the Lagrangian particle dispersion model FLEXPART. Weekly 24-hour measurements were collected from September 2009 to October 2010, using a high volume air sampler. Glass-fiber filters and polyurethane foams from the sampler were extracted, analyzed and quantified for PCBs using GC/MS. The FLEXPART-model was used for simulations of atmospheric transport and was run backward in time to identify the source regions of POPs at the sampling site. Here we present concentrations of PCBs and compare our results with data from other EMEP background stations in Europe and additional sites. The more likely source regions affecting the measured levels of PCBs in Leova are finally identified and discussed on the basis of model calculations.

**WE186 Size fraction distribution and seasonal trends of particulate-bound persistent organic pollutants** L. Melymuk, Research Centre for Toxic Compounds in the Environment RECETOX / RECETOX; K. Okonski, Masaryk University / Faculty of Science RECETOX; C. Degrendele, L. Landlova, Masaryk University; P. Kukucka, J. Klanova, Masaryk University / RECETOX. Size distributions of atmospheric particulate matter (PM) and associated persistent organic pollutants (POPs) have been investigated on a seasonal basis for a range of compounds, including PCBs, organochlorine pesticides, flame retardants and dioxins/furans. This study addressed the need for a more complete understanding of particle-bound POPs to better characterize long-range transport and to improve estimates of toxicity. Seasonal resolution provided additional information on temperature-dependent differences in particle-size distribution, reflecting the seasonal variability of both POPs and PM. Weekly samples were collected at two sites (urban, rural) in the Czech Republic in 2009-2010 using a high volume air sampler equipped with a cascade impactor, separating atmospheric particles into 6 size fractions (< 0.49, 0.49-0.95, 0.95-1.5, 1.5-3.0, 3.0-7.2, and 7.2-10 µm). Target analytes (PCBs, PCDD/Fs, PBDEs, OCPs) were extracted in toluene and fractionated with silica and carbon columns. All compounds were analysed via GC-MS. In general, POPs were associated with the two smallest particle size fractions (< 0.49 and 0.49-0.95 µm), which accounted for 50-80% of the particle-bound POPs. Exceptions were selected banned pesticides, such as HCHs, as well as BDE-209, both of which were more uniformly distributed amongst size fractions. Seasonal and site-specific variability was also observed in the distributions. In summer, lower-molecular weight POPs (e.g. PCBs, tri-PBDEs, OCPs) were distributed more evenly amongst size fractions, which may suggest volatilization and redistribution of POPs amongst aerosols at warmer temperatures. The dominant size fraction also differed between the urban and rural sites, with the smallest (< 0.45 µm) size fraction dominating at the urban site and the 0.49-0.95 µm fraction dominating at the rural site. These results can be interpreted in the context of two main influences: sources and temperature (season). Differences exist in sources of both POPs and PM between urban and rural sites, with a higher proportion of fine PM at the urban site, and higher concentrations of most POPs at the urban site. Similarly, temperature influences bulk POP concentrations, the particle-bound

fraction of POPs, and PM concentrations. These two influences combine to drive the particle size fraction distributions of POPs and provide a framework for better understanding the larger impact on POP transport and toxicity.

**WE187 Temporal trends of POPs in sediments from a high mountain lake in the Tyrolean Alps** G. Garriga, J. Grimalt, IDAEA-CSIC; K. Koinig, Innsbruck University; P. Pechan, Ludwig-Maximilians-Universität; R. Psenner, Innsbruck University **B. van Drooge**, . European high mountain lake systems serve as sensitive environmental indicators to determine the speed and direction of changing of the environmental quality on the continent. Persistent organic pollutants (POP), among them the organochlorines, such HCB, PCBs and DDTs, are xenobiotic chemicals that were introduced to the environment in the second half of the 20<sup>th</sup> century. These toxic compounds arrive to the high mountains after atmospheric transport, where part of them gets buried in lake sediments [1, 2]. Under the Stockholm Convention the production and use of most of the POPs are restricted and prohibited, with the goal to reduce the emissions of POPs to the environment [3]. The objective of the present study was to gain insight on the temporal profile of POPs in a high mountain lake sediment from the Tyrolean Alps (Gossenköllesee, 2417 m.a.s.l.; 47°13' N, 11°1' E), and to see whether a reduction of the POPs is visible in the samples from the most recent decade (2000-2010). In summer 2010, a 15 cm sediment core was sampled, and sub-samples of 0.5 cm were analyzed on 17 POPs. HCB, 4,4'-DDE, 4,4'-DDD, PCB#52, #101, #118, #153, #138, #180 were above the LOD (>0.02 ng/g dw) and the LOQ (>0.07 ng/g dw) in all the samples. In the deeper layers (>8 cm depth; pre-1950s) "field blank" levels (~0.1 ng/g dw) were observed. Then, the POP levels increase to maximum levels for ?DDTs (2.7 ng/g dw) in the layer at 4.5 cm depth (~ 1970s), while PCBs (?PCBs = 3.5 ng/g dw) peak in the sediment layer at 2 cm depth (~ 1990s). For ?DDTs a constant decrease of about 0.05 ng/g dw per year was observed over the past 35 years ( $r^2 = 0.9$ ), while no significant decrease could be observed for HCB and the PCB congeners in the past 15 years. These results indicate that the input of POP to this Alpine area by atmospheric transport has decreased in the recent decades for 4,4'-DDE and 4,4'-DDD, while the input of PCBs has not changed. References [1] Grimalt et al. 2001. Environ. Sci. Technol. 35, 2690-2697. [2] Grimalt et al. 2004. Chemosphere 54, 1549-1561. [3] www.pops.int/ *Acknowledgement* - The authors thank Paul acknowledge the financial support from the EU-ComEnvironment project. Inma Fernandez is acknowledged for the analytical assistant.

**WE188 Dioxin-like activity of composts from European countries** M. Benisek, Masaryk University Faculty of Science / RECETOX; P. Kukucka, Masaryk University / RECETOX; B. Gawlick, EC Joint Research Centre (JRC); L. Blaha, Masaryk University, Faculty of Science / RECETOX. Persistent organic pollutants (POPs) and also other chemicals (such as PAHs) acting via AhR receptor and causing the dioxin-like activity can be found in various environmental matrices including composts, which are widely used in agriculture. In the present study, AhR-active POPs in composts were measured by biological and chemical methods. At first, crude organic extracts of various types of compost (separated biowaste, green waste, sewage sludge and others) from several European countries were tested by H4IIE-luc reporter gene bioassay for dioxin-like activity (in total, 97 samples were investigated). Based on the screening results, 20 samples were selected for further analyses and extracts were treated with sulfuric acid to remove less persistent compounds such as PAHs. In addition, levels of polychlorinated dioxins/furans (PCDD/Fs), polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs) were analyzed by HRGC-HRMS in these selected samples. Most of the 97 samples showed pronounced dioxin-like effects with the highest levels 12 ng TEQ-bio/g dry matter (TEQ-bio - TCDD toxicity equivalents derived from the bioassay). However, samples treated with sulfuric acid did not show any significant effects in H4IIE-luc bioassay, which indicate that PAHs and other less stable compounds were responsible for the effects observed. Chemical analyses revealed relatively low concentrations of POPs in selected samples. In conclusion, some studied extracts of

composts were found to have dioxin-like potential. However, the effects were not related to POPs but rather less-persistent compounds such as PAHs and their metabolites.

**WE189 Sediment profiles of natural and anthropogenic organohalogen compounds in a Baltic sediment core** K. Löfstrand Stockholm University / Department of applied environmental science; L. Asplund, Stockholm University / Department of Materials and Environmental Chemistry; P. Haglund, Umea University / Department of Chemistry. Polychlorinated and polybrominated dibenzo-*p*-dioxins (PCDDs and PBDDs) and dibenzo-furans (PCDFs and PBDFs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), hydroxylated and methoxylated polybrominated diphenyl ethers (OH-PBDEs and MeO-PBDEs) and polychlorinated and polybrominated phenols (PCPs and PBPs) were analyzed in one sediment core from Kvädöfjärden, a remote bay on the Swedish coast of the Baltic proper. The sediment profiles of chlorinated and brominated compounds showed two different distributions. The classic POPs (PCDD/Fs and PCBs) approximately follow the trend of usage of PCBs in Sweden, while the data indicates an exponential increase in the levels of naturally produced brominated compounds (PBDDs, OH-PBDEs, MeO-PBDEs and PBPs) with time. Interestingly, PBDE, dominated by BDE-209, also show an exponential increasing trend with time. Whether this trend really reflects a true trend, showing the increasing usage of BDE-209 and elevated natural production, is debatable. Another explanation may be degradation of the brominated compounds in the sediment. Based on congener profiles of the brominated compounds there are no indications of debromination or degradation of either the natural products or the PBDEs within the dataset. Thus, the possibility of an increase in natural production cannot be discarded.

**WE190 Residues of organochlorine pesticides (OCPs) in arable soils from the Eastern part of Poland** B. Smreczak, Institute of Soil Science and Plant Cultivation / Department of Soil Science Erosion and Land Conservation; B. Maliszewska-Kordybach, Institute of Soil Science and Plant Cultivation – State Research Institute; A. Klimkowicz-Pawlas, Institute of Soil Science and Plant Cultivation State Research Institute / Department of Soil Science Erosion and Land Protection. Organochlorine pesticides including DDTs and HCHs have a long history of agricultural use worldwide. The persistence of OCPs in the environment and their bioaccumulation in tissues of living organisms led to the cancellation of their production and use in many countries, in Poland during 1970s and 1980s. Agriculture production is a very important sector in Polish economy especially for regions situated in the East part of the country e.g. Lubelskie voivodeship where rural areas cover over 90% of their territories. The control of the residue content of OCPs in arable soils in such regions is of special concern to produce pollution-free and high quality products. Polish policy for soil protection includes national soil quality standards introduced in 2002 by the Ministry of Environment related to the land use, soil depth and soil hydraulic permeability. Limit values specified for contaminants like e.g. OCPs has been set on a very low level, very often below the guidelines applied for agricultural soils in other countries. The aim of the study was to evaluate the residual concentrations of selected OCPs in arable soils from Eastern part of Poland represented by Lubelskie voivodeship. The data set embraced 20 soil samples collected from the surface soil horizon. The organochlorine pesticides: p,p'-DDT, p,p'-DDD, p,p'-DDE, a-HCH, b-HCH, and g-HCH (lindane) were determined by GC-?ECD and GC-MS techniques. Polish (*Dz.U.02.165.1359*) and Canadian (CCME,) soil quality standards were applied for the assessment of contamination status of investigated soils. The data indicated that DDTs residue concentrations in soils expressed as the sum of three individual compounds were in the range of 0.8-106.5 mg·kg<sup>-1</sup> (median of 8.6 mg·kg<sup>-1</sup>). Over 75% of the data set were below both Polish: 0.025mg·kg<sup>-1</sup> and Canadian: 0.7 mg·kg<sup>-1</sup> requirements for agricultural soils. The parent p,p'-DDT isomer was on the level expected for aged pollution but the dominance of p,p'-DDE metabolite over p,p'-DDD pointed out on the aerobic transformation of investigated pesticides. Concentration of the sum of HCHs were much lower than DDTs and

ranged between 1.3-7.2 mg·kg<sup>-1</sup> with the g-HCH isomer as the most abundant (median of 2.1 mg·kg<sup>-1</sup>). Generally, the level of OCPs in the soils under study was on the level corresponding to other European countries and indicated on dispersed pollution as the main source of those contaminants.

**WE191 Do temporal trends of persistent organic pollutants in Arctic ringed seals reflect global trends?** D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division; X. Wang, A. Sett, E. Barresi, E. Sverko, Environment Canada; S. Ferguson, Dept of Fisheries and Oceans; A. Fisk, University of Windsor; R. Addison, Addison Environmental Consulting. The ringed seal is the most abundant Arctic pinniped with a circumpolar distribution and has been a key biomonitoring animal for examining spatial and temporal trends of persistent organic pollutants (POPs). In the Canadian Arctic temporal trends of legacy POPs and new/emerging POPs can be assessed using sample collections that started in the 1980s in Hudson Bay (Arviat) and Cumberland Sound (Pangnirtung), in the 1970s in Lancaster Sound (Resolute) and in the southeastern Beaufort Sea (Sachs Harbour and Ulukhaktok). Results for new POPs, polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), perfluorinated chemicals (PFCs), and endosulfan, along with carbon and nitrogen stable isotope data, have been added to samples collected since 2001 and on selected archived samples from the 1970s and 1990s. Sample collections consisting of 10 to 25 adult ringed seals are carried out by hunters each year (June-October) as part of their traditional hunting. Sample analysis follows previously published methods for neutral POPs in blubber and liver (for PFCs only). At most locations only blubber of females and juveniles are analysed to limit the influence of age. Results show that there are declining trends for legacy POPs in all locations with the relative magnitude of ?DDT> ?HCH> ?10PCB> ?CHL. Largest declines are in Hudson Bay (?10PCB =5%/yr; ?DDT =7%/yr) and lowest in Sachs Harbour (?10PCB =1.5%/yr; ?DDT =3.3%/yr). An exception is ?-HCH which has been increasing particularly at Sachs Harbour and Ulukhaktok. PBDEs increased in concentrations in the 1990s to 2010 at Resolute and Sachs Harbour while in Hudson Bay they have declined over the period 2005-2011. PFCs also increased until 2003-2005 in all locations but have declined over 2005-2011. Endosulfan, HBCD, and bis(tribromophenoxy)ethane are present at low concentrations in seal blubber (0.01-2.0 ng/g) and appeared to increase in concentration over the period 2005-2011. Proximity to North American source regions for the Hudson Bay population, and the influence of Pacific Ocean seawater transport through the Canadian archipelago for the Sachs Harbour/Ulukhaktok and Lancaster Sound populations, may explain regional differences. Overall the trends of legacy POPs, PBDEs and PFCs in ringed seals do appear to reflect predicted declines in use and emissions resulting from past bans and phaseouts in the Northern Hemisphere.

**WE192 Determination of PCB content in fish tissue and exposure assessment among sports fishermen in Sanski most, Bosnia and Herzegovina** A. Marjanovic, J. djedjibegovic, M. Sober, K. Sinanovic, Faculty of pharmacy, University of Sarajevo; M. Grung, E. Fjeld, S. Rannekleiv, C. Harman, S. Rognerud, T. Larssen, Norwegian Institute for Water Research (NIVA); S. Burnic, Faculty of pharmacy, University of Sarajevo. Persistent organic pollutants are extremely stable substances which are mostly chlorinated derivatives of aromatic and polyaromatic compounds. These compounds represent a special problem, because they are very stable in nature, they accumulate in fat tissue of most living organisms and can exhibit significant toxic effect on humans and animals, including malignant changes. Nowadays investigations focus on the effect of low concentrations of polychlorinated biphenyls (PCBs) exposure, including increased incidence of hypertension and cardiovascular diseases, as well as dysfunction of endocrine system, reproductive organs and neurological changes. The most significant source of PCB for humans are animal products, fish being the most important single source of PCBs. The objective of this work was to determine content of PCBs in edible fish tissue using ELISA kit. Fish (n=28) were collected from three sites on

River Sana, Bosnia and Herzegovina during April and May 2012. The frequency of fish consumption among sports fishermen were assessed by the questionnaire. The most commonly consumed fish: trout (*Salmo trutta* L.), grayling (*Thymallus thymallus* L.), and chub (*Leuciscus cephalus* L.) were chosen for the analysis. Homogenized fish filets were pretreated with tetrabutyl ammonium sulphate and sulphuric acid and analyzed with immunoassay based commercial kit for highly chlorinated PCB according to the procedure provided by the manufacturer. The PCBs content (as Aroclor 1254) was in range of 0.39-207 ppb wet weight (6.80-1260 ppb lipid weight). According to US EPA guidance, 6 samples (21%) and 7 samples (25%) could not be considered as safe for consumption in regard to chronic non-carcinogenic effects and carcinogenic effects, respectively if consumed more than once per month. Since most of the fishermen and their family members consume this fish once a week, the possible health effects due to PCB exposure can not be ruled out.

**WE193 The total PCB-task: A comprehensive HRGC-HRMS method for analysis of all 209 PCB congeners in fish matrices** E. Neugebauer, Eurofins GfA GmbH / R&D Department; C. Ast, Eurofins GfA Lab Service GmbH / R&D Department; M. Opel, Eurofins GfA Lab Service GmbH. Polychlorinated Biphenyls (PCB) have been known as being toxicologically relevant for a long period of time. There are different toxicological action modes of PCB, comprising dioxin-like (dl) and non-dioxin-like (ndl) activities, depending on specific chlorination patterns of the respective congeners. Derived from these facts, there have been ongoing discussions and activities in order to minimize human PCB exposure as far as possible and to regulate maximal allowed levels and intakes. One of these activities resulted in the "safe harbour levels" for PCB, issued by the Californian government in legislative Proposition 65. This issued level describes a maximum daily intake considered as being safe for human health, expressed as "total PCB". This legislative demand resulted in a question about the PCB content of food additives, as e.g. otherwise healthy fish oil products. It therefore implies the ability of precisely analysing the "total PCB" content in terms of determining all 209 PCB congeners. There have been several different approaches for respective methods and calculation modes, reaching from quantification against technical PCB mixtures over fractionation of PCBs to complete generic templates as e.g. US-EPA method 1668. With all these approaches showing difficulties to different extents, we developed a completed approach using a specialised multistep-cleanup in combination with a modern HRGC column and HRMS detection, which enables a comprehensive analysis of all PCB congeners with around 180 peak separations. We present results for this method as it has been applied on biota and biota-related samples, e.g. fish oils from South American origin from different fish species (anchovy, tuna). We are able to show patterns of all PCB congeners as well as congener groups, opening access to examine preferences in transport and enrichment as well as metabolic behavior.

**WE194 Searching for potential POP candidates – a strategy approach** J. Koch-Jugl, Federal Environment Agency / International Chemical Management; M. Scheringer, ETH Zuerich / Institute for Chemical and Bioengineering; C. Rauert, Umweltbundesamt / International Chemical Management; S. Hukari, ETH Zurich; M. Blepp, Öko-Institute e.v.. Persistent Organic Pollutants (POPs) are chemical substances that are not readily degradable in the environment, that bioaccumulate and have harmful effects on human health or the environment; furthermore, POPs have the potential for long-range transport. Once released, they spread around the globe via air and water, and also through the food chain. Thus, POPs pose a global risk to man and the environment. When the Stockholm Convention was adopted in 2001, initially twelve substances were listed in its Annexes in order to eliminate or restrict these substances or to reduce their emissions. In 2009, nine more POPs were included in the Convention. At the fifth Conference of the Parties (COP 5) in April 2010, Endosulfan was included in the convention, as the 22nd substance listed there. In addition, five additional substances are currently under review by the POP Review Committee (POPRC) of the Convention. Have the most

relevant substances been identified and banned, or are there others that should be looked at closely? To answer this question, the German Federal Environment Agency (UBA) has contracted Öko-Institute and ETH Zürich to develop a strategy for identifying further potential POP candidates. In order to develop an iterative procedure (i.e. strategy), the evaluation of current methods to assess and validate POP-properties of chemicals was conducted by an ex-post analysis of the substances defined as POPs so far. The strategy reveals the relevant steps that lead to the identification of possible new POP candidates. It also identifies gaps that need further consideration. In this context, the poster aims at discussing a possible way forward to identifying new POP candidates.

**WE195 Resource Efficiency and Future Resource Requirement in Photovoltaic (PV) System; Case Study of South Korea** J. Lim, Inha University / Environmental Engineering; J. Kim, University of Technology of Troyes / CREIDD; Y. Hwang, Inha University / Environmental Engineering. The worldwide demand for fossil and minerals are growing continuously. Also the demand for a number of metals and rare materials are forecast to double over the next 50 years (Muilerman and Blonk 2001). This resource depletion is also related with technologies for harvesting adequate amounts of sustainable energy. Renewable energy technology and systems such as photovoltaic (PV) and wind system are consuming rare materials and using available land as well as providing direct benefits at national and local levels. In South Korea, intensive effort started in 1988 under Promotion Act for New and Renewable Energy Development. According to the 3rd National Plan for Energy Technology Development, the Government is aiming at the supply of 6% of total energy demand by new and renewable energy by 2020 and 11% by 2030. In case of the PV system, the goal is targeted from 59,000 TOE production in 2008 to 1,364,000 TOE production in 2030 (about 2,311% increased). In this study, based on 1 m<sup>2</sup> PV module production (Single-crystal silicon (SC-Si), Multi-crystal silicon (MC-Si), CI(G)S thin-film (CI(G)S), CdTe thin-film (CdTe)), the life cycle resources requirement (e.g., types and amount of input ferrous and nonferrous metals and rare materials), resource efficiency and land use are calculated by using material balance data and Eco-invent life cycle inventory data. Also by using the photovoltaic energy production target in South Korea by 2030, future requirement resources and land use amount calculated. As a result, the consumption of ferrous and nonferrous metals, rare earth and critical materials as well as land use were quantified. In the ferrous and nonferrous metal, aluminium (SC-Si; 23kg, MC-Si; 23kg, CI(G)S; 17kg, and CdTe; 16kg) was the most consumed metal and followed by iron and zinc. In the precious materials, cadmium (SC-Si; 9.20E-06 kg, MC-Si; 9.30E-06 kg, CI(G)S; 6.54E-01 kg, and CdTe; 7.70E-01 kg), chromium (SC-Si; 1.60E-01 kg, MC-Si; 1.60E-01 kg, CI(G)S; 1.50E-01 kg, and CdTe; 1.60E-01 kg), manganese, gallium were the most used metals. Also uranium (SC-Si; 4.60E-02 kg, MC-Si; 4.90E-04 kg, CI(G)S; 9.20E-04 kg, and CdTe; 2.40E-03 kg) and cerium were the most consumed metals in rare earth materials. The amount of land use by 2030 is 942 km<sup>2</sup> for SC-Si system, 1,077 km<sup>2</sup> MC-Si system, 311 km<sup>2</sup> CI(G)S system and 606 km<sup>2</sup> CdTe system. Our research approach can be used and applied for future sustainable resource and land use management in South Korea.

**WE196 Ecotoxicity of rare earth elements (REEs): Are they a uniform group beyond chemistry?** V. Gonzalez, LIEC, CNRS UMR 7360; D. Vignati, C. Leyval, J. Ferard, Université de Lorraine / LIEC, CNRS UMR 7360. The rare earth elements (REEs) include scandium (Sc), yttrium (Y), lanthanum (La) and the lanthanides cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb) and lutetium (Lu). These elements form a chemically uniform set. Because their many applications (e.g., aerospace, green energy or defense industries) and their limited supply, they are considered as strategic metals. Moreover, REEs are used in fertilizers, at least in East Asia. The raising use of REEs has triggered renewed interest in exploring new sources which will likely result in increasing public and environmental exposure to these elements. Several reviews regarding

agricultural significance, factors governing the behaviour of these elements in soil-plant systems or their use to trace pedogenetic processes have been published. However, studies about toxic effects following environmental exposure as well as the mechanism of action of these elements are much less known, except for lanthanum and cerium that are relatively well studied. Lanthanides are most abundant than other elements such as Hg or Cd and as abundant as Cr, Zn or Mo. It is well documented that high abundance of elements in nature usually results in weak biological toxicity, but some studies suggest that this relation does not appear to hold true for the lanthanides. However, early evidences exist about similarities in ecotoxicity effects of these less studied elements (LSE). We assume that given their chemical similarities, many of LSE should exhibit ecotoxicological similarities. In order to test this hypothesis, a review of ancient and recent literature dealing with REEs ecotoxicity is addressed and data discussed, allowing us to detect future needs of research in terms of REEs ecotoxicity data.

**WE197 LCA and criticality of raw material: relationship and potential synergies** S. Sala, Joint Research Centre European Commission / Sustainability Assessment Unit - Institute of Environment and Sustainability; L. Mancini, F. Ardente, Joint Research Centre / Sustainability Assessment Unit - Institute of Environment and Sustainability; M. Goralczyk, D.W. Pennington, Joint Research Centre / Institute of Environment and Sustainability. Life cycle assessment (LCA) is increasingly adopted for the appraisal of products as the methodology accounts for environmental impacts of resource use. European Commission recently has been listing a number of critical raw materials (CRM), based on their supply risk and economic importance. Depletion, scarcity and criticality are key issues under discussion both in the LCA community and in the wider resource debate. A bidirectional relationship between CRM evaluations and LCA could be considered since strategies of resource efficiency could be applied both for reducing environmental burdens, supporting a sustainable choice of substitute materials and boosting economic benefit. The LCA community is currently debating how these aspects should be considered and modeled within LCA: e.g. whether criticality should be part of the impact category "resource depletion" and of an area of protection "natural resources"; how to use results of LCA for comparing CRMs or for identifying a potential substitute material; whether socio-economic implications should be part of the LCA; if current LCA indicators for resources provide governments and business with the most appropriate information for decision support. The present study is based on the results of 3 initiatives developed by the Joint research Centre of the EC: (1) LC-indicators project, providing an overview of the impact associated with the resource use in EU, based on account of material flows in the EU economy in a life cycle perspective (<http://lct.jrc.ec.europa.eu/assessment/assessment/publications#indicators>). (2) Ecodesign project focusing on the assessment of the impact and benefit associated with strategies to reduce resource use, e.g. via improvement of reuse and recycling (<http://lct.jrc.ec.europa.eu/assessment/projects>). (3) Results of a recent workshop (<http://lct.jrc.ec.europa.eu/assessment/ResourceSecurity-SecuritySupply>) on a methodological framework for sustainability assessment referring to security of supply and scarcity of raw material. The aims of the present study, are, therefore, related to the evaluation of the potential relationship between LCA and CRM appraisal in order to identify major gaps in knowledge and potential synergies. In particular, the study aims at assessing: coverage of CRM in Life cycle inventories and life cycle impact assessment methods, environmental profiles of CRMs with focus on ecotoxicity and human toxicity

**WE198 New Environmental Risk Limits for uranium for the Dutch aquatic environment** R. van Herwijnen, Centre for Safety of Substances and Products; E. Verbruggen, RIVM Expertise Centre for Substance / Centre for Safety of Substances and Products. The Dutch National Institute for Public Health and the Environment (RIVM) has derived new Environmental Risk Limits (ERLs) for uranium. The ERLs were derived according to the new WFD methodology. Not only direct ecotoxicity, but also exposure via secondary poisoning and human fish

consumption were covered in this new ERL. Uranium is a natural element which is mainly known for its use in nuclear power plants and in nuclear weapons. Another (civilian) use is as counter weight in airplanes. Its natural presence in rocks and soil, and anthropogenic activities like mining, ore processing, agriculture (phosphate fertilizers) and coal combustion can also contribute to an increased presence of uranium above its natural background concentration. Because of the many sources and the fact that uranium is regularly found in Dutch surface water, uranium is listed as a so-called 'specific pollutant' and included in the Dutch national legislation under the Water Framework Directive (WFD). For this purpose, new Environmental Risk Limits (ERLs) according to the methodology of the WFD were required for uranium. Public literature on aquatic ecotoxicity and bioaccumulation of uranium was reviewed with respect to scientific reliability and relevance for this purpose. Most available studies were performed with uranyl nitrate and uranyl sulfate but also studies with other uranium species like uranyl chloride and uranyl phosphate were reviewed. The resulting proposal for new water quality standards will be presented. For naturally occurring compounds like trace metals, ERLs are expressed as maximum concentrations that may be added to the background level. Background concentrations for uranium in the Netherlands are reported ranging from 0.087 to 0.97 µg/L. This information is however not specified enough to determine a general natural background concentration for the Netherlands. Therefore, for compliance check the effective background concentration is assumed to be zero. Nevertheless it is likely that background levels will have a significant influence when compliance to the new ERLs needs to be evaluated.

**WE199 Changes in Hormone Production and Steroidogenic Gene Expression in H295R Cells by Anthropogenic Micropollutants in Drinking Water** R. Redelstein, J. Kuckelkorn, RWTH Aachen University / Institute for Environmental Research; S. Maletz, RWTH Aachen University / Inst. for Environmental Research (Biology V); T. Grummt, A. Eckhardt, German Federal Environment Agency; T. Seiler, H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V). The presented study is part of the joint research project "Tox-Box - Risk assessment of anthropogenic micropollutants to assure the drinking water supply" (BMBF FKZ 02WRS12821) which aims at a harmonized, hierarchic test strategy to assess the toxicity of micropollutants that may occur in drinking water. As one part of this project, endocrine activity of several drinking water relevant substances, which are released into the environment via different pathways, is assessed with a set of bioassays. One of these assays is the H295R Steroidogenesis Assay with the human adrenocortical carcinoma cell line H295R. Effects on the production of the hormones 17 $\beta$ -estradiol and testosterone are determined by means of a competitive ELISA while effects on the expression of different steroidogenic genes will be determined using quantitative real-time PCR. First results indicate that the pharmaceutical diclofenac, the flame retardant tris (1-chloro-2-propyl) phosphate (TCPP), the radio contrast agent diatrizoic acid (DTA) and the industrial chemical perfluorooctanoic acid (PFOA) have no effects on hormone production when applied at their water solubility limit. Further substances to be assessed with the H295R Assay are benzo[a]pyrene,  $\beta$ -sitosterol, atrazine, tributyltin, carbamazepin and sulfamethoxazol. Effects on the expression of steroidogenic genes will be assessed for substances with strong effects on hormone production in H295R cells or high environmental relevance. Correlation between hormone production and expression of steroidogenic genes will particularly be of interest in order to understand the mode of action of these substances. The results will be compared to results from other bioassays of the test battery such as the ER/AR-Calux<sup>®</sup> assay and the reproduction toxicity assay with the snail *Potamopyrgus antipodarum* (cf. poster presentation Kuckelkorn et al.). Results of the entire Tox-Box project, which is part of the BMBF funding measure "Risk Management of Emerging Compounds and Pathogens in the Water Cycle (RiSKWa)", will be used to establish a new guideline for the risk assessment of anthropogenic micropollutants in drinking water including the health-related indication value concept (HRIV-concept).

**WE200 Rapid cost effective in vitro mode of action-based tools for early detection of pollutants and mixtures with endocrine disruptive activities** S. Jarque, J. Dansova, J. Javurek, E. Sychrova, M. Benisek, L. Blaha, K. Hilscherova, Masaryk University / Faculty of Science, RECETOX. Reporter gene assays are becoming widespread tools for screening of interference of pollutants and environmental mixtures with nuclear hormone receptors, which play a crucial role in endocrine regulation. The sensitive models are usually based on in vitro mammalian or fish cell lines, thus for testing require continuous cell culture under sterile conditions that can be rather costly and dependent on special equipment for sterile work. They might also require longer exposure. Recombinant yeast bioassays can serve as a very useful alternative because of their high sensitivity, reproducibility and relatively low costs. During this project we aim to optimize especially yeast *Saccharomyces cerevisiae* based assays for the fast assessment namely of anti/estrogenic, anti/androgenic and glucocorticoid potencies of environmental samples and compare their applicability in comparison with more elaborate, expensive and equipment-demanding vertebrate-cell based models. The study includes detailed characterization and comparison of the sensitivity, robustness and reproducibility of the two types of models on a set of model compounds and comparison of their relative potencies (REP). We tested the suitability of the yeast models for testing of both agonistic and antagonistic effects. Important part of the research is the preparation of ready-to-use version of the assays to provide fast and cost-effective response in a few hours without continuous culture of the model. In the present work we consider new approaches to improve existing procedures, being the assays optimized and modified for best sensitivity and applicability for field samples. The study includes the discussion on interpretation of the potencies and specific toxic equivalents determined in various in vitro models in relation to potential pollution risk assessment.

**WE201 Toxicological profiling of organic pollutants in river Bosna by passive sampling in combination with a battery of bioassays and chemical analysis** Z. Rabova, Masaryk University / Faculty of Science, RECETOX; B. Vrana, Water Research Institute; J. Slobodnik, Environmental Institute; M. Dzajic-Valjevac, S. Milanolo, Hydro-Engineering Institute of Civil Engineering Faculty Sarajevo (HEIS); K. Hilscherova, Masaryk University / Faculty of Science, RECETOX. Integrated assessment using passive sampling and chemical analysis along with a set of specific bioassays was used to characterize river basin specific organic pollutants, their fate and associated environmental risks in River Bosna in Bosna and Herzegovina. Non-polar and polar organic contaminants were sampled in autumn 2012 for 3 weeks at 10 localities by SPMD (Semipermeable Membrane Device) and POCIS (Polar Organic Chemical Integrative Sampler), respectively. Passive samplers were exposed upstream and downstream of major point source pollution discharges to investigate their effects on contamination levels in the main river. The investigated pollution sources include five industrial sites (coal thermo power plant, metal industry, leather industry, pulp and paper industry, crude oil and derivatives refinery), two regional landfills (Sarajevo and Zenica) and three municipal wastewater discharge points (Sarajevo, Zenica and Dobo). A battery of *in vitro* bioassays based on reporter-gene cell lines was used to assess dioxin-like, anti-/estrogenic, anti-/androgenic and glucocorticoid potential as well as non-specific toxicity of extracts from passive samplers. Specific toxicity profiles from 10 localities will be supplemented with results of target (for priority substances) and non-target chemical analyses to identify the most contaminated areas and the major pollution sources. The study also investigates to which extent chemical analysis of prioritised compounds correlates with the more complex response of bioanalytical tools to sampled contaminant mixtures. This research was supported by project CETOCOEN (CZZ.1.05/2.1.00/01/0001) and EDA EMERGE Project.

**WE202 In silico methods as a basis for identification of potential thyroid disrupting chemicals** J. Zhang, Umea University / Chemistry Department; J.M. Weiss, VU University Amsterdam / IVM; P.L. Andersson, Umea University / Chemistry Department. Thyroid

hormones (TH) play an important role in the maintenance of a normal physiological state and are essential for normal metabolism, CNS development and body growth. Thyroid disrupting chemicals (TDCs) that interfere with the physiological function of THs may lead to hormone related diseases and disorders especially during early childhood. Humans can be exposed to TDCs indoors through dust particulates where children are among the most heavily exposed groups. Identification of TDCs through in vitro and in vivo tests is costly, laborious and time-consuming. A Swedish research project, MISSE, was recently initiated aiming at unravelling mixture effects from dust exposure with special focus on thyroid hormone related effects. One part of the projects aims at developing in silico models for identification and prioritization of industrial chemicals as potential TDCs. In the first phase of the MISSE project, the thyroid hormone system structure has been analysed in order to identify potential targets for TDCs. Ten targets have been identified including the thyrotropin-releasing hormone receptor, thyrotropin receptor, Na<sup>+</sup>/I<sup>-</sup> symporter (NIS), pendrin (PDS, I-/Cl<sup>-</sup> porter), thyroperoxidase, thyroxine-binding globulin, transthyretin (TTR), iodothyronine deiodinases, thyroid hormone receptors, and UDP-glucuronosyltransferase (UGT). Their physiological function and congenital ligands are well described in the literature. Industrial chemicals acting on each target together with their IC50 values have been collected through literature searches. The chemical variation of potential TDCs acting on various pathways are characterized using computational chemistry tools in combination with multivariate statistics. All these data gives us better knowledge of the mechanism-of-action of TDCs disturbing effects on the normal function of the thyroid hormone system. According to our preliminary searching outcomes, TTR might be the most-studied target; over 200 industrial chemicals including their metabolites have been collected. In future steps, using collected data as input, we will focus on the biophysical features of binding targets and the interaction between selected industrial chemicals and targets, by introducing computer-aided drug design tools, such as molecular docking, pharmacophore studies and 3D QSAR.

**WE204 Bioactive compounds from cyanobacteria as potential endocrine disruptors in aquatic environment** E. Sychrova, Faculty of Science, RECETOX; K. Novakova, O. Adamovsky, T. Prochazkova, Masaryk University / Faculty of Science, RECETOX; J. Giesy, University of Saskatchewan / Veterinary Biomedical Sciences and Toxicology Centre; K. Hilscherova, Masaryk University / Faculty of Science, RECETOX. According to numerous scientific findings, wide range of chemicals has potency to cause endocrine disruption. Besides anthropogenic compounds natural bioactive compounds could contribute to the endocrine disruptive effects in the environment. Our results document that cyanobacteria could be producing endocrine disruptive compounds directly to aquatic ecosystems. These bioactive compounds are namely important in relation to the possible mass expansion of cyanobacteria under favorable conditions, often caused by human activities (eutrophication). Our research has demonstrated endocrine disturbing potential of samples prepared from complex cyanobacteria biomasses collected in the environment and also from laboratory cultured cyanobacterial species. Cyanobacterial species were chosen according to their occurrence in European surface waters and their endocrine disruptive potencies were assessed in transgenic cell lines containing reporter luciferase gene under control of selected steroid receptors. The results document namely production of estrogenic compounds, which was confirmed on two independent in vitro reporter gene trans-activation models. The total potency of the mixture of compounds from cyanobacteria was quantified as estrogenic equivalents. In this work we summarize the findings related to the variability of production of the estrogenic compounds by cyanobacteria and algae. Important part of the study is also discussion of the implications of the findings for the environmental risk of cyanobacterial water blooms and potential contribution of bioactive compounds from cyanobacteria to endocrine disruptive effects observed in surface waters. The research was supported by the Czech Science Foundation grant No. P503/12/0553.

**WE205 Distribution character and potential risk of Phenols in surface water from schistosome-endemic areas in different periods** G. Zhao, H. Zhou, K. Li, L. Yu, China IWHR. Pentachlorophenol (PCP) and its sodium salt (Na-PCP) were widely used to kill mollusk in these areas where schistosomiasis is epidemic. To investigate the pollution characteristics of phenols in surface water samples from classic schistosome-endemic areas, 81 water samples were collected from Songli spillway, Ouchi river downstream, Tuojiang River in Nan County in low-flow period, medium-flow period and high-flow period. 14 phenols were measured in water samples using Varian CP3800/300 GC-MS/MS technique, two-hybrid yeast bioassay was used to detect thyroid disrupting effects in the samples extracts. The results show that the highest concentration of phenols (1362.64±2436.53 ng·L<sup>-1</sup>) were detected in the samples from the three rivers in the medium-flow period, there is significant difference between the concentrations of phenols in the samples from the same river in the three different period ( $P < 0.05$ , T-test); there is no significant difference between the concentrations of phenols in different rivers in the same period ( $P > 0.05$ , T-test). Phenol, 2,6-Dichlorophenol, 2-Nitrophenol and PCP are the predominant compounds, the concentrations of Phenol, 2,6-Dichlorophenol and 2-Nitrophenol were much lower than these limits listed in Chinese environmental quality standards for surface water and drinking water, however, the concentration of PCP in the samples were moderate and compared with the results reported in the other schistosome-endemic areas. Bioassay results show that inhibition activity to T3 reach 30.7%, the thyroid receptor (TR) antagonistic activities of these extracts were calibrated regarding to a known TR-inhibitor NH-3, which is equal to  $2.93 \times 10^{-3}$  g L<sup>-1</sup> NH-3. The facts suggest there exist potential TR disrupting risk due to the pollution of the phenols in these samples; therefore, more in-depth investigations of phenols contamination in these schistosome-endemic areas are needed.

**WE206 Microorganisms on Steroids** T. Schwarz, Cefas; I. Katsiadaki, Cefas / Environment and Animal Health. The presence of endocrine disrupting chemicals (EDCs) in surface waters has been linked to a number of adverse effects in aquatic organisms; an increasing body of literature attempts to link EDCs with human endocrine and developmental disorders too. Therefore the need to improve the removal of EDCs from wastewater in a cost-effective manner has become increasingly important. Ethinyl-oestradiol (EE2) is by far the most potent and recalcitrant EDC to date; as the active ingredient of the contraceptive pill it is also highly ubiquitous. Hence the focus of this project is to achieve higher removal rates of EE2 from wastewater in an environmentally friendly and affordable manner using fungal and bacterial biodegrading abilities. Laccases are multi-copper oxidases well known for degrading phenolic and aromatic compounds. However there are still gaps in the knowledge in terms of this degradation process (e.g. chemical structure of the metabolites). In order to assess the bioremediation potential of laccases our main aim is to elucidate the structure of these metabolites. Part of this assessment is ensuring that produced metabolites don't present significant oestrogenic activity themselves. Since the nature of the metabolites is unknown, we are employing bioassays that measure oestrogenic activity. These bioassays include the well-defined yeast estrogen screen (YES)-using the human oestrogen receptor-and a newly developed reporter-gene assay using a teleost oestrogen receptor. The latter is highly important because the detrimental effect of EE2 exposure to fish populations is well documented. Here we present our results on the comparative ability of six laccases of bacterial and fungal origin to degrade EE2, along with the oestrogenic profile of the metabolites produced. Where possible the chemical structures of major metabolites will be also included.

**WE207 Detection of endocrine effects by anthropogenic micropollutants in drinking water** J. Kuckelkorn, R. Redelstein, S. Maletz, RWTH Aachen University / Institute for Environmental Research; T. Grummt, A. Eckhardt, Federal Environment Agency; P. Waldmann, Incos Boté GmbH; H. Hollert, T. Seiler, RWTH Aachen University / Institute for Environmental Research. The joint project "Tox-Box – Risk assessment of anthropogenic micropollutants to assure the

drinking water supply" (BMBF FKZ 02WRS1282I) aims for a harmonized, hierarchic test strategy to assess the toxicity of micropollutants that may occur in drinking water by means of the health-related indication value (HRIVconcept). This concept offers four health-related values ranging from  $0.1 \mu\text{g L}^{-1}$  to  $3 \mu\text{g L}^{-1}$  depending on availability and completeness of toxicological data, including genotoxicity, neurotoxicity and germ cell damages. As one part of this project the Institute for Environmental Research at RWTH Aachen University will analyze and establish endocrine activity as an important, additional toxicological mode of action within this concept using a set of bioassays. The *in-vitro* ER/AR-Calux® test (Estrogen-/Androgen-Responsive Chemical-Activated Luciferase gene eXpression) will detect receptor-mediated endocrine activity in the human cell line ER- $\beta$ -CALUX and the *in-vivo* reproduction toxicity assay with the mud snail *Potamopyrgus antipodarum* will provide data of endocrine disruption on an individual level. A second *in-vitro* assay, the H295r test, will identify alterations in the steroidogenesis of the human adrenocortical carcinoma cell line H295R (see poster presentation Regine Redelstein). First results indicate that the combustion product benzo[a]pyrene, the pharmaceutical diclofenac, the flame retardant tris (1-chloro-2-propyl) phosphate (TCPP), the radio contrast agent diatrizoic acid (DTA) and the industrial chemical perfluorooctanoic acid (PFOA), applied at their water solubility limit, have no effects on the estrogen receptor-mediated hormone production. Lab work on the androgen receptor-mediated effects and the reproduction toxicity assay is still in progress. Further substances, namely  $\beta$ -sitosterol, atrazine, tributyltin, carbamazepin and sulfamethoxazol will be assessed with the entire set of biotests. Finally all results of the endocrine biotest battery will be compared and assessed including the H295r assay and the recombinant yeast assays R-YES-/R-YAS conducted by Incos Boté GmbH. The results from the entire Tox-Box project, which is part of the BMBF funding measure "Risk Management of Emerging Compounds and Pathogens in the Water Cycle (RISKWa)" will be used to establish a new guideline regarding the risk assessment of anthropogenic micro pollutants in drinking water.

#### **WE208 Estrogenic hormones in river waters of a rapidly industrialising: differences of India and United Kingdom conditions.**

Z. Shaikh, Newcastle University / School of Civil Engineering and Geosciences; W. Mroziak, Newcastle University / School of Civil Engineering Geoscience; T. Sreekrishnan, Indian Institute of Technology Delhi / Department of Biochemical Engineering and Biotechnology; R. Davenport, Newcastle University / School of Civil Engineering and Geosciences. Endocrine Disrupting Chemicals (EDCs) such as the natural and synthetic estrogenic hormones (estrone (E1), 17 $\beta$ -estradiol (E2), estriol (E3) and 17 $\alpha$ -ethinylestradiol (EE2)) may enter the natural environment via direct release of sewage and effluents from wastewater treatment plants. It has been well documented that aquatic fauna are at major risk of exposure from these compounds *i.e.* hormonal disruption in wild fish. Most of this information has been gathered in the developed industrialised nations such as those of North America and Europe. Relatively little is known about the extent of these chemicals in those countries that are undergoing rapid economic expansion, urbanization and industrialization and where there is little regulation of chemicals or treatment of wastewaters. The aim of this study was to estimate the extent of pollution by estrogenic hormones in two major rivers in India; the River Ganges and the River Yamuna in New Delhi, both of which are the subject of important governmental plans to improve water quality. The sampling sites were at the upper Ganges basin in Rishikesh – Haridwar City and within the city limits of New Delhi for the River Yamuna, where only a proportion of the city's wastewater is treated. The values obtained were compared with those from samples collected from the River Tyne near Newcastle upon Tyne (United Kingdom). The study may be considered a baseline with which to compare the efficacy of the action plans currently in place to improve the treatment of wastewaters prior to discharge into these receiving waters. **Keywords:** Endocrine Disrupting Chemicals, river waters, estrogenic hormones, wastewater

#### **WE209 Weight of evidence review to determine endocrine**

**disrupting properties of active substances** M. Gross, WCA Environment; D. Maycock, WCA Environment Limited; P. Simpson, WCA Environment Ltd; P. Whitehead, WCA Environment. The European legislation on plant protection products (Regulation (EC) No. 1107/2009) sets out new, stricter, criteria for approval of active substances and includes the assessment of endocrine disrupting properties. Active substances that are endocrine disruptors will not be approved under the new regulation unless there is negligible exposure to humans and non-target species. In addition to this, endocrine disrupting substances approved because of negligible exposure will become candidates for substitution with less hazardous substances during the authorisation stage at Member State level. The burden of proof is placed on the notifier. The specific criteria for the definition of an Endocrine Disrupter are being discussed by the European Commission and are due to be published by December 2013. One way of determining whether a substance is an endocrine disrupter is to use a weight of evidence (WoE) approach to evaluate all available data. We have reviewed two published WoE frameworks for the assessment of endocrine disrupting properties of chemicals (CEFIC EMSG 1999, Brown et al. 2001) and combined elements of each into a practical WoE evaluation for active substances in plant protection products. The combined framework consists of four evaluation steps: 1) Study reliability — quality of work undertaken, 2) Study relevance — endpoint relevance to endocrine disruption, 3) Study significance — based on the earlier assessments made for reliability and relevance, and 4) Balance of the weight of evidence, coherence and gap assessment. The method makes full use of endpoints available in standard regulatory toxicity and ecotoxicity studies for indications of endocrine effects and any relevant information in the open literature. Experience indicates that regulatory studies required for product approval will identify indicative endocrine disruption, but targeted supporting *in vitro* and *in vivo* studies (either from open literature or commissioned) are required to confirm an endocrine mode of action for any effects that are observed. This approach provides substantive, robust evidence for notifiers and regulators to prioritise substances under the new regulatory requirements.

**WE210 Assessing the effects of endocrine disruptors with the freshwater snail *Potamopyrgus antipodarum*** K. Ruppert, Goethe Universität / Aquatic Ecotoxicology; A. Sieratowicz, K. Bender, C. Geiss, U. Schulte-Oehlmann, J. Oehlmann, Goethe University. Molluscs have widely been ignored in environmental risk assessment schemes for chemicals, mainly due to the lack of standardised test guidelines. However, they are known to be uniquely sensitive to a number of endocrine disrupting chemicals (EDCs) and other reproductive toxicants. Therefore the OECD test guideline programme has been extended to cover reproduction effects of chemicals in molluscs, funded by the German Federal Environment Agency and the Department for Environment, Food and Rural Affairs of the United Kingdom. A *Detailed Review Paper on Molluscs Life-cycle Toxicity Testing* was prepared proposing *inter alia* the parthenogenetic snail *Potamopyrgus antipodarum* as standard test organism. In the *P. antipodarum* reproduction test the number of embryos in the broodpouch, reflecting the individual reproduction effort, and mortality serve as main endpoints. The aim of our investigations is to demonstrate that apical effects of EDCs can be assessed by an increased or decreased embryo production. Snails have been exposed in a four-week semi static test design to known EDCs including triphenyltin (TPT; 30-250 ng Sn/L), methyltestosterone (MT; 10-1000 ng/L), 17 $\beta$ -ethinylestradiol (EE<sub>2</sub>; 6.25-100 ng/L) and 4-tert-octylphenol (OP; 1-100  $\mu\text{g/L}$ ) at 16°C. The mean embryo number in snails exposed to TPT and MT decreased with increasing concentrations. Significant differences between controls and groups exposed to the test chemicals were observed for the two highest TPT concentrations (125 and 250 ng Sn/L) and for snails exposed to MT at 30, 300 and 1000 ng/L. Exposure to the estrogen EE<sub>2</sub> resulted in a significant increase of embryo numbers at 50 and 100 ng/L. Snails exposed to OP showed an inverted u-shaped response. The results demonstrate the sensitivity of *P. antipodarum* to EDCs. Exposure to androgenic substances like TPT and MT results in a decreased reproduction whereas estrogenic substances cause an increased embryo



production. Currently, the influence of different breeding and test conditions (e.g., snail density) and of the seasonal reproduction cycle on the sensitivity to chemicals is investigated. For reproducible results with low variability between laboratories it is essential to standardise not only test conditions but also the breeding of snails and to define an appropriate range of embryo numbers in animals at the start of reproduction tests. *Acknowledgement* - The authors thank the German Federal Environment Agency for funding.

**WE211 The use of yolk protein as biomarkers for endocrine disruption in molluscs** H. Holbech, University of Southern Denmark / Department of Biology; K.L. Kinnberg, Department of Biology; J.E. Morthorst, K.L. Pedersen, P. Bjerregaard, University of Southern Denmark / Department of Biology. Invertebrates and especially molluscs have received increasing attention in relation to endocrine disrupting chemicals (EDs) during the last few years and the development of OECD test guidelines to assess the effect of EDs with molluscs are in progress. One of the main problems with the development of standardized tests in molluscs is that no specific biomarkers or endpoints for endocrine effects have been validated. Some attempts have been made to transfer biomarkers developed for vertebrates – e.g. from fish to molluscs to investigate ED effects. One example is the vertebrate yolk protein vitellogenin that is known to be oestrogen dependent in fish. The yolk proteins in molluscs have been proposed to have the same oestrogenic dependence and used as biomarker for oestrogenic EDs. The present work investigates the possible usability of the main yolk protein in three species of molluscs to function as biomarker for oestrogenic exposure. We have developed specific antibodies against the main yolk protein from the three species (*Unio pictorum*, *Unio tumidus* and *Lymnaea stagnalis*) and with specific ELISAs (enzyme linked immune-sorbent assay) and immune-histology we investigate the distribution and concentration of the proteins in molluscs of different sex and life-stage. The main yolk protein was purified from gonads containing eggs of the freshwater bivalves *U. pictorum* and *U. tumidus* and from eggs dissected from egg clutches of the pulmonate gastropod *L. stagnalis*. The ELISAs were used to quantify the concentration of the yolk protein in juveniles and adult male and female *U. pictorum* and *U. tumidus* and in the hermaphroditic *L. stagnalis*. With use of immune-histology, the distribution of the proteins in the tissue of the three species was investigated. The ELISAs revealed that the normal sex specific concentration distribution seen in fish, where vitellogenin is normally seen in very low concentrations in male and juveniles was not seen in the molluscs. The concentration of the protein did not differ among the sexes and was a factor of approximately 10000 higher in male *U. tumidus* and *U. Pictorum* than in male fish. Based on The results the authors do not support the use of mollusc yolk protein as biomarker for oestrogenic exposure because it seems to have more than one function in the investigated species. We have shown that the content and distribution of the proteins are not sex specific.

**WE212 Baseline growth and reproductive parameters in *Lymnaea stagnalis* for OECD test guideline development: optimization of diets and culturing conditions** J. Tasker, C. Veauvy, S. Morris, C. Askem, Cefas; V. Ducrot, L. Lagadic, D. Azam, M. Coke, INRA; R. Brown, AstraZeneca; H. Holbech, University of Southern Denmark; K. Ruppert, J. Oehlmann, Johann Wolfgang Goethe University; L. Weltje, BASF; M. Roberts, DEFRA; T. Hutchinson, School of Biological Sciences Plymouth University. The OECD has successfully validated reproductive toxicity test guidelines with fish and frogs for diverse chemicals (including endocrine disrupters). Since molluscs are especially sensitive to chronic exposure to a number of chemicals, the OECD supports the complementary development and validation of a mollusc reproduction test guideline. An *ad hoc* mollusc expert group has been formed in Europe to validate methods that can meet this need. Currently, a key species for use in this context is the freshwater gastropod *Lymnaea stagnalis*. An important aspect of this work is to first develop a specific pathogen free defined strain of *L. stagnalis* and second to establish a historical database of growth and reproductive rates under defined culturing conditions. A mass culture of the

RENILYS® strain of *L. stagnalis* have been established at INRA (France) since 2002 and has been distributed to research laboratories in Denmark, Germany and the UK for the OECD pre-validation work to date. Laboratory cultures of *L. stagnalis* are traditionally fed fresh (preferably organic) lettuce; however, interrupted supplies of fresh lettuce in some countries in 2011 highlighted a potential problem for the draft OECD test guideline. Therefore, we have evaluated other diets based on a review of the published literature and report here the results for different feeds: namely, cabbage leaves, fish flakes, lettuce leaves or sliced sweet potato. For the feeding trial, 5 snails (shell size ca. 2.6 cm) were held in 1 L freshwater. Aeration was provided in all vessels, and for all the vegetable diets treatments concentrations of ammonia, nitrate and nitrite, the dissolved oxygen concentrations (>6 mg/L), pH (6.5-8.5) and temperature (20±1°C) ranges remained acceptable. However, the fish flake diet gave low dissolved oxygen levels (< 10% saturation). In this 28-d study, mean specific growth rates were highest for snails fed lettuce *ad libitum* (0.09 mm/snail/day) followed by snails fed fish flake>sweet potato>cabbage leave diets (0.03 mm/snail/day). Similarly, in a 56-d ring-test study with 7 laboratories, the lettuce diet gave mean specific growth rates of 0.06-0.09 mm/snail/day. Fecundity in the same test ranged from 519-1424 total embryos per snail. These data will be used towards developing a reference database of growth and reproduction for *L. stagnalis* and to aid the statistical optimization of the draft OECD test guideline for partial and full lifecycle testing of chemicals.

**WE213 Is reproduction of the snail *Physella acuta* affected by EDCs? An in situ bioassay in three Mediterranean basins** N. De Castro, Departament d'Ecologia; J. Lopez-Doval, Universitat de Barcelona / Departament d'Ecologia; M. Gorga, IDAEA- CSIC / Department of Environmental Chemistry; M. Petrovic, Catalan Institute for Water Research ICRA; I. Munoz, Universitat de Barcelona. *In situ* bioassays are desirable tools to study the effects of fluvial pressures in aquatic communities. In order to test the relationships between river pollution and invertebrate community function an *in situ* bioassay was carried out in three Mediterranean basins: Ebro, Llobregat and Júcar. Adult individuals of the freshwater snail *Physella acuta* were transplanted in specially designed cylindrical cages. End-points included mortality, number of clutches, eggs per clutch, total number of eggs and egg development after 8 days. Results were contrasted with lab temperature controls (16°C and 23°C) and normalised by degree-days (DD). The organisms tested reflected high resistance and adaptability to the different environmental conditions found in the studied basins, making them appropriate to test sub-lethal responses. Significant changes in *P. acuta* reproduction parameters were detected in all rivers. The number of clutches (clutches number·snail<sup>-1</sup>·DD<sup>-1</sup>) decreased in the Ebro and Llobregat respect to control, and towards downstream. On the other hand, the number of eggs per clutch (egg number·clutch<sup>-1</sup>·DD<sup>-1</sup>) increased significantly in the Llobregat and in the Júcar respect to control, and increasing towards downstream. The total number of eggs was lower respect to control in the most polluted site of the Llobregat, and in two sites of the Ebro. The results were contrasted with specific data of chemical compound concentrations (Endocrine Disrupting Compounds) in water to check correlations between reproduction parameters and possible stressors by multivariate analyses tools. Redundancy analyses revealed that bisphenol A accounted for 21% of the total variance in the reproduction endpoints in the Llobregat, and, together with estrogens, for 12% of the variance in the Ebro. Although these EDCs have been found at a ng·L<sup>-1</sup> concentration level in the three studied basins, they can have direct effects in the reproduction of snails because of its high estrogenic capacity (Jobling *et al.*, 2004; Oehlmann *et al.*, 2006; Schmitt *et al.*, 2010). This study provides evidence of the usefulness of *in situ* bioassays as a tool to test possible relationships between specific pollutants and physiological responses in order to assess the environmental toxicological risk (Schmitt *et al.*, 2010).

**WE214 Comparative proteomics of the freshwater amphipod *Gammarus fossarum*: focus on endocrine regulation** I. Trapp,

Irstea / Laboratoire d'écotoxicologie; O. Geffard, Irstea; J. Gaillard, A. Davin, CEA / Laboratoire de Biochimie des Systemes Perturbés; G. Imbert, CEA / Laboratoire de détection et de caractérisation des agents du risque environnemental; a. chaumot, Irstea; J. Armengaud, CEA / Laboratoire de Biochimie des Systemes Perturbés. Among all contaminants present in the aquatic ecosystems, endocrine-disrupting chemicals (EDCs) are currently a great concern because of their high potential induction of alterations in reproductive function and consequently population dynamics. Their mode of action and associated effects on hormonal regulation have been intensively studied for fish, resulting in robust, specific and reliable biomarkers for risk assessment. Meanwhile, similar tools are lacking for invertebrates, especially crustaceans, while they are major biocenose components, playing key role in ecosystemic processes. While disruption of endocrine regulation is documented *in-situ*, causality between these adverse effects and their cause can not be established due to the lack of knowledge on hormonal regulation for these models. A few biomarkers have been transposed from vertebrates. However, different evolution paths restraint this type of approach, involving that candidate biomarkers must be specifically identified and characterized. The recent trends in high-throughput proteomics allow large-scale characterization of the whole proteome of model organisms. We started to document the reproductive proteome of the freshwater amphipod, *Gammarus fossarum*, an ecologically and ecotoxicology relevant species. For this, we used the most advanced approaches in shotgun proteomics for an extensive comparison of the female and male reproductive tissues of *G. fossarum*. Tandem mass spectrometry analysis of a large pool of peptides arising from trypsin digestion of both tissues was done with a high resolution LTQ-Orbitrap XL hybrid mass spectrometer (ThermoFisher). This led to the identification of 37,167 peptides. A total of 1,336 proteins were identified with high confidence and quantified using the spectral count approach. The cross-comparison of the relative abundance of these proteins in both tissues has shown that 66 and 103 proteins were preferentially expressed in the female and the male reproductive tissues, respectively. The functions of these sex-specific proteins were tentatively assigned by PSI-BLAST searches but functional annotation resulted quite scarce with the identification of numerous orphans. As gammarids reproductive biology is well described, involvement of each sex-specific protein in reproduction processes will be assessed by studying their modulation throughout the reproductive cycle. Our results should facilitate further biomarker development of EDCs exposure for crustaceans.

**WE215 Guidance on test concentration setting for fish in vivo endocrine screening assays** J.R. Wheeler, Syngenta Ltd; G.H. Panter, Brixham Environmental Lab; L. Weltje, BASF SE / Agricultural Centre; K.L. Thorpe, CRI UPEI / Department of Biology. Fish *in vivo* screening methods to detect endocrine active substances, specifically interacting with the hypothalamic-pituitary-gonadal axis, have been developed by both the Organization for Economic Co-operation and Development (OECD) and United States Environmental Protection Agency (US-EPA). In application of these methods, i.e. regulatory testing, this poster provides guidance on the setting of test concentrations using all available acute and chronic data and also discusses the importance of avoiding the confounding effects of systemic toxicity on endocrine endpoints. This guidance is aimed at reducing the number of false positives and subsequently the number of inappropriate definitive vertebrate studies potentially triggered by effects consequent to systemic rather than endocrine toxicity.

**WE216 Endosulfan alters fish gonadal steroidogenesis in vitro** R. Da Cuña, UBA & CONICET / Laboratorio de Ecotoxicología Acuática; E. Rodríguez, CONICET; F.L. Lo Nostro, UBA CONICET / PhD. Research assistant and teaching ass.. The use of the organochlorine insecticide endosulfan (ES) is in the process of being phased out worldwide after its inclusion in the list of Persistent Organic Chemicals by the Stockholm Convention. However, it is still used extensively in several countries as a broad spectrum insecticide in crops of high commercial value despite showing high toxicity to non-target animals

and acting as an endocrine disruptor in fish. Our previous studies with the South American freshwater cichlid fish *Cichlasoma dimerus* showed waterborne exposure to ES (active ingredient; AI) caused decreased FSH pituitary content and histopathological alterations in testes. The aim of this study was to analyze and compare the *in vitro* effect of two commercial formulations (CF) of ES (Master® and Zebra Ciagro®) and the AI alone on gonadal steroidogenesis of *C. dimerus* to narrow down possible points of disruption. Testes and ovaries from adult fish were dissected and divided into equal pieces. Portions from the same gonad were randomly assigned to one experimental condition and incubated for 4 h in Krebs medium with the addition of DMSO (control), ES 100 µM, LH 0.5 µg/mL, ES 100 µM + LH 0.5 µg/mL, dehydroepiandrosterone (DHEA) 1 µM or ES 100 µM + DHEA 1 µM. Testosterone (T) and estradiol (E2) levels were measured in media from incubated testes or ovaries using RIA or EQLIA, respectively. ES alone had no effect on T or E2 levels, either when using the CF or the AI. LH caused an increase in the synthesis and release of both sex steroids. The co-incubation of LH with ES -for both CF and the AI- caused a reduction in the level of sex hormones when compared with LH alone. When incubating with the 3βHSD enzyme substrate DHEA, precursor in the biosynthetic pathway of both T and E2, a marked increase in both steroids was observed. Joint incubation of DHEA with ES -either CF or the AI- inhibited the increase in T and E2 when compared with DHEA alone. Based on these results, ES is capable of inhibiting the production of sex steroids from testes and ovaries *in vitro*, thus acting as an endocrine disruptor. As ES shows an inhibitory effect even when adding DHEA, it is likely to act downstream the synthetic pathway -i.e. interfering with 3βHSD or 17βHSD. No differences were found between the use of the AI alone or in combination with excipients in either CF, suggesting that the effect on steroidogenesis (Leydig or thecal cells) is caused by ES and not the remaining components of the mixture. **Key Words:** cichlid fish, endocrine disruption, endosulfan, gonadal steroidogenesis.

**WE217 Intersex in wild fish from French rivers: preliminary results of a large monitoring campaign** W. Sanchez, E. Chadili, INERIS; C. Blanchard, ONEMA; C. Minier, Univ Le Havre; J. Porcher, INERIS. Intersex is the condition whereby both testicular and ovarian tissues are observed within the same gonad. The phenomenon traduces the exposure to endocrine-disrupting chemicals and can predict adverse reproductive effects for wild fish and populations. The occurrence of intersex is documented in several aquatic ecosystem around the world but few data is available in French rivers. Also, due to the ecological relevance of this parameter, a large monitoring campaign is performed. For this purpose, wild Cyprinid fish were electrofished in rivers characterised by various levels of contamination and impact in fish population. Gonads were dissected and prepared for histological analysis. During microscopic observations, fish gender is noticed and sex-ratio is determined. For intersex fish, the ovotestis severity index previously developed in European flounder and recommended by OSPAR is calculated. Preliminary results of this large monitoring campaign will be presented. First results obtained in 13 sites show that intersex is observed in 8 rivers where 4 to 38% of fish are affected. To complete, two sites exposed to industrial pressures, levels of occurrence up to 50% are observed. Complementary works are in progress to analyse intersex occurrence in other sampled sites (n=15) and to explore relationships between intersex and environmental pressures or fish assemblage quality. *This work is supported by INTERREG IV A-DIESE project, the French ministry for ecology (MEDDE, program 190 and 181) and the French office for water and aquatic ecosystems (ONEMA)*

**WE218 Altered gene expression in zebrafish embryos as an early detection marker for androgen and anti-estrogen mediated endocrine disruption** M. Macherey; V. Schiller, Fraunhofer Institute for Molecular Biology and Applied Ecology; R. Kriehuber, Forschungszentrum Jülich / Department of Safety and Radiation Protection; H. Hollert, RWTH Aachen University / Institute for Environmental Research (Biology V); M. Fenske, Fraunhofer Gesellschaft IME / Institute for Molecular Biology and Appl. Ecology. Estrogenic and androgenic hormones regulate the sexual development

and the reproduction of vertebrates. Environmental pollutants can mimic these sex hormones and interfere with the endocrine system and the regulation of sexual differentiation and behaviour. This can severely impact reproduction and endanger wildlife populations. For this reason, endocrine disruption (ED) has become a high-priority issue for environmental safety. \nChemical risk assessment requires testing for endocrine disruptive effects, and currently adult animals and their offspring are used to identify population relevant effects. These tests are cost- and time- intensive and disregard the 3R's (reduce, refine, replace) principles and should therefore be reduced to a minimum. Regarding fish tests, the use of embryos promise a good alternative as they are not subject to European regulation on animal experiments. Recent studies also show that endocrine markers are already detectable in 48 h old embryos. We therefore investigate the suitability of the zebrafish embryo (*Danio rerio*) assay for the assessment of ED. In this study, we focused on androgenic and anti-estrogenic synthetic substances and evaluated the impact on the embryonic development and gene expression. \nZebrafish embryos were exposed to 17 $\beta$ -methyltestosterone (MT), trenbolone (TB), fadrozole (FAD) and ICI 182,780 (ICI) for 48h and morphological abnormalities were assessed. After exposure, total RNA was extracted from the embryos and subjected to microarray analysis. Subsequent gene set analysis was applied to identify the molecular mechanisms of actions of the examined endocrine disrupting chemicals (EDCs). \nExposure to MT and TB affected numerous pathways, including steroid biosynthesis and metabolic processes and TB also promoted the down-regulation of cytochrome P450-genes (e.g. *cyp19alb*). Pathways linked to steroid hormone receptor activity were found upregulated by FAD and down-regulated by ICI, which also repressed steroid binding. \nThe results demonstrate that our approach can provide important information on androgenic and anti-estrogenic mechanisms of action, suggesting that transcriptomics in zebrafish embryos may be a viable alternative for the testing of endocrine disrupting chemicals. The goal is now to validate the assay with further endocrine active substances and to identify specific gene expression markers, which could be used for the screening and categorising of EDCs.

**WE219 Biomarker responses to endocrine disruptors in females and juveniles of the estuarine fish *Pomatoschistus microps*** L. Dias, C.S. Santos, University of Aveiro / department of Biology & CESAM; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; M.S. Monteiro, Aveiro University / Department of Biology. The presence of chemical compounds, in the most diverse aquatic ecosystems it was not a recent subject. However, in spite of the prohibition of the use of many of these compounds, their presence in the environment keeps being detected even at low concentrations. The coastal areas, namely the estuaries, are object of great concern. Due to their great ecologic and economic value and, also, the fact of being the final destination of a lot of compounds mainly from anthropogenic sources, become necessary its monitoring and the development of methods with autochthonous species that allow a better evaluation of the impact of those compounds. Thus, the main objective of this work consisted in the determination and evaluation of the biomarker responses to selected endocrine disruptor compounds (EDCs) using the estuarine fish *Pomatoschistus microps* as organism test and in the assessment of the viability to use vitellogenin quantification (vtg) in this species as a biomarker of exposure to these compounds. This evaluation was realized after 21-days exposure, both in juveniles (whole body) and females (liver and gonads). In addition to vtg were analysed the hepato and gonadosomatic indexes (HSI and GSI, respectively) for endocrine disruption assessment and acetylcholinesterase (AChE) for exposure to neurotoxicants. The EDCs tested, at sub-lethal level, were the 17 $\beta$ -estradiol (E2) and the pesticides p-p'-DDE and PCB-77, all considered endocrine disruptors with estrogenic and/or antiestrogenic activity. The results showed, in juveniles, an increase in vtg-like proteins by action of 17 $\beta$ -estradiol and a decrease in its values by action of PCB-77. In females, it was found significant results with an increase in liver vtg-like proteins after exposure to PCB-77 and no significant results in the other endpoints.

The p,p'-DDE exposure did not induce any significant alterations in the endocrine endpoints analyzed. Relatively to AChE, the compound PCB-77 seems to increase its activity in juveniles and the opposite result was observed in females. In turn, p-p'-DDE seems to not affect the AChE activity in females. In conclusion, the juveniles of *P. microps* seem to respond to EDCs contamination at environmental relevant concentrations of E2 and PCB-77 and the use of vtg in this life stage seems appropriate to track EDCs contamination in field biomonitoring studies. The female fish, in general, does not seem to be clearly affected at the concentrations used of the tested EDCs.

**WE220 Teratogenic effects of 17 $\beta$ -estradiol and other environmental chemicals in eelpout *Zoarces viviparus*** E. Morthorst, University of Southern Denmark / Department of Biology; K.L. Madsen, University of Southern Denmark / Institute of Biology; N. Brande-Lavridsen, B. Korsgaard, University of Southern Denmark; P. Bjerregaard, University of Southern Denmark / Department of Biology. In recent years increased frequencies of malformations among eelpout fry living in coastal areas with high anthropogenic input have been observed. Eelpout (*Zoarces viviparus*) is the only viviparous fish species in Northern Europe, which makes this species very suitable for investigation of mother-offspring interactions and effects in the offspring upon maternal exposure to various chemicals. In oviparous fish species malformations can be induced by exposure to chemicals, including endocrine disrupting substances. Hence the malformations observed in wild eelpout could be due to anthropogenic chemicals. The specific chemicals or group of chemicals causing the malformations observed in nature are not known, but similar malformations upon exposure of pregnant eelpout to octylphenol (OP) and 17 $\beta$ -estradiol (E2) in high concentrations have recently been observed in the laboratory. In the current two experiments pregnant eelpout with newly fertilized eggs were exposed to 5.7-133 ng E2/L (autumn 2011) and 6.25-50  $\mu$ g 4-t-OP/L, 500 ng pyrene/L or 5 ng EE2/L (autumn 2012) for 6 weeks in order to establish no effect concentrations for the teratogenic effect (fry malformations) of E2 and OP in eelpout and to investigate if two environmental chemicals with known endocrine disrupting effects, the PAH pyrene and the synthetic hormone 17 $\beta$ -ethinylestradiol (EE2), could induce similar teratogenic effects. Exposure of female eelpouts to environmentally realistic concentrations of E2 during early pregnancy increased the abundance of larvae malformations and the amount of ovarian fluid was significantly reduced. Plasma levels of E2 and vitellogenin increased with increasing E2 exposure. Similar endpoints were investigated in the experiment with OP, pyrene and EE2. The results also indicate that there is a sensitive window for induction of malformations in eelpout fry.

**WE221 Using molecular tools to evaluate the endocrine disrupting impacts of paper mill effluent exposure on the Eastern Mosquitofish (*Gambusia holbrooki*)** E.K. Brockmeier, University of Florida / Physiological Sciences; W. Pine, University of Florida; N.D. Denslow, University of Florida / Physiological Sciences. The Eastern Mosquitofish (*Gambusia holbrooki*) is a freshwater fish species that exhibits sexual dimorphism. Male mosquitofish have an elongation of the anal fin (termed the gonopodium) which is used for internal fertilization of females. While females do not normally exhibit anal fin growth, abnormal elongation can be induced by androgen exposure, and masculinized female mosquitofish have been found downstream of pulp and paper mills. Masculinized female mosquitofish continue to reside at one site in the state of Florida—downstream of the Buckeye Paper Mill near Perry, FL—30 years after their initial discovery. However, it is still unknown what effects paper mill effluent exposure is having on wildlife inhabiting this site. The objective of this study is to use molecular tools to evaluate the impacts of paper mill effluent exposure on *G. holbrooki*. To address this objective, female *G. holbrooki* were collected downstream of the Buckeye Paper Mill (Fenholloway River) and a clean control site (Econfina River) during Summer 2012. Body measurements (body length and anal fin elongation) were obtained while livers, gonads, and anal fins were collected for gene expression analyses. Gonads were used for quantitative polymerase chain reaction (qPCR)

analysis of aromatase, livers for vitellogenin (vtg), and anal fin tissues for sonic hedgehog (shh) mRNA quantification. In addition, a subset of the liver samples was used for microarray analysis. Results from this study indicate that anal fin elongation is still occurring in female mosquitofish at the paper-mill impacted site compared to the control site (t-test,  $p < 0.001$ ). *G. holbrooki* in early stages of oocyte development downstream of the paper mill have increased expression of vtg and decreased expression of aromatase (Mann-Whitney rank sum test,  $p = 0.017$ ; t-test,  $p = 0.016$ ). Significantly higher expression of shh is found in females with masculinized anal fins that live downstream of the paper mill (Mann-Whitney test,  $p = 0.028$ ), providing additional impetus for developing this gene as a biomarker of androgen exposure. The results of the microarray analysis give further insights into the mechanisms of action of the chemicals present in this system. These data provide information on the biological impacts of paper mill exposure in Florida and provide a foundation for future incorporation of molecular and omics data for evaluating the ecological impacts of pollutants in the environment.

**WE222 Characterization of a protein potentially implied in the action mode of aquatic pollutants: the protein disulfide isomerase PDI from *Danio rerio*** E. Tinti, C. Michaux, E.A. Perpete, University of Namur / Department of Chemistry; P. Kestemont, F. Silvestre, University of Namur / Department of Biology. The accumulation of anthropogenic chemical agents like endocrine disrupting compounds (EDC) in aquatic environment and their potential deleterious effects on both wild species and human is an increasingly large concern. In this context, sensitive early life stages (ELS) are choice targets as they need extensive developmental changes for an effective adaptation to new environmental conditions. The ELS physiological adaptations and mechanisms involved in the responses brought to a changing and stressful environment require a precise description in order to allow the control of new chemical agent production and use. During the last decade, many studies at the molecular level were conducted to assess the EDC impact on organisms. Recently, a proteomics meta-analysis by our team noticed organochlorine and polyphenol chemicals affect during a toxic exposure the expression of protein disulfide isomerase (PDI). PDI is a membrane protein mostly localized in the endoplasmic reticulum. As a chaperone enzyme, PDI catalyses the formation of disulfide bonds. As a carrier, PDI interacts with thyroid hormones, as a T-binding protein. The thyroid system plays a key role in various biochemical and physiological processes. When happening during the development, its alteration by EDC such as PCBs and triclosan (TCS), causes permanent morphological, neurochemical and neurobehavioural deteriorations. Indeed, the massive use of EDC in household and healthcare products, coupled to their release in aquatic environments expose the thyroid system (and more broadly the general development of individuals) to serious damages from widely unknown nature. In this context, PDI was chosen as a model protein because of the modification of its expression pattern in many "omics" studies during a toxic exposure stress. Zebrafish *Danio rerio* was selected as a model organism; on the one hand because of its whole sequenced genome and annotated PDI gene region, and on the other hand for practical reasons such as breeding facilities and pre-existing experience. The overall output of the present work is to understand the molecular mechanisms of endocrine disrupting activity of TCS on *Danio rerio* PDI. To do so, we isolate the nucleotide sequence of PDI in order to overproduce, purify and characterize its structure. In parallel to this experimental study, molecular modelling is used to obtain 3D structures and to stimulate molecular interactions between TCS and PDI.

**WE223 Effects of Progestin and Estrogen Mixtures: A partial life cycle study on sex differentiation** C. Berg, Dept of Environmental Toxicology; M. Safholm, E. Jansson, Uppsala University / Dept of Environmental Toxicology; J. Fick, Umea University / Department of Chemistry; I. Brandt, Uppsala University / Department of Environmental Toxicology. A large number of endocrine disruptors with different modes of action are present in the environment leading to simultaneous exposure to several kinds of endocrine disruptors.

Comparatively little information is available on combination effects of mixtures of endocrine disruptors with different modes of action. The estrogen ethynylestradiol (EE<sub>2</sub>) and the progestin levonorgestrel (LNG) are potent hormones in the environment, that both target the developing reproductive organs. Induction of vitellogenin (Vtg) synthesis is a sensitive biomarker for estrogenic activity in oviparous species. Our previous research suggests that LNG inhibits vitellogenesis in adult female amphibians at low environmental concentrations (0.01 nM). Here, we examined the hypothesis that environmental progestins antagonise estrogenic effects on sex differentiation and mRNA levels of Vtg and the receptors for estrogen (ER), progesterone (PR) and androgen (AR). *Xenopus tropicalis* tadpoles were exposed to LNG (0.1 nM) alone, or EE<sub>2</sub> (0.1 nM) alone or in combination with LNG (0.01, 0.1, 1.0 nM) via the water in duplicate tanks until metamorphosis. At metamorphosis, levels on Vtg, ER $\alpha$ , ER $\beta$ , AR, and PR mRNA in the liver were quantified. The levels were normalised to the level of mRNA for Arnt2 (aryl hydrocarbon receptor nuclear translocator 2), whose expression was unaffected by the test substances. All groups exposed to EE<sub>2</sub> showed female-biased gonadal sex ratios and a strikingly increased Vtg mRNA level in the liver. No antagonistic effect of LNG of the estrogenic effects was detected. The results show that induction of Vtg mRNA is a sensitive biomarker for estrogen, whereas none of the tested endpoints proved to be sensitive for progestin exposure in juvenile *Xenopus tropicalis*. In conclusion, in the present setting combined exposure to estrogen and progestin did not reveal any mixture effects.

**WE224 The endocrine disruptive potential of phytoplankton exudates** A. Jonas, Masaryk University RECETOX / Faculty of Science, RECETOX; S. Scholz, Helmholtz Centre for Environmental Research / Department of Bioanalytical Ecotoxicology; K. Novakova, Masaryk University / Faculty of Science RECETOX; E. Fetter, Department of Bioanalytical Ecotoxicology; E. Sychrova, J. Kohoutek, Masaryk University / Faculty of Science, RECETOX; J. Ortmann, Helmholtz centre for environmental research - UFZ / Department of Bioanalytical Ecotoxicology; K. Hilscherova, Masaryk University Faculty of Science RECETOX / Faculty of Science, RECETOX. Phytoplankton is an important group of organisms inhabiting most of the water bodies. Toxicity of certain cyanobacteria metabolites was shown in various studies. The potential of phytoplankton to produce and release endocrine disrupting compounds, particularly estrogens, have been addressed only recently. This study focuses on the endocrine disruptive potential of exudates. Exudates are of high environmental relevance since they represent compounds excreted by the phytoplankton into the surrounding environment during normal physiological processes. A transgenic zebrafish strain (tg:cyp19a1b-GFP) was chosen to assess the estrogenicity in fish embryos. In this strain green fluorescent protein (GFP) is expressed under the control of the aromatase B (cyp19a1b) promoter and GFP expression is induced by estrogens. Furthermore, mortality, malformations and neurotoxicity was analysed. Several cellular reporter assays were used to detect estrogenic, anti/androgenic, glucocorticoid and anti/retinoid effects. Research was initiated to identify the potential estrogenic compounds by chemical analysis of selected known phytoestrogens in the exudates. Based on previous data we selected three species: the cyanobacteria *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*, and green algae *Scenedesmus quadricauda* for analysis of endocrine disrupting activity. These species were grown under controlled laboratory conditions. Exudates of all three phytoplankton species caused estrogenicity in zebrafish embryos and *in vitro*. The estrogenic effect was covered by deformations and mortality of zebrafish embryos at greater concentrations of cyanobacteria exudates. The study documents that compounds produced by cyanobacteria and algae and released via exudates to their environment can contribute to endocrine disrupting effects in aquatic environment. This would be of particular relevance in case of water eutrophication and subsequent massive algal blooms. **Acknowledgement** - We greatly acknowledge Prof. Olivier Kah (INSERM, Rennes, France) for providing the cyp19a1b-GFP transgenic zebrafish strain to the UFZ and Prof. John P. Giesy for providing the MVLN cell line. Analysis of transgenic embryos has been performed at the UFZ. The work was

supported by the Czech Science Foundation grant No. GACR P503/12/0553, project CETOCOEN (CZ.1.05/2.1.00/01.0001).  
Keywords: Endocrine disruption, phytoplankton and zebrafish embryo.

**WE225 Models that ignore oil droplet uptake are sufficiently accurate to predict the bioaccumulation of oil-associated PAHs.** Viaene, Ghent University; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; L. de Hoop, Radboud University Nijmegen; J.A. Hendriks, Radboud University Nijmegen / Department of Environmental Sciences; F. De Laender, Université de Namur ASBL / Lab of Env.Tox&Appl.Ecol.. During an oil spill, aquatic organisms are exposed to the water soluble fraction of the oil dispersion. Because the size range of oil droplets (< 100 µm), also present in the oil dispersion, overlaps with the size range of food items of certain aquatic organisms, the dietary uptake of oil components via oil droplets has been suggested as an additional exposure pathway. By extending an allometric bioaccumulation model with oil droplet uptake, we tested if and to what extent oil droplets contribute to the bioaccumulation of oil components using the case of polyaromatic hydrocarbons (PAHs). Six studies covering six species (four fish, one crustacean and one shellfish) were selected to test whether the inclusion of oil droplets led to better model predictions. The model without oil droplets was able to predict the PAH body burdens within one order of magnitude for all studies. Including oil droplets led to slightly better model predictions for some studies, possibly related to the feeding mechanism of the species. Given the large variation on the predictions of the model with oil droplets, the only slight improvements of model predictions when oil droplets are included (maximum 1,6-fold increase in PAH body burden) and the accuracy of the model without oil droplets, we conclude that oil droplets probably are a negligible exposure route for PAHs and that the bioaccumulation model without oil droplets is sufficient to predict PAH body burdens.

**WE226 Comparative study on oil fouling from chemically and mechanically dispersed oil on the cold water copepod *Calanus finmarchicus*** T. Nordtug, SINTEF Materials Chemistry / Applied Environmental Biology and Chemistry; A.J. Olsen, Norwegian University of Science and Technology / Department of Biology; K.E. Reed, SINTEF Materials and Chemistry; I. Salaverria, Norwegian University of Science and Technology; I.B. Overjordet, SINTEF Materials and Chemistry; D. Altin, BioTrix; B. Hansen, SINTEF Materials and Chemistry / Applied Environmental Biology and Chemistry. Chemical dispersants are used to break up oil spills and aid the process of dispersion into the water column. In general this will increase the amount of oil in the water and consequently, at least locally, potentially increase the risk of adverse effects on marine biota. Chemical dispersants act by reducing the surface tension of the oil-water interface. This also makes the oil droplets less sticky. Thus, one would expect that chemically dispersed oil would cause less fouling on marine organism than mechanically dispersed oil. This hypothesis was tested on the cold water copepod *C. finmarchicus*. Adult copepods were exposed to three different concentrations of mechanically and chemically dispersed oil with similar droplet size (mean size 14 µm) and oil concentration (4, 0.8 and 0.2 mg/L), for four days. Body burden of semi-volatile oil components were recorded after the exposure period and 15 and 25 days into the recovery period. During the exposure period the body burden analyses of intact individuals is expected to reflect both absorbed (bio-concentration) and adsorbed oil droplets (oil fouling). Based on the chemical composition of the analyzed components from whole animal non-soluble oil components were used to calculate the fraction of oil in the samples.

**WE227 Comparative study on the effects of chemically and mechanically dispersed oil on reproduction in the cold water copepod *Calanus finmarchicus*** B. Hansen, SINTEF Materials and Chemistry / Applied Environmental Biology and Chemistry; A.J. Olsen, Norwegian University of Science and Technology / Department of Biology; K.E. Reed, SINTEF Materials and Chemistry / Marine Environmental Technology; I. Salaverria, Norwegian University of

Science and Technology; I.B. Overjordet, SINTEF Materials and Chemistry; D. Altin, BioTrix; T. Nordtug, SINTEF Materials Chemistry / Applied Environmental Biology and Chemistry. Young and presumably fertilized female *C. finmarchicus* were exposed to three different concentrations of mechanically and chemically dispersed oil with similar droplet size (mean size 14 µm) and oil concentration (4, 0.8 and 0.2 mg/L), for four days. After the exposure 150 females from each group was transferred to clean seawater (total 28 groups) in where the reproductive output was recorded daily for approximately three weeks. Body burden of semi-volatile oil components were recorded after the exposure period and 15 and 25 days into the recovery period. The survival rate after the exposure was above 90% in all exposure groups. There was a slightly increased lethality in the exposed groups during recovery, but no significant differences between exposure groups. Reproductive output was recorded daily during 25 days of recovery from subsamples from the recovery tanks and counting the density of eggs and the various developmental stages. The results indicate that a short-term exposure for oil-polluted water after an oil spill can temporarily suspend reproduction. However, the copepods recover and produce viable offspring soon after cessation of exposure. The reproductive output during the egg-laying period was not significantly affected by the presence of chemical dispersant. The results may imply that for *C. finmarchicus* populations the impact from short term exposure to an oil spill may be predicted from acute mortality and that delayed effects have limited contribution to population decrease.

**WE228 Monitoring the biological effects of an offshore oil platform in the North Sea as part of the Norwegian Water Column monitoring programme** S. Brooks, NIVA / Ecotoxicology and Risk Assessment; D. Pampanin, IRIS; C. Harman, Norwegian Institute for Water Research (NIVA); R.C. Sundt, International Research Institute. The Norwegian Water Column Monitoring (WCM) performs investigations into the potential biological effects of offshore oil and gas activity on the biota living within the water column of the Norwegian sector of the North Sea. Oil companies in the Norwegian sector with produced water discharges are obliged by the Norwegian authorities to perform water column monitoring offshore. The objective of the WCM in 2011 was to assess the biological effects of the produced water (PW) discharged from Gullfaks C platform. The study was designed to monitor bioaccumulation and biomarker responses in mussels held in cages in the vicinity of the PW outlet, with supporting information from passive sampling devices. Significantly greater bioaccumulation of PAH and NPD compounds was found in mussels from the two stations positioned 500 m from the platform, with concentrations significantly higher in mussels from one of the 500 m stations (i.e. station 2). All other mussel stations positioned 1000 m and 2000 m from the platform had PAH-NPD bioaccumulation typical of offshore background concentrations. There was very good agreement between the biomarker responses and the chemical concentration data. The calculated integrated biological response (IBR/n) was markedly higher in mussels from station 2, indicating poorer health. The IBR/n was also slightly raised in mussels from station 3 (1000 m), which was considered to be due to other chemicals within the PW. Alkylphenols and naphthenic acids were detected in all POCIS placed at selected mussel stations from 500 to 2000 m, with mussel station 2 (500 m) and 3 (1000 m) showing highest concentrations of these compounds. Overall chemical bioaccumulation and impaired health to caged mussels was observed in mussels exposed to the PW plume located 500 m downstream from the platform.

**WE229 Extension of the Target Lipid Model to Soils and Sediments with the Equilibrium Partitioning Model applied to laboratory and field datasets** A.D. Redman, Exxon Mobil Biomedical Sciences; M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences, T&ES Division; T. Parkerton, ExxonMobil Biomedical Sciences Inc; C.V. Eadsforth, Shell International; D.M. Di Toro, University of Delaware / Dept. of Civil & Environmental Engineering. The Target Lipid Model (TLM) provides a framework for deriving predicted no effect concentrations (PNEC) for nonpolar organic chemicals. This approach has been used to perform environmental risk

assessment of individual hydrocarbons as well as complex petroleum substances. The TLM is based primarily on data for aquatic test organisms and this work evaluates the potential for extending the TLM to soil and sediment using Equilibrium Partitioning (EqP) theory. Literature data for nonpolar organics were compiled for short- and long-term exposures to invertebrates in soils and sediments. The default TLM and TLM-derived PNEC were applied to these data using EqP to develop critical body burdens (CBB) for ecologically relevant endpoints (e.g., mortality, growth) including the associated uncertainty in the model application. Comparison of the CBBs for soil and sediment species to CBBs from the larger TLM database for aquatic organisms showed no difference in the relative sensitivity between the two groups of species within the uncertainty of the model and experimental data. Acute-to-chronic ratios (ACRs) for soil and sediment tests were within the range of ACRs for aquatic organisms exposed to nonpolar organic chemicals. The TLM-derived PNEC applied to these data, also, demonstrated sufficient level of protection (e.g., ~95% of data above PNEC) even for chemicals up to  $\log K_{ow}$  6. This modeling approach was able to successfully describe the observed toxicity of field contaminated sediments. Furthermore, the TLM-derived PNEC appears to be protective of ecological quality indicators at contaminated sites without application of additional assessment factors. In conclusion, the application of EqP to TLM-derived environmental quality guidelines is suitable for risk assessment purposes.

**WE230 Metals and hydrocarbons in soils and sediments near a major in situ bitumen extraction centre in Alberta** M. Blais, E. Skierszian, J.M. Doyle, G. Irvine, University of Ottawa; L. Kimpe, University of Ottawa / Biology; P. White, Health Canada. The Cold Lake region is a major center for Alberta's in-situ bitumen extraction industry, accounting for nearly half of Alberta's total in-situ bitumen production. Concerns over contamination by metals and hydrocarbons from in situ bitumen extraction prompted an analysis of contaminants in the areas adjacent to drilling wells near Cold Lake Alberta. In general, concentrations of metals and hydrocarbons analyzed in soils were higher near the drilling wells than areas more distant, but were all below the federal (Canadian Council of Ministers of the Environment or CCME) soil quality guidelines for residential and agricultural soils. For example, nickel and vanadium, which are found in Cold Lake bitumen at 69 and 90 ug/g, respectively, never exceeded 12 and 25 ug/g in soils, respectively. Lake sediment cores were dated radiometrically and analyzed to revealed only modest surface enrichment of metals or hydrocarbons since the 1970s, when these installations began operations. Results suggest that bitumen extraction alone, in the absence of nearby upgraders and refineries, does not lead to high contamination of metals and PAHs in soils and sediments in the region.

**WE231 Biodegradation of a crude oil by an identified bacterial consortium isolated from the Western Baltic Sea** J. Rubarth, J. Jeschek, Leibniz Institut für Baltic Sea Research; S. Koenig, E. Safonova, University of Leipzig. While it is well known that numerous bacterial species are able to degrade crude oil not much is known about the potential for oil degradation by organisms in the Baltic Sea. To study this more than a hundred pure bacterial cultures were isolated from coastal areas of the Baltic Sea. Various selection criteria were considered to find the most robust bacteria tolerating crude oil as well as varying temperature and salinity. To identify the most appropriate species they were sequenced. Different consortia of these identified bacteria species are examined regarding their oil degradation capacity to identify the most efficient mixed culture. A gravimetric method was used to measure degradation of the total crude oil. For more detailed information on the degradation process we determined the before/after concentrations of common target compounds in the field of analytical oil studies. Using internal standards and a validated method we quantified the biodegradation of 38 alkanes and 24 polycyclic aromatic hydrocarbons (PAH). In addition to the 16 compounds declared by the US EPA as "Priority Pollutants" we determined another 8 alkylated PAH. The measurements were performed by GC-FID and GC-MS. Additionally, we used FT-ICR-MS measurements to compare the

heterocyclic fingerprint of oil samples before and after the incubation period. This comparison provided information concerning the preferred biodegradation of heteroaromatic oil compounds. As the availability of nutrients could be a limiting factor of microbial oil degradation in the Baltic Sea we also examined the nutrient consumption during the incubation period with a special focus on phosphate concentrations. We determined oil degradation rates of the most efficient consortia as a function of temperature and salinity ranges common in the Baltic Sea. All these analytical results were used to discuss the fate and behaviour of crude oil in the environment.

**WE232 Water and Air Quality Issues Surrounding the Use of Hydraulic Fracturing in Natural Gas Extraction** R. Jeffries, ENVIRON UK Ltd.; D.A. Kaden, ENVIRON International Corporation; C. Holman, ENVIRON UK Ltd. Exploration of European shale gas reserves, and in particular the use of hydraulic fracturing, is receiving increasing public attention. Public concerns have been voiced surrounding potential environmental impacts on air and water resources. In the last few months the European Parliament narrowly voted against a ban and the European Commission has pledged to develop an environmental protection framework. Due to its nature, shale gas is more difficult to extract and process than conventional gas. Hydraulic fracturing uses a combination of water, sand, and chemicals injected into the ground under high pressure to release natural gas or oil from tight shale plays. Large volumes of water and sand are required by the process, which may strain natural resources in some areas. Waste water contains solids and chemical additives including biocides, is highly saline, may also contain metals and naturally occurring radioactive material characteristic of the formation, and includes petroleum hydrocarbons. Exploratory drilling is underway in several EU countries, most notably Poland, but also in France, Sweden, Germany and the UK. The development of shale gas in Europe will follow from the significant experience of assessing the impacts in the US. As knowledge has increased, voluntary and legal controls have been introduced at both State and Federal levels, although there remains a lack of transparency regarding the chemicals used. Provided Europe learns the lessons from across the Atlantic and puts in place sufficient safeguards before there is significant development it is unlikely that significant environment impacts will occur. This paper provides a review of the water and air quality issues, as well as the impacts on communities and ecosystems from intensive shale production. It draws on experience from the United States of working with the shale gas industry to understand these issues and find solutions.

**WE233 Remote Sensing Research Project: Satellite Imagery Evaluation for Environmental Classification in Qatar** E.J. Febbo, ExxonMobil Research Qatar; C. Richard, CREOCEAN. Qatar is facing unprecedented development both inland and its surrounding waters. While the natural environment is not yet fully characterized, there is a need for understanding baseline conditions and a mechanism for detecting changes so that managers have an accurate decision making tool and a basis for monitoring and assessing future changes. Remote sensing techniques are cost effective in their ability to cover wide areas and to provide information in a time and cost efficient manner. Development of new sensors such as the WorldView2 satellite and airborne hyperspectral sensors provides highly accurate data including habitat, and superficial soil characterizations. Finer scale techniques such as acoustic surveys can be deployed to complement study areas of particular interest. In addition, data have to be verified and validated by visual field observations. Implementation of these new techniques for producing a large scale GIS data set for Qatar would highly improve the current knowledge and provide a powerful decision making tool for environmental management and policy decisions. In this context, WorldView2 satellite images have been evaluated as a first step in a remote sensing research program to test the potential of such data for coastal mapping. The objectives of this project were to: test the standard strategies generally used for high resolution imagery processing taking advantage of the new specifications of the sensor (8 spectral bands), and to develop innovative methods for bathymetry estimation and sea

bottom characterization based on automated processes. Preliminary results indicate that accurate classifications are possible; habitats such as coral patch reef structures, seagrass and soil classifications have been identified in agreement with the validation field surveys. This is the first step in a multi-faceted approach to utilize the latest remote sensing technologies.

**WE234 Assessing the variability of highly complex mixtures of organic contaminants from oil industry produced waters using GCxGC-ToF-MS** A. Scarlett, C. West, Plymouth University / Petroleum and Environmental Geochemistry Group, Biogeochemistry Research Centre; R. Frank, M. Hewitt, Environment Canada / Aquatic Contaminants Research Division/Water Science & Technology Directorate; S. Rowland, Plymouth University / Petroleum and Environmental Geochemistry Group, Biogeochemistry Research Centre. Produced waters from oil industries represent a huge challenge in terms of complexity and risk assessment. Some of the most complex mixtures are derived from the oil sands process-affected waters (OSPW) of Alberta Canada which contain tens of thousands of polar acidic organic compounds usually referred as 'naphthenic acids' (NAs). Until recently, individual structures of these NAs were unknown but analyses by tandem gas chromatography with time of the flight mass spectrometry (GCxGC-ToF-MS) have now begun to reveal the individual structures of alicyclic, aromatic and sulphur-containing acids within OSPWs contained within tailings ponds. Recently some of the acids have also been identified within ground water samples outside of the tailings ponds. One obstacle to understanding whether such acids from environmental samples, as ground waters, are associated with particular tailings ponds is the lack of knowledge of the variability of OSPW within and between ponds. GCxGC-ToF-MS has now been used in the present study to compare such acids in OSPWs, both temporally and spatially, using specific, known compounds. These techniques can now be applied to studies of organic acids in other oil industry produced waters. Knowledge of individual chemical structures within these highly complex mixtures also facilitates toxicological testing/modelling and aids risk assessment.

**WE235 Critical Evaluation of USEPA's Toxicological Assessment of Benzo(a)pyrene and PAH Mixture Toxicity** B.H. Magee, ARCADIS; K. Connor, ARCADIS / Risk Assessment and Environmental Science; D.W. Chin, ARCADIS. USEPA's (EPA) draft Integrated Risk Information System (IRIS) Assessment of Benzo(a)pyrene (BaP) is due to be released for public review in early 2013. The document will present a new Oral Slope Factor that will replace the current value. In addition, EPA will present four new values that are not currently on EPA's IRIS database: an Inhalation Unit Risk, a dermal Cancer Slope Factor, an oral Reference Dose, and an inhalation Reference Concentration. In advance of the document's release, the authors have performed a literature search to identify the available studies that could potentially meet EPA's study quality criteria as critical studies in the IRIS program. In addition, the authors have performed dose-response modeling using EPA's benchmark dose model to identify *point of departure* doses and composite uncertainty factors to derive the five toxicological reference values in accordance with standard EPA policy and guidance. The authors have also evaluated alternative approaches to characterize the uncertainties in deriving the above toxicological reference values. In so doing, the authors have identified the toxicological reference values likely to be presented in EPA's draft document. This research will be used to prepare detailed comments to the EPA docket on EPA's toxicological assessment of BaP. If the document is released as scheduled, the presentation will compare the authors' proposed toxicological reference values to the EPA's proposed values and critically evaluate the scientific merit of the EPA's toxicological assessment of BaP. Specifically, the presentation will summarize the comments prepared for and submitted to the EPA docket. Additionally, the presentation will discuss the implications for the regulated community of the proposed toxicological reference values coupled with the Relative Potency Factors (RPFs) that are due to be issued by EPA in final form in late 2013. Updated whole mixture

validation exercises of carcinogenic endpoints will also be presented.

**WE236 Toxicity of mixture herbicides used in sugarcane crops to juvenile tilapia (*Oreochromis niloticus*)** M.A. Moura, Instituto Biológico / Laboratório da Ciência das Plantas Daninhas; M.J. Ranzani-Paiva, Instituto de Pesca, APTA, SAA; E.A. Oliveira, Embrapa - Brazilian Agricultural Research Corporation; Z. Clemente, Embrapa Meio Ambiente / Embrapa Environment; J.H. Valim, Embrapa - Brazilian Agricultural Research Corporation; C.M. Jonsson, Embrapa - Brazilian Agricultural Research Corporation / Embrapa Environment. Tilapia (*Oreochromis niloticus*) is the main specie cultivated in São Paulo State, Brazil, due to its high aquaculture potential. The predominant culture in this region is the sugarcane, which demands the intensive use of equipment and pesticides that have the potential to contaminate water bodies adjacent to the cultivation areas. Herbicides ametryn and tebuthiuron are commonly used in mixture in the spray tank in sugarcane crops, although it is prohibited by law. Possible interactions between different herbicides used simultaneously are not understood and the impact of this practice on non-target organisms is not well known. In this work we evaluated the prolonged toxicity (14 days) of herbicides ametryn + tebuthiuron (control, 0.1076 e 1.076 mg L<sup>-1</sup> active ingredient) to juvenile tilapia. Both herbicides were used in the form of commercial products in concentrated suspension containing 50% a.i.. Tests were conducted in duplicate, corresponding to 1/100 e 1/10 of LC<sub>50,96h</sub> previously determined. Fish (Ls = 9.98 ± 0.56 cm; Wt = 33.48 ± 6.15 g) (n=36) were fed *ad libitum* and maintained in tanks of 115L with aeration, in a density around 3.8 g L<sup>-1</sup>, in room with temperature (26.0 ± 2 °C) and photoperiod (16:8h, light: dark) control. At seven and 14 days of exposure, fish were sacrificed (benzocaine solution 8%) and blood and liver were collected. Blood was drawn by caudal puncture, with the help of a needle and syringe previously heparinized. The blood specimens were assayed for: number of erythrocytes (RBC), counted in a Neubauer chamber; hematocrit (Ht) by the microhematocrit technique; and hemoglobin level (Hb) by the cyanomethemoglobin method. After these procedures, the following RBC indices were calculated: MCV (mean corpuscular volume) and MCHC (mean corpuscular hemoglobin concentration). Assays were performed to analyze enzymatic activities (GST, CAT, GPx and SOD) in fish liver. These samples were stored at - 80 °C until enzymatic analysis. The data from this study will allow a better understanding of the mode of action and toxicity of this mixture to tilapia. Also, would be useful for the establishment of maximum concentration limits in water bodies. For various pesticides, these parameters are not yet determined in Brazilian resolutions. Since it is almost impossible to found these pesticides isolated in nature, the real risk would be underestimated by the use of data from a single substance.

**WE237 Assessment of lethal and sublethal toxicity of disinfectants mixtures to *Daphnia magna*** L. Queiros, F.T. Jesus, University of Aveiro / department of Biology & CESAM; A.J. Nogueira, University of Aveiro / Departamento de Biologia & CESAM. Disinfectants are chemicals with the property to eliminate viruses, bacteria and fungi in a short period of time. These chemicals are increasingly used for clinical and veterinary purposes and also in personal care products. After use they reach the aquatic environment through effluents, forming complex mixtures. Chlorhexidine digluconate (ChD) and benzalkonium chloride (BKC) are among the most common disinfectants. In this work we assessed lethal and sublethal toxicity of binary mixtures of ChD and BKC to the crustacean *Daphnia magna*. Lethal and sublethal effects were determined as immobilization and feeding inhibition, respectively. Both disinfectants are highly toxic to the daphnids, with median effective concentrations in the order of micrograms per liter. The appropriate prediction model for the lethal and sublethal toxicity of the mixtures is presented, using the model that best fits the data (independent action and concentration addition).

**WE238 Toxicity of carbendazim and triclosan on *Daphnia magna* and species sensitivity distribution on aquatic organisms** A.R. Silva, University of Aveiro / department of Biology & CESAM; P.V.



Silva, department of Biology & CESAM; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; S. Loureiro, Universidade de Aveiro / Biology. Pesticides have become an indispensable tool for the sustainability of Humanity through crop protection and pest control. Carbendazim and triclosan are examples of widely used chemical compounds: carbendazim is a fungicide largely used in agriculture and triclosan has a biocide action and it's used in a variety of personal care products (e.g. toothpaste, shampoos, cosmetics, etc). However, these compounds end up in the aquatic system, affecting negatively a large number of organisms. This work aimed to assess the effect of carbendazim and triclosan on the freshwater cladoceran species *Daphnia magna* and to evaluate the ecological risk to aquatic ecosystems by constructing Species Sensitivity Distributions (SSDs) based on organisms' traits. Different bioassays were performed: acute immobilisation test, feeding inhibition test and reproduction test (where the number/size of broods and number of aborted eggs per animal; and the body length of the parent animal were measured). SSDs and the hazardous concentration at 5% (HC<sub>5</sub>) were estimated considering different organisms traits. A decrease in the number of neonates per animal and increase in the number of aborted eggs (reproduction test) was noticed with increasing concentrations of carbendazim and triclosan. Also, in the feeding inhibition test, a decrease on feeding rate was observed, as well as mortality as the concentrations increase. SSDs revealed to be a promising method to assess ecological risk, however further information about species traits is needed to increase the accuracy of this approach.

#### **WE239 Waste water harmful effect detection by using biological methods – bioassays and benthic macroinvertebrate metrics**

Putna, Latvian Institute of Aquatic Ecology / Experimental Hydrobiology; A. Skuja, E. Parele, Institute of Biology University of Latvia; L. Muzikante, E. Seile, M. Balode, I. Purina, Latvian Institute of Aquatic Ecology. One of the main reasons of the Baltic Sea pollution is leaching waste water from the waste water treatment plants along the sea and also from inland. With HELCOM Baltic Sea Action Plan the Baltic Sea countries have committed themselves to achieve Baltic Sea with life undisturbed by hazardous substances hence reaching good environmental quality status. The aim of this study was to evaluate harmful effect of biologically treated waste water (WW) from four WW treatment plants (WWTP) by using several bioassays and benthic macroinvertebrate metrics. Samples for acute bioassays to detect WW harmful toxic effect were collected 5 times (from May 2011 to May 2012). Toxicity was determined by using 4 different acute toxicity standard methods: freshwater - *Daphnia magna* immobilization test (EN ISO 6341:1996), luminescent bacteria inhibition test (*Vibrio fischeri* LVS EN ISO 11348-3:1998), freshwater algae test (*Desmodesmus communis* LVS EN ISO 8692:2005) and saltwater - *Artemia salina* test (ArtoxKit M standard method). To calculate benthic invertebrate metrics in order to detect WW negative impact on benthic fauna of watercourses where WW are discharged, zoobenthos samples were collected in May 2012 50 m upstream and downstream from the WW discharge place by modified AQEM (A comprehensive method to assess European streams using benthic macroinvertebrates) method. The acquired bioassay results evidences about periodic WW acute toxicity. Main reasons for that are seasonal changes, WW day and night inflow intensity, abundance of hazardous substances, active sludge activity etc. Comparing testorganism sensitivity of applied bioassays it can be assumed that the most sensitive testobject against biologically treated WW is *D.magna*, but the most toxicoresistant organism - *V.fischeri*. *D.communis* and saltwater organism *A.salina* can be characterized as intermediate sensitive. Besides in *V.fischeri* and *D.communis* tests it was possible to observe bioluminescence and growth stimulation evidencing about more organic and inorganic than toxic substance abundance. It proves that in these cases WW can promote or cause eutrophication. Benthic macroinvertebrate metrics showed similar results in two WWTP cases - most of the metrics evidenced about organic pollution increase downstream the WW discharge place. Study revealed that it is necessary to improve treatment processes in Latvia's WWTPs. This study was

performed in the frame of ESF projects  
2009/0226/1DP/1.1.1.2.0/09/APIA/VIAA/080.

#### **WE240 Predictions gone wrong: mixture toxicity of a repellent and a pyrethroid on *Daphnia magna***

R. Wolf, Goethe University / Aquatic Ecotoxicology. In the past decades, combinatory effects of substances have been of growing interest. Still, knowledge on the "cocktail-effect" of compounds is limited. For the prediction of mixture toxicity, the models of concentration addition (CA) and independent action (IA) are well established. CA's main assumption is a common mode of action (MoA) of substances, while IA assumes different molecular targets. Repellents and insecticides are both regularly detected in aquatic ecosystems. In addition to the most common and effective repellent, N,N-diethyl-m-toluamide (DEET), pyrethroids (e.g. permethrin) are often used to impregnate cloth. DEET's MoA is a competitive inhibition of acetylcholine esterase, whereas permethrin irreversibly opens pre-synaptic, voltage dependent sodium channels. Both result in elevated levels of acetylcholine within the synaptic cleft and hence increase nervous excitation, ultimately killing target organisms. The water flea *Daphnia magna* was chosen as pelagic non-target organism to evaluate the effects of a combined exposure to DEET and permethrin. Mixture toxicity was predicted using single substance data and applying CA and IA models. As expected, the toxicity of the mixture is more severe than those of DEET and permethrin alone. With increasing exposure time, the observations diverge from the predictions provided by the CA and IA models. The failure of the models' predictions could be due to indirect effects of the chemicals. The iso-effect plot results in a predicted "no effect concentration" (NEC) equalling 50 µM of the mixture. Interestingly, testing for 48 hours would have been insufficient for an assessment on long term effects. With regard to future assessments of mixture toxicity, it should be considered that *Daphnia magna* acute toxicity assays may not provide ideal terminal effects for testing ecotoxicological relevant mixtures. Biomarkers, however, would be a welcome alternative. Additionally, we think it is necessary to extend testing periods. In the future, a more detailed mixture toxicity testing, including biomarkers, and an appropriate test duration would be desirable

#### **WE241 Mixture toxicity assessment within the biocidal products authorisation process**

A. Kehrer, Federal Environment Agency. Biocidal products are usually multi-component mixtures of one or more active substances and a range of co-formulants that serve different purposes e.g. anti-foaming agents, stabilisers, pigments, emulsifiers, solvents or diluents. Additionally, metabolites and degradation products might be formed during and after use of a biocidal product. Therefore the overall ecotoxicity of a biocidal product might be significantly different from that of each individual ingredient(s) alone and hence, needs to be assessed during the product authorisation. In fact, the assessment of the mixture (eco-) toxicity is foreseen by the new Biocidal Products Regulation (BPR; Regulation (EU) 528/2012) which will replace the current Biocidal Products Directive (BPD, Directive 98/8/EC) in September 2013. There article 19(2) states that "the evaluation [...] shall take into account the following factors: [...] (d) cumulative effects, (e) synergistic effects." This is further elaborated in Annex VI (common principles for the evaluation of biocidal products) which states that the risks associated with the relevant individual components of the biocidal product shall be assessed, taking into account any cumulative and synergistic effects. However, only very limited details on how mixture effects should be considered during the authorisation of a biocidal product are provided in the current Guidance Documents and no specific guidance is at hand on how potential combination effects of active substance(s) and other ingredients should be accounted for during the environmental risk assessment of biocidal products. Therefore a tiered assessment scheme for an adequate consideration of mixture effects during the environmental risk assessment (ERA) of biocidal products was developed to assess the mixture toxicity of the products and/or, where relevant, also of the environmental relevant mixtures derived from the use of the products as well as synergistic effects as required by BPR and BPD. The scheme

presented was discussed and finally agreed among the European Member states at the Biocides Technical Meetings III/2012 and I/2013. More detailed information on the tiered assessment approach can be found in the Guidance Document on mixture toxicity assessment during biocidal products authorisation prepared by the German Federal Environment Agency (Umweltbundesamt, UBA), which will be made available after final discussion at member state level in summer 2013.

#### **WE242 Risk assessment of chemical mixtures on the Swedish west coast using biotic and water quality monitoring data**

Gustavsson, . Currently chemical monitoring on the Swedish west coast is carried out in a slightly fragmented manner. The monitoring is carried out in a large number of different programs. For instance a national monitoring program exist which primarily look for persistent organic pollutants and heavy metals in biotic as well as sediment samples. Also, a number of regional programs with differentiated focuses between programs, as well as years, survey for compounds with everything from agricultural to consumer product origins. These regional programs deal with a number of different sampling matrices such as biota, sediments and water. But also sewage treatment effluent and sludge is sampled in some campaigns. Finally, so called screening programs are carried out yearly in a multitude of different sampling matrices. These screening programs generally focus on a different set of compounds between each campaign, trying to act as early warning systems for emerging contaminants. Thus, all of these campaigns are in essences geared towards the single compound, single risk paradigm. Mixture toxicity and risk assessment of chemical mixtures has been receiving increasing attention over the last few years and the need to get an overview of the combined exposure has become even more apparent. By merging all of the available data from the monitoring campaigns mentioned, a more in depth view of the exposure scenario on the Swedish west coast becomes possible. Combining this combined database with knowledge of the sensitivity of algae, crustaceans and fish enables the risk assessment of the combined exposure scenario on the Swedish west coast. A number of suggestions on how monitoring can be performed in the mixture toxicity paradigm is also provided; hopefully enhancing the power of each individual sample taken from a risk assessment point of view.

#### **WE243 Use and interpretation of bioanalytical tools in the drinking water production chain – casus Ames fluctuation test**

van Wezel, KIWA Water Research. Over 100.000 chemical compounds are in daily use. Many compounds are found in low concentrations in sewage effluents, groundwater and surface waters. Only for some official drinking water quality guidelines do exist. Consistently it is concluded that there is a large margin of safety and human health risks are unlikely. Nevertheless, the possible presence of chemical contaminants in drinking water is a major concern for citizens. Bioanalytical tools can give a clue on possible mixture effects, without knowing all individual compounds in the mixture. Within the drinking water sector, concentrations of chemicals occurring in (sources for) drinking water are very low and hence the focus is thus upon hormonal disruption and reactive toxicity, both possibly relevant modes of toxicological action for human health at chronic exposure to mixtures of compounds in low concentrations. As the use of bioanalytical tools within the context of drinking water production is relatively recent, these are not yet embedded within any legislation. Guidelines for the interpretation of results gathered with help of bioanalytical tools have not been developed yet within this context, neither decision support schemes for subsequent steps. Here, we describe a first attempt for such interpretation and subsequent steps, based on a extensive discussion within the Dutch drinking water sector. The ‘*Threshold of Toxicological Concern*’ (TTC) is helpful for risk assessment of data-poor chemicals. For drinking water, conservative TTC-values are deduced of 0,1 µg/L for non-genotoxic (sum 1 µg/L) and 0,01 µg/L for genotoxic compounds (sum 0,05 µg/L). The response in the bioanalytical tests expressed in term of equivalents can be compared with the TTC-values, to conclude on potential relevance of the response. After a repeated relevant response: -on the drinking water production site evaluate and possibly adapt treatment and sources used - discover the cause of the effect; is it a natural or anthropogenic cause,

can it be induced during treatment? -combine bioanalytical and analytical-chemical tools to identify responsible (mixture of) compounds, followed by human toxicological risk assessment -in case that forementioned approaches do not yield enough information and the effects are measured in the final product, a risk assessment based on in vivo experiments is possible

#### **WE244 Review of studies assessing the impact of Bt-maize events expressing Cry3Bb1 on non-target organisms**

A. De Schrijver, Biosafety and Biotechnology Unit; Y. Devos, European Food Safety Authority (EFSA) / GMO Unit; P. De Clercq, Ghent University / Department of Crop Protection; J. Kiss, Szent István University / Plant Protection Institute; J. Romeis, Agroscope Reckenholz-Tänikon Research Station ART. Western corn rootworm (*Diabrotica virgifera virgifera*; WCR) (Coleoptera: Chrysomelidae) is a major maize pest and a serious threat to agriculture in North America and the European Union (EU). Pest management options for WCR are usually directed towards larval feeding and consist of crop rotation, the use of maize seed coated with systemic insecticides and the application of soil insecticides. Foliar broad-spectrum insecticides are sometimes applied to suppress adult populations. Genetically modified (GM) maize expressing insecticidal *Bacillus thuringiensis* (Bt) toxins against corn rootworms offers an additional means of control against WCR larvae. To ensure that *Diabrotica-resistant Bt-maize* cultivation does not harm other organisms than the pests it targets, termed non-target organisms (NTOs), an environmental risk assessment is carried out as part of the regulatory approval process for commercial cultivation of GM plants. Here, we report on a review of studies assessing the potential adverse effects of the cultivation of the Cry3Bb1-expressing *Bt-maize* events MON 88017 and MON 863 causes on non-target organisms and the ecological and anthropocentric functions they provide[1]. Available data do not reveal adverse effects of Cry3Bb1 on various NTOs that are representative of potentially exposed taxonomic and functional groups, confirming that the insecticidal activity of the Cry3Bb1 proteins is limited to species belonging to the coleopteran family of Chrysomelidae. The potential risk to non-target chrysomelid larvae ingesting *Bt-maize* pollen deposited on host plants is minimal, as their abundance in maize fields and the likelihood of encountering harmful amounts of pollen in and around *Bt-maize* fields are low. Non-target adult chrysomelids, which may occasionally feed on *Bt-maize* plants, are not expected to be affected due to the low activity of the Cry3Bb1 proteins on adults. Impacts on non-target organisms caused by potential unintended changes in *Bt-maize* MON 88017 and MON 863 are not expected to occur, as no differences in composition and phenotypic characteristics and in interactions between non-target organisms and the GM plant were observed between *Bt-maize* and their near-isogenic lines. [1] Devos Y, De Schrijver A, De Clercq P, Kiss J, Romeis J. 2012. *Bt-maize* event MON 88017 expressing Cry3Bb1 does not cause harm to non-target organisms. Transgenic Res, 1191-1214 DOI:10.1007/s11248-012-9617-z

#### **WE245 Formulating specific protection goals in environmental risk assessments for GM crops: a practical approach**

M. Garcia-Alonso, Estel Consult Ltd. As part of the regulatory process for approval of plant protection products and genetically modified (GM) plants, an environmental risk assessment (ERA) is conducted in order to evaluate whether adverse environmental effects may occur once the product is commercialised. For both types of products, the ERA methodology used is similar and usually follows a conceptual framework starting with problem formulation. This first step requires a clear understanding of the protection goals and data requirements set by the regulatory framework. Protection goals set in the legislation are, however, often formulated in very broad terms and are too vague to be scientifically useful for ERAs. Since the main purpose of an ERA is to address key regulatory concerns using scientific information to allow decision-making, it is important that these broadly formulated policy protection goals are translated into concise and concrete measurable endpoints and scientifically testable hypothesis. This talk will explore practical ways in which generic protection goals can be translated into operational protection goals that allow the formulation of assessment

and measurement endpoints and focus the ERA of GM crops to those questions meriting detailed risk characterisation. The current regulatory framework in the EU will be used as an example.

**WE246 MIRALBERSAGLIO ('aim to the target'): implementing low drift nozzles in Italy for orchards and vines for chlorpyrifos containing products**

C. Vaj, M. Bradascio, E. Tescari, V. Bosco, Dow AgroSciences Italia s.r.l.; S. Norman, Ridgeway Eco. Chlorpyrifos is one of the most important and well-known broad-spectrum insecticides on the market, its mechanism of action allows the management of anti-resistance strategies, and it falls in many protocols for Integrated Pest Management of crops particularly important for the Italian market as apples, pears, grapes and stone fruits. It is often at the center of the strategy of farmer pest management for its effectiveness and costs. Actually the products containing chlorpyrifos are under re-registration process. Spanish authorities have assessed the dossier submitted by the members of the Consortium of registration holders (Dow AgroSciences, Maktheshim Agan and Cheminova). The conclusions were provided to the authorities of other countries in Southern Europe. In Italy the re-registration process of chlorpyrifos based products is still in progress. The result of the risk assessment for aquatic organisms due to load by drift required the identification of appropriate mitigation measures to prevent drift load into water bodies. In 2011 in UK the project SAYNOTODRIFT (<http://www.saynotodrift.co.uk/>) was launched to promote the use of Low Drift Nozzles (LDN) by farmers applying chlorpyrifos products in both fruit and arable crops. The aim of the project was to promote the use of LDN in all the treatments as soon as possible and consisted in a communication plan, promotion and dissemination for growers, consultants and technicians. In one year activity, from 2011 to 2012, the use of LDN increased from 12% to over 88%, with great farmers' satisfaction. In Italy, a specific research project was started with the University of Turin, in summer 2012, to be finished by 2013. The project was focused on two pilot areas in Northern Italy (Emilia Romagna and Trentino Alto Adige) on two important crops such as apple trees and vines. The results of the study provided the technical and scientific basis for the MIRALBERSAGLIO project, which represented the communication promotion and dissemination plan, complying with the characteristics of the Italian market and farmer expectations. The MIRALBERSAGLIO project aimed to promote the use of LDN for applying chlorpyrifos based products in Italy, by demonstrations for farmers and technology transfer initiatives. It was accompanied by some efficacy trials that assessed the possible influence of different nozzles on the efficacy, as required by the Italian guidelines for mitigation measures of the Ministry of Health (2009).

**WE247 Spatially differentiated risk mitigation measures for spray drift are part of the pesticide risk management of terrestrial ecosystems in Germany**

B. Golla, Institute for Strategies and Technology Assessment; R. Neukampf, Julius-Kühn-Institut, Federal Research Centre for Cultivated Plants / Institute for Strategies and Technology Assessment. Based on the ecological concepts of recovery and recolonization, the capability of non-target organisms to regenerate from adverse effects due to pesticide spray drift depends on the amount of natural habitats and the intensity of agriculture in a landscape. Since 2002 regionalized label requirements concerning off-target pesticide spray drift are part of the risk management in Germany. The proportion of semi-natural and extensively used habitats in agrarian landscapes within 13'000 administrative communities are assessed in a GIS-based landscape analysis. Whereas low drift nozzles requirements are not regionalized, buffer zone requirements are reduced according to the calculated proportion of semi-natural habitats (SNH) of the community. Recently the methodology was reviewed, underlying data were updated and results cross-checked against terrestrial survey data (e.g. from the High Nature Value Farmland project). Major methodological adjustments are (1) an increase of the minimum required proportion of semi natural habitats (2) an upper limit for connected agrarian landscape not fulfilling SNH requirements (3) additional terrestrial survey data on qualitative and quantitative aspects of SNH. Technical advancements

include computer-routines for the integration of refined spatial data (e.g. from LPIS-LE) and a web-based mapping tool. This poster demonstrates the details on the reviewed methodology, shows results of the recent landscape analysis and the cross-check against terrestrial survey data.

**WE248 Sugarcane and Rice Paddy Pesticides Usage in the Kilombero Ramsar Site: A Quest for Sustainable Management**

S.F. Materu, Hamburg University of Applied Sciences / Institute of Ecology; S. Heise, Hamburg University of Applied Sciences; B. Urban, Leuphana University of Lüneburg. The Tanzanian government is putting emphasis on attracting more investors on the agricultural sector to boost performance, combat poverty and increase food security. The fertile land of the southern Tanzania corridor where the Kilombero Valley Ramsar Site is located has been earmarked for large scale crop production. Already a significant part of this valley has been offered to the investors for sugarcane and rice plantations. A sustainable use of wetland resources by maintaining the ecological health while contributing to improved people's livelihood is required. This study focuses on assessing the potential impacts of agrochemicals from sugar and rice companies on the ecological integrity of the Kilombero flood plain. In the first screening sampling survey, 80 water and sediment samples were collected in seven plantations in spring 2012 during rainy season. The samples were transferred to Germany and ecotoxicity was measured applying 3 standardized bioassays (Algae growth inhibition, sediment contact test with *Arthrobacter globiformis*, luminescence bacteria test). Results were classified using an integrated fuzzy-logic based classification scheme (Keiter, et al 2009) in order to get a first overview over toxic responses in the area. Sediment samples from 3 sites showed moderate toxicity while other stations are classified as no or low toxic. Specific sites will be selected for dry season sampling in January 2013 on the basis of these first screening results. Another rainy season sampling is anticipated for later in the project. Future samples will undergo detailed chemical analysis; the ecotoxicological test battery will be extended by organisms that are sensitive to fungicides and insecticides. Ultimately, results of rainy and dry season sampling surveys will be compared and an integrated risk assessment carried out of the rice and sugar plantations on this Ramsar site. This study shall provide comprehensive information on the impact of toxic agrochemicals on the health status of this Ramsar site ecosystem. It will help in raising awareness towards the ecological and human-related risks of excessive pesticide application, and develop wetland management recommendations for a more efficient and less environmentally adverse use. Overview results of the extent of contamination and toxicity levels in comparison to the pesticides analytical data will be presented.

**WE249 Impact of drift mitigation measures on national aquatic risk trends analyzed with the GIS-based indicator SYNOPS using a network of reference farms**

J. Strassmeyer, Federal Research Centre for Cultivated Plants / Institute for Strategies and Technology Assessment; B. Freier, V. Gutsche, Julius Kühn-Institut / Institute for Strategies and Technology Assessment. The use of pesticides in agriculture causes environmental risks that must be carefully managed. An overall goal of the current German National Action Plan on the Sustainable Use of Plant Protection Products (NAP) is to reduce the environmental risk by 25%. One of the data sources to assess the risk development in agricultural production are annual surveys of pesticide use in main arable crops (winter wheat, winter barley and oilseed rape) which are conducted on representative farms. This Network of Reference Farms, which is a joint project of the Federal Ministry for Food, Agriculture and Consumer Protection (BMELV), the State Plant Protection Services and the Julius Kühn-Institut, was established in 2007 as part of the German NAP. From 2007 to 2011, 45,000 uses were investigated in four different survey regions. In 2011, pesticide treatments in 762 fields on 85 arable cropping farms were analyzed. Within the NAP the risk indicator model SYNOPS-GIS is applied for regional risk analysis and the detection of hot spots assuming a random spatial distribution of the surveyed of pesticide use patterns together with an extended GIS based datasets on land use, slope, soil types and climate. This spatial database was established by merging information from an extended geographical

dataset on land use (ATKIS), a digital soil map (BÜK1000), a digital elevation model (DGM25) and a set of 430 climate stations and >2000 precipitation stations (DWD). Furthermore a database of all registered pesticides in Germany was linked to the tool giving information on formulation, active ingredient content and product specific drift and run-off mitigation requirements. Assessments of the aquatic risk potential of pesticide use with the indicator-model-SYNOPS were conducted on field level and aggregated on regional and national level. These aggregated values showed a decrease of the risk potential for winter wheat by 48% and for winter barley by 43% related to the average risk of survey period from 2007 to 2011. The aquatic risk values for winter rape did not follow a clear risk trend. The effects of the labeled drift mitigation measures and fixed buffer zones on these national risk trends are demonstrated.

**WE250 Harmonized risk mitigation measures for the authorization of disinfectants** S. Wieck; S. Gartiser, Hydrotex GmbH; K. Wege, E. Petersohn, Federal Environment Agency, Germany. According to Article 19 of the European Biocidal Product Regulation (EU) No 528/2012 biocidal products shall not be authorized if they amongst other conditions have unacceptable effects on the environment. This has to be proven in the frame of the environmental risk assessment (ERA) during the authorization process of biocidal products. If the ERA indicates unacceptable risks from the respective product, risk mitigation measures (RMM) might be proposed to reduce the risk and allow an authorization. Authorizations of biocidal products can be mutually recognized in all European member states (MS) if once been granted in another MS. This regulatory process has very short time frames. First experiences with wood protection products and rodenticides have shown that harmonized RMM are a valuable instrument to facilitate this process. However, harmonized RMM are only available for rodenticides. This will especially be a problem for product types (PT) containing a lot of different products where a lot of authorizations will have to be mutually recognized at the same time. This will be the case for disinfectants. Out of the approx. 33 000 products that are registered in the German Registry for Biocidal Products ("BAuA-Melderegister") approx. 15 000 are disinfectants (PT 1-5). The German Federal Environment Agency funded a project on the harmonization of RMM for PT 1-5. The project aim was to elaborate PT-specific guidance documents and to make them available to the national authorities during authorization and mutual recognition processes. The sources were RMM catalogues from other regulatory areas as the EuPhraC-catalogue, specific national RMM collections as for the Dutch "Legal Instructions for Use" (LIU), currently available Competent Authority (CA)-reports for active substances in PT 1-5 or recommendations of the supplier and professional user associations. The guidance documents account for the PT-specific use patterns and each provide a collection of RMM indicating the environmental compartment where the RMM is heading. The guidance documents were discussed with the members in the "Technical Meeting on Biocides" in 2012 and will be finalized in 2013. This poster will show the developing process of these guidance documents, the results, and might give support for potential follow-up activities for other PTs.

**WE251 Exposure scenarios and risk mitigation measures for ornamentals** E. Kohlschmid, Agroscope ACW; M. Gandolfi, Research Station Agroscope; A. Aldrich, Agroscope ACW. Nurseries produce and sell different kinds of ornamental plants (e.g. trees, shrubs, flowers), fruit trees, berry plants and forest plants. In Switzerland around 1370 nurseries exist on an area of approximately 2000 ha. Culture techniques and plant protection measures including the application of plant protection products (PPP) in nurseries are very diverse. Exposure scenarios and risk mitigation measures do not fully take into account this diversity and complexity at the moment. Although nurseries cover a relatively small area compared to the total area of arable land and permanent cultures, they can contribute to PPP emissions in the environment. Therefore detailed knowledge of how PPP are applied and what kind of exposure routes exist in these different ornamental cultures, is important to define the risk to non-target organisms. Future improvements aim at categorizing nursery applications of PPP based on

selected culture types: trees/shrubs, flowers/green plants, flower bulbs, roses, lawn and fallow. In the poster we will describe parameters of PPP application, exposure scenarios and models as well as pertinent risk mitigation measures for the selected culture types in nurseries. A comparison will be made in how far risk mitigation measures derived from orchards, vineyards and arable crops are suitable to protect non-target organisms from the application of PPP in ornamental cultures. Similarities and differences are analyzed, information gaps are identified and proposition for amelioration are suggested.

**WE252 PPP applications in Italy: reducing drift by improved maintenance of equipment and low drift nozzles use** P. Balsari, P. Marucco, University of Turin / Department of Agricultural, Forest and Food Sciences; C. Vaj, V. Bosco, Dow AgroSciences Italia s.r.l.; S. Norman, Ridgeway Eco. During the application of Plant Protection Products (PPP), a certain amount of droplets can be transported out of the treated field by spray drift, especially for 3D crops, which may lead to undesired exposure in non target compartments. This is considered in the PPP registration process, which may provide specific mitigation measures. Drift can be mitigated implementing specific measures. Some techniques that mitigate drift by acting on the droplets production are already available on the market, i.e. Low Drift Nozzles (LDN) or low drift sprayers. Also the correct calibration of the sprayer, depending on the canopy geometry and the volume of PPP solution to be applied, has an influence in reducing drift. The good maintenance of the equipment is essential in the correct calibration and use of the sprayer itself. This is confirmed by a number of works conducted in field. A recent activity carried out in Emilia Romagna (North Italy) in 2012 confirmed the influence of LDN, compared to conventional nozzles, and the correct sprayer calibration. ISO 22866 guideline was followed and 3 theses were tested: conventional nozzles with correct sprayer calibration, LDN with appropriate calibration and conventional nozzles without an appropriate calibration that is often the field reality. The applications were made using a colored tracer (Tartrazine E102) and the droplets deposited by drift were sampled at increasing distances from the field margin. The results confirmed the influence of the tested mitigation techniques and are shown as example. The major critical points in the dissemination of LDN and other measures that can mitigate drift are discussed. The dissemination of LDN and other equipments that mitigate drift is still very low in Italy especially in the South, farmer awareness of the problem is generally low and users are not trained on how to reduce drift. Despite the activities carried out on the topic, only few experimental data carried out in Italy are available. Spray drift values are derived by consolidated tables (Ganzelmeier tables) that however are not representative of the Italian environmental and field/crops conditions. Also the reference spray technique necessary to estimate the percentage amount of drift reduction of the proposed new technology/nozzle, that is a key parameter in risk assessment, has not yet been defined. This should also be reflected in the mitigation measures indications on PPP labels in a way that is implementable by farmers.

**WE253 Quantifying greenhouse gases from the production, transportation and utilization of charcoal in developing countries, a case study of Kampala, Uganda** O. Ekeh, Ecology. This paper aims to estimate greenhouse gases (GHGs) and other air pollutants from the production, transportation and utilization of charcoal in Kampala, Uganda. This work was done in accordance with ISO 14040 methodology for Life Cycle Assessment (LCA), using GABi 4.0 – a commercially available software for Life Cycle Assessment. A cradle-to-grave study was conducted, excluding emissions arising from machinery use during biomass cultivation and harvesting. The distance from charcoal production locations to Kampala was estimated using ArcGIS 10.0 software and a GPS tool. Emission data from a modern charcoal production process (Pyreg methane-free charcoal production equipment) which complies with the German air quality standards (TA-Luft), was compared with emissions from a traditional charcoal production process. Four coupled scenarios were modeled to account for differences in the quantity of greenhouse gases emitted from the traditional charcoal production phase; improved charcoal production phase (biomass

feedstock sourced sustainably and unsustainably); transportation phase; and utilization phase. Data for this study was obtained via literature review and onsite measurements. The results showed that greenhouse gases emitted due to demand of charcoal (production; transportation; and utilization) in Kampala was 304,486,926 tCO<sub>2</sub> eq, with the transportation phase accounted for approximately 99.5% of greenhouse gases emitted. The utilization phase (charcoal cookstoves) emitted 723,985 tCO<sub>2</sub> eq. Changing the charcoal production technology from a traditional method to an improved production method (Pyreg charcoal process) resulted in reductions of 230,747 tCO<sub>2</sub> eq (0.8tCO<sub>2</sub> eq/kg charcoal produced), however, with biomass feedstock sourced sustainably; this resulted in reductions of 801,817 tCO<sub>2</sub> eq (2.9tCO<sub>2</sub> eq/kg charcoal produced). Additionally, the study showed results of biomass consumed; greenhouse gases and air pollutants emitted from the production and utilization of charcoal for the entire country –Uganda – for year 2004 and 2011. Projections for 2015 and 2020 were also calculated.

**Keywords:** Greenhouse-gases; methane-free-equipment; charcoal-cookstove; charcoal; life-cycle-assessment, earth-mound kiln.

**WE254 Case study illustrating use of the ‘Sustainability Business Assessment’ tool (SBA) to enable sustainable decision making within the tobacco industry** N.G. Rehman, British American Tobacco / Product Stewardship. The sustainability business assessment (SBA) tool has been developed within British American Tobacco (BAT) to consider the risks and impacts of our business by taking a long term product lifecycle view. Consideration of the 3 ‘P’ elements i.e. Planet (reducing harm to the environment), People (impacts and benefits on society) and Prosperity (business viability of the product/process for today and the future) helps to make informed decisions from the early design stage of product development to support sustainable design and thinking, and where possible reducing negative impacts. This is achieved by collaboration within the organisation, and through dialogue with our suppliers. The SBA is a checklist of areas of risk that should be considered within any project and is applied during the feasibility, development and commercialisation of a new material, process or product throughout the life cycle of the project. i.e. in the case of our product from ‘seed to stub’. The environmental impacts identified in the SBA i.e. the use of energy, water, greenhouse gas emissions and carbon footprint are more specifically addressed by conducting life cycle analysis (LCA) studies using Gabi software. We describe a life cycle study which has been conducted to compare two different forms of sugar used in our products. The results of this LCA study are presented to identify and compare the impacts of these at key stages of the product lifecycle to enable informed business decision making.

**WE255 EXPERTISE AND SUPPORT TO IMPLEMENT ENVIRONMENTAL FOOTPRINT LABELLING INITIATIVE IN THE TEXTILE INDUSTRY** J. Payet, I. Radtke, Cycleco. The « Grenelle 2 » law[1], published in France in 2010, provides that consumers will be informed through an environmental display about the environmental impacts of mass market products (JORF, 2010). The environmental footprint of products, will thereby become a new purchase criterion. Within firms with higher standards for performance, ecolabels are no longer seen as a regulatory nuisance but as a competitive advantage in the long run. Indeed, more than 168 firms have participated in the French experimentation for LCA-based mandatory environmental communication scheme[2]. This paper will focus on a pilot project that involved 15 partners in a joint operation to test the feasibility of systematic LCA for textiles. The project brought together representative of the entire industry: from spinners to distributors and textile institutions, thus ensuring representativeness of the distribution channels, in order to quantify the performance of production processes and identify key points to be dealt with. More than 48 attributional LCA studies were conducted and the following points were identified: - The need for a reference database specific to the sector, providing default values to allow any firm, regardless of their knowledge of the supply chain to assess the impact of their product, while giving a competitive advantage to the firm making the effort to collect specific data. -The need, in order to mainstream LCA, for those data and methods to be

accessible easily and integrated within a simplified environmental impact calculator enabling collaborative work. -The importance of expert representation within the ADEME-AFNOR work groups to represent and protect SMEs interest and ensure adequate consideration of LCA methods and guidelines. In conclusion this paper will describe recent and foreseen development within the strategic planning of those SME's and show how sector specific LCA tools empowers non-experts so they can lead environmental change within their industry. [1] LOI n° 2010-788 du 12 juillet 2010 portant engagement national pour l'environnement, Affichage environnemental : Titre VI - Chapitre 1 - Article 228, Journal Officiel de la République Française (JORF) du 13 juillet 2010, version consolidée au 10 septembre 2011 [2] BP X30-323-0 June 2011 General principles for an environmental communication on mass market products : part 0 : general principles and methodological framework, AFNOR

**WE256 Estimating of life cycle inventories of land use of Japan** K. Horiguchi, Tokyo City University / Research Division in Env and Infor Studies; N. ITSUBO, Tokyo City University. Land use is a fundamental elementary flow for assessing damages on biodiversity in life cycle assessment. Recently, several inventory databases includes elementary flows of land use as an inventory, moreover Koellner et al. (2012) showed the principles for life cycle inventories of land use. On the other hand, these data and principles are compiled mainly European datasets, therefore Japanese statistics or information of land use cannot always be available. In this presentation, the result of estimation of elementary flows of land use inventory in Japan will be showed.

**WE257 Effect of land-use change and CO<sub>2</sub>-eq emissions due to sugarcane crop expansion and abrogation of burnings on field in the state of São Paulo, Brazil** R.R. Sallaberry, TU Darmstadt / Industrial Material Cycles; K.R. Nunes, Technische Universität Darmstadt / Institute IWAR Industrial Material Cycle; L. Schebek, Technische Universität Darmstadt / Industrial Material Cycles. The burning of sugarcane fields is a current practice to both reduce the quantity of straw and facilitate the manual cane cutting. The gradual abrogation of burning of sugarcane in the state of São Paulo (SP) has been implemented, forced by a new law in 2002, and reinforced by agreement with the sugarcane union in 2007. This decision is important, since it is pioneer in change an age-old practice in Brazil. Additionally, SP planted 54% of Brazilian sugarcane in harvest-year 2010-11. Concomitantly, Brazil experiences a large expansion of sugarcane crop due to the growing demand of sugarcane products, e.g. sugar, ethanol, plastics, biofuel, electricity, among others. This expansion triggers displacement of former occupations of land to the agriculture frontier and over natural vegetation, leading to not only direct but also indirect land-use change. In order to verify the effect of gradual abrogation of burnings and simultaneously sugarcane expansion, national surveys and forecasts were confronted. The basis scenario presents a determined land cover of SP, including land occupation and carbon stock. Harvest data and sugar and ethanol production, as well as expansion forecast, were acquired from Brazilian Ministries of Energy and Agriculture, and from global carbon map dataset. Therefore, the carbon stock of different crops and natural vegetation, as well as the difference between burning and other alternatives to harvest sugarcane were considered in a time period of five years until 2020. The result is presented in carbon stock loss or gain. This variation in carbon stock is than converted to carbon dioxide equivalent emissions, which is indicator of global warming potential. This study aims at to find out if the gradual abrogation of burnings on sugarcane crop could reduce the environmental impact of global warming potential, even with the increase of mechanization. However, its direct and indirect land-use change due to expansion would affect strongly the overall environmental impact account. The use of cropland and rangeland would trigger the indirect effect of deforestation related to expansion of agriculture frontiers. Although this phenomenon could happen, the state of SP would have possibility to support until certain limit the sugarcane crop expansion.

**WE258 Roles of LCA experts in policy-making** T. Ekvall, IVL

Swedish Environmental Research Institute. Results from life cycle assessments (LCAs) have been used to justify and put in question policies aiming at, for example, increased recycling. The LCA results depend heavily on the choice of system boundaries, etc. These methodological choices are subjective. They depend on the values and perspectives of the individuals making the choices. The question is what perspectives should the system boundaries reflect? Who should make this decision, thereby influencing the LCA results and, possibly, the policy? There are at least two schools of thought here. One argues that the system boundaries should reflect the perspectives of the policy-makers. Researchers and other LCA practitioners should only deliver objective information to guide the policy-maker in this decision. This makes the LCA expert a tool in the policy-making process. The other school argues that the LCA expert should decide on the system boundaries, as an independent researcher. The LCA results then reflects the values and perspectives of the researcher, and the researcher becomes an active part in the policy-making process: an activist. There are advantages to both of these roles. As a tool, the LCA expert can facilitate an efficient learning process, resulting in more knowledgeable policy-makers. As an activist, the LCA expert can often send messages that are clear enough to be conveyed by journalists and understood by the general public. As a result, the public will be better informed. There are also risks involved in both roles. A tool and an activist can both misuse the credibility of research and academic institutions, if they are intellectually dishonest. Even when intellectually honest, a tool can be regarded as a lackey of the powerful. The activist, on the other hand, wields power not given through democratic procedures. Luckily, there are safeguards to both of these risks. A tool can involve not only policy-makers in the LCA, but also stakeholders through reference groups, etc. An activist, if too extreme or unruly, will have difficulty finding funding for further research.

**WE259 Assessment of Luxembourgish ecological deficit and loss of natural capital using time dependent EE-IO model** B. Rugani, University of Siena / Resource Centre for Environmental Technologies (CRTE); D. Roviani, P. Hild, E. Benetto, CRP Henri Tudor (CRPHT) / CRTE. The Ecological Footprint (EF) accounts for the impact of human activities in terms of demand for land area. It also calculates the biocapacity in the same units. While an EF assessment is worthwhile to address the decision-making policies of pressure mitigation on land resources, the indicators based on EF could underestimate the problem of human carrying capacity, since EF does not consider the work of untouched nature in productivity and ecosystems services. In this connection, the Emergy Evaluation (EME) method can be considered a more robust tool than EF, because it allows taking into account other fundamental flows that can influence the systems sustainability (i.e. energy flows used to dilute internal wastes, soil erosion, deforestation, water acidification, among others). This work focused on the calculation of EF and Emergy for the case study of Luxembourg, with the final aim of combining the two methods in order to enhance the perspective of biocapacity and natural capital evaluation in the country. Based on the most recent and appropriate literature findings and statistics datasets, we built an Environmentally Extended Input-Output (EE-IO) model for Luxembourg, considering time-series (from 1995 to 2009), and implemented specific characterization factors for EF and EME in the LCA software SimaPro®. The EF and Emergy values related to the goods and services imported and produced in, and exported from, Luxembourg were then compared to the available (land) resources in the country (i.e. biocapacity in EF terms and natural capital in Emergy terms). Results showed a quite linear increase in the EF and Emergy during the 15 years of analyzed economic trend, with corresponding growth of, respectively, ecological deficit and loss of natural capital. The increase is basically in relation to the demographic growth and the high dependency from fossil fuel resources, which are all imported. The economic sector that mostly influenced (both directly and indirectly) the results was the financial services activity, which is essentially the largest and most relevant sector in the economy of Luxembourg. The strenght of the developed framework, which is compliance with the worldwide interest and exponential proliferation of EE-IO studies, allowed the

identification of consumption patterns in Luxembourg as well as the footprint responsibility to be awarded among the imported (indirect), produced (indirect + direct) and exported (direct) vectors of the economy.

## **WE260 INCORPORATING ECODESIGN INTO THE DECISION MAKING PROCESS BY APPLYING ISO 14006. A CASE STUDY**

V. Perez-Belis, Universitat Jaume I. Dpt. Mechanical Engineering & Construction; M.D. Bovea, Universitat Jaume I Department of Mechanical Engineering Construction; D. Bernad-Beltran, Universitat Jaume I. Dpt. Mechanical Engineering & Construction / Dpt. Mechanical Engineering & Construction; V. Ibáñez Forés, Universitat Jaume I. The ISO 14006 aims to incorporate environmental requirements into the design and development of products in a systematic way, reducing their environmental impact and improving their environmental performance. The implementation of this standard in the design departments of companies could be a way to integrate the environmental aspect of the product with other design requirements, incorporating it into the decision making process. However, to make this inclusion efficiently, companies need specific sectorial tools which enable them to apply the methodology of Life Cycle Assessment to their products in a regular and precise way. This paper presents a tool adapted to the toy industry whose application provides environmental indicators for different impact categories, making it possible to identify designs with better environmental performance. In addition, the results obtained can be used to develop an Environmental Declaration according to ISO 14025 and to communicate the environmental profile of the product to consumers or businesses.

## **WE261 Comparison of the life cycle impacts of six common British chilled ready-made meals** X. Schmidt; N. Namy Espinoza Orias, A. Azapagic, The University of Manchester. Ready-made meals are

convenience food products, the market for which is growing fast due to the modern lifestyle. The UK is the largest consumer of these products in Europe and only behind the USA globally. For instance, convenience food now constitutes more than a third of the British food market with approximately 8.8 kg of chilled and frozen ready-made meals consumed per capita per year and with a market valued at £2.7 billion in 2010. In terms of variety, a recent survey indicated that the most preferred ready-made meals are the Italian and British with 24.2% and 20.1% people expressing their preferences for them; the Indian and Chinese cuisines are next with 17% and 13.5% votes. Whilst ready-made meals provide much-needed consumer convenience, they are also increasing the environmental pressures in the food industry which already contributes 18% of the national greenhouse gas (GHG) emission and 14% of industrial energy consumption. Yet, there is currently little information on the life cycle environmental impacts of convenience food, and particularly ready-made meals. Whilst numerous LCA studies exist of single food items, there are few studies of complete meals with most focusing on global warming potential (GWP) or on a limited number of environmental impacts. To date, only two studies have considered the full LCA impacts of ready-made meals, both based in Spain. As far as the authors are aware, only one LCA study has been carried out for ready-made meals in the UK. This paper builds on the latter and evaluates the life cycle environmental impacts of six common British chilled ready-made meals. The LCA methodology follows the ISO 14040/44 standards. GaBi software has been used to carry out the LCA, applying the CML 2 method. The following six common British chilled ready-made meals prepared industrially are considered: lamb masala curry, chicken korma curry, cottage pie, roast pork and four vegetables, chilli con-carne and lasagne. The scope is from 'cradle to grave' and the functional unit is defined as 'preparation and consumption of a ready-made meal for one person (360 g)'. The results suggest that Indian meal of chicken korma curry is environmentally more sustainable than the British and Italian recipes such as roast pork and veg, cottage pie and lasagne. The main reason for this is the ingredients, especially the type and quantity of meat. *Keywords: convenience food; ready-made meals; environmental impacts; LCA*

**WE262 Wooden pallets in Italy: environmental sustainability of the open pallet exchange pool** by LCA G. Dotelli, Politecnico di Milano

/ Chemistry, Materials, and Chemical Engineering "G. Natta". The objective of this study was to assess the environmental sustainability of the good logistic system supported by the open pallet exchange pool. The work was commissioned by Comitato Nazionale EPAL del Consorzio Servizi Legno-Sughero, EPAL European Pallet Association in collaboration with Assoimballaggi/Federlegno Arredo with the aim of performing the *Life Cycle Assessment* (LCA) of pallet EUR/EPAL *from cradle to grave*. The study tries to highlight the environmental advantages of the open pallet exchange pool with respect to disposable one-way pallets; the work refers to the Italian panorama only. The pallet is a flat transport structure that supports goods in a stable fashion. In this work wooden pallets are considered, but on the market are also available plastic, metallic, cardboard pallets with different uses. Pallet whose quality is certified are said "standard pallets", otherwise they are said "non-standard". Among them there is the Pallet EUR-EPAL, which typically measures 800 by 1200 mm, and is a four-way non-reversible pallet. The LCA study has been done in accordance with ISO standards: ISO14040 e ISO14044. Primary data were gathered through direct interviews with the representative stakeholders (producers and repairers), then via questionnaires to be filled in by other stakeholders of the field. Impacts have been assessed using two different eco-indicators: IPCC2007 GWP100 and ECOINDICATOR 99 –HIERARCHIST (H/A). One major benefit of the use of the EUR/EPAL pallet comes from the open pooling systems, but to highlights this fact is necessary to choose a proper functional unit which takes into account the role of the pallet in the industrial sector, i.e. to facilitate goods transport. So the distance covered by the pallet seems to be the more appropriate unit to select. In this work 100 km has been chosen, but of course any other distance would have been equally appropriate. The great advantage of this choice is that it enables to compare on proper grounds different pooling systems. So, disposable vs. returnable systems have been compared. Among others results, it has been highlighted that the use phase is the most impacting one whichever of the two eco-indicators is used.

**WE263 Decision making methodology for evaluation of greener synthesis options in pharmaceutical industry** P. Yaseneva,

University of Warwick / School of Engineering; A. Peace, BRITEST LTD.; R. Woodward, AstraZeneca; A. Lapkin, University of Warwick / School of Engineering. The pharmaceutical industry traditionally has the worst E-factor, defined as a ratio of mass of waste generated per unit mass of product (1). This waste is mainly composed of organic solvents used in synthesis and reactor cleaning. The amount of waste solvents is a concern due to their toxicity, high cost of recovery and treatment, and environmental impact of manufacture (1,2). Hence, there is a significant interest in developing of alternative (greener) solvents, and in process-intensification (including reduction in solvent use). One approach to PI is via a transition from batch to flow reactors. However, such a transition must be supported by a multi-objective and multi-stage decision-making processes, including short-cut environmental assessment, LCA and evaluation of sustainability. A large-scale integrated project SYNFLOW (FP7), aims to develop more sustainable chemical processes based on novel catalytic technologies and processes exploiting greener solvents, such as ionic liquids, supercritical fluids, etc. In order to justify that the developed processes are indeed more sustainable, the SYNFLOW project has developed a methodology of evaluation of sustainability for the demonstration case studies in order to support its decision-making process. Within the SYNFLOW project the development of novel chemical processes is considered as a synergistic development of chemical routes, catalysts and processes. Assessment of sustainability of the developed technologies is built-in as an integral part of the SYNFLOW R&D process and initial results of this integral approach will be discussed in this presentation. References [1] Sheldon, R.A. 2000. Atom utilisation, E factors and the catalytic solution. C.R. Acad. Sci. Paris, Serie IIc, Chimie 3: 541-551. [2] Slater C.S., Savelski M. 2007. A method to characterize the greenness of solvents used in pharmaceutical manufacture. Journal of Environmental Science and Health Part A 42: 1595-1605.

**WE264 Assessment of impact on water resource and aquatic ecosystems in Life Cycle Analysis (LCA): case study on three rinse off cosmetic products** P. Martz, L'Oréal Research Innovation; C.

SPEICH, L'Oréal Research & Innovation; J. Lharidon, L'OREAL / Life Sciences Direction. Along with biodiversity and climate change, water is one of the major environmental issues to deal with nowadays. However, unlike the emission of greenhouse gases, water is rather a local / regional problem. During the last years, many LCA methods have been developed to assess the impact of products on human health, ecosystems and water resource. At the same time, more and more studies on water footprints of products and organizations are being conducted and published in companies' sustainable development reports as well as in TV broadcastings for people at large. LCA has shown that rinse off products are the ones that contribute the most to L'Oréal's water footprint. Preservation of water resource being one of L'Oréal's key sustainable development commitments, Quantis and their Water Database helped conduct LCA study on three rinse off cosmetic products: two shower gels and one face cleansing product. According to this study, the three products show similar environmental profile. For sake of clarity, we decided to present our results only for one shower gel. It emerges that: 1) a lot of impacts linked to water cannot be observed with a mere assessment on withdrawn water; 2) availability, local water stress and origin of the water as well as the quality of the water discharged into the environment have also to be taken into account. However, the application of some methods integrated into the Quantis Water Database remains limited for our purposes, due to relevance issues and lack of regionalized data. Some results, or units used, seem inappropriate for our objectives, which are 1) cosmetic product ecodesign, and 2) education of consumers and stakeholders to water issues using cosmetic products.

**WE265 Water footprint of margarine: impacts on water quantity and quality** H. Franceschini, Unilever; L. Muñoz, Unilever / Safety

and Environmental Assurance Centre SEAC; L. Lessard, S. Vionnet, Quantis; D. Jefferies, Unilever. Water is becoming a priority issue for companies in terms of product sustainability. The water footprint concept developed by the Water Footprint Network emerged as a suitable method to address impacts of products on freshwater resources. Up to recently, a common problem was the lack of water inventory data, especially for activities other than agriculture. The Quantis Water Database offered for the first time a broad set of inventories, based on the ecoinvent database, allowing practitioners to fill common data gaps at the water accounting stage. We present the application of water footprinting to a Unilever product, namely a margarine manufactured and consumed in Germany. A cradle-to-grave analysis of the margarine product was carried out, using as functional unit of a 500-g pack of margarine sold in Germany. The activities included were the production oil crops (rapeseed, sunflower, palm fruit, maize), the extraction and refining of the vegetable oils, the manufacturing of the margarine, distribution, consumer use (refrigeration), and packaging disposal. An effort was made to regionalise as much as possible the inventory, by defining the locations where activities take place, from the agricultural stage to the consumer stage. The foreground data used (product and packaging composition, manufacturing) was based on internal Unilever data and on previous analyses of margarine. The Quantis Water Database was used for the production of raw materials and for providing the background data. The indicators used to assess the product included impacts on quantity and quality, all of them at the midpoint level: the blue water footprint, the water stress index (WSI), aquatic acidification according to Impact 2002+, aquatic eutrophication according to Recipe, and aquatic ecotoxicity according to USEtox. The contribution analysis showed how raw materials, namely seed production is the main contributor to all indicators, although the extent of the contribution is indicator-dependent. The main contributors to water stress are sunflower oil, grown in Ukraine and Argentina, and palm oil from South-East Asia. In terms of water quality impacts, rapeseed cultivation appears as the main contributor, as it is the main ingredient in margarine, and contributes through fertilisers and energy use. In USEtox contributions



are mainly due to the use of pesticides in agriculture and heavy metals associated with energy use.

**WE266 Effects of Marine Microbial and Meiofaunal communities on CO<sub>2</sub> exposure** J. Lee; B. Gim, NeoEnBiz Co.. Carbon Capture and Storage (CCS) is one of the feasible options to mitigate atmospheric CO<sub>2</sub> increase. On the basis of detailed investigations to date, it has been widely recognized that the CCS in the marine is a promising option from the points of view of its capacity, economy and effectiveness. The impacts of the leaked CO<sub>2</sub> from sub-seabed storage site on benthic communities are of great concern because they play an important role in the biogeochemical cycles. A carbon dioxide exposure experiment (CO<sub>2</sub>-EXP) was conducted to study the responses of marine microbial and meiofaunal communities to different pH levels acidified by CO<sub>2</sub>. Seawater and sediment was delivered to proposed site for marine CO<sub>2</sub> storage in Korea. The exposure concentrations of CO<sub>2</sub> were 750 ppm for Control and 1500, 3000 and 6000 ppm for Treatments. Chemical and biological parameters were analyzed to evaluate the impacts of CO<sub>2</sub> on the benthos communities. The effects of CO<sub>2</sub> on benthos communities were also confirmed using molecular biological methods. To clarify the impact of CO<sub>2</sub> on marine ecosystem, CO<sub>2</sub>-EXP should be planned to take interactions of variety of marine benthos species into account. Meiobenthos and microbenthos play important role in benthic environments under where CO<sub>2</sub> is to be injected.

**WE267 Influence of the cell division cycle on effects and bioaccumulation of lead (II) in *Chlamydomonas reinhardtii*** A. Kroll, Environmental Toxicology; M. Burkard; R. Behra, Eawag / Department of Environmental Toxicology; L. Sigg, Eawag / Environmental Toxicology. Toxicity studies on algae are usually performed with non-synchronous cultures under continuous illumination while natural populations are synchronized by the day-night-cycle. It is largely unknown whether bioaccumulation and effects of stressors in algae vary with individual physiology leading to a selection towards certain traits. Thus, we studied effects of lead (Pb) in synchronized populations of the alga *Chlamydomonas reinhardtii* over 3 culture generations (S0-S2). Pb is a non-essential trace metal that elicits acute and chronic effects in many freshwater organisms. Our aim was to elucidate the influence of cell cycle on the bioaccumulation and effects of Pb as well as recovery from exposure. *C. reinhardtii* cultures were synchronized by light/dark cycles (14:10h). A fixed cell number was exposed to 10<sup>-6</sup> or 10<sup>-7</sup> M [Pb<sup>2+</sup>] and re-diluted to the starting cell number after 24h and 48h. Cells were exposed either (a) continuously over 3 generations or (b) at the beginning of the cell growth phase (G) or of the synthesis and cell division phase (S/M). Flow cytometry was used to determine growth rate, cell size, chlorophyll *a* (Chl *a*) fluorescence, and DNA content. DNA was stained with propidium iodide in fixed cells. Pb bioaccumulation was determined by ICP-MS. Cellular Pb distribution was visualized with the Pb-specific fluorescent dye LeadmiumGreen and confocal laser scanning microscopy. Continuous exposure to Pb inhibited growth and Chl *a* fluorescence up to 40% in S1 and 70% in S2, while no effects were detected in S0. S/M phase was prolonged and DNA content varied from control cells in S1 and S2. Short-term exposure in S0 during G or S/M phase also resulted in a prolonged S/M phase in S1 and S2 with specific DNA content patterns depending on the time of exposure. Growth rates decreased by 40% in S1 but almost recovered in S2. Bioaccumulation of Pb was independent of the phase algae were exposed in. Intracellular Pb was visualized by a Pb specific fluorescent stain which revealed that Pb is mostly located in granular structures in the cytosol. Conclusions: (a) DNA content revealed effects of Pb not reflected in growth rate or Chl *a* fluorescence. (b) *C. reinhardtii* takes up Pb at a certain rate and detoxifies it in cytosolic granules, both independent of the cell cycle state. (c) Although Pb bioaccumulation and localization were indistinguishable between treatments, Pb affects processes related to S/M phase dependent on the time of exposure.

**WE268 Time-variable exposure and effects of pesticides in an alpine catchment** A. Ippolito, University of Milano-Bicocca / Department of

Environmental and Earth Sciences DISAT; C. Bruno, Fondazione Edmund Mach - Research and Innovation Center; A. Di Guardo, University of Insubria / Department of Science and High Technology; S. Endrizzi, Fondazione Edmund Mach - Research and Innovation Center; R. Giacchini, University of Milano-Bicocca / Department of Environmental and Earth Sciences (DISAT); M. Morselli, University of Insubria / Department of Science and High Technology; S. Villa, University of Milano-Bicocca / Department of Environmental and Earth Sciences (DISAT); M. Vighi, University of Milano / Earth and Environmental Sciences. One of the most important source of uncertainty in assessing risk for plant protection products (PPP) regards the characterisation of the exposure, and the relative link with the effects. It is widely acknowledged that in site-specific risk assessment particular attention should be posed to time-variable exposure patterns. However, an evaluation of the induced effects is less commonly carried out with the sufficient time resolution, since response patterns at the biological community level may significantly vary along with the exposure. In this work the preliminary results of a study on a small alpine catchment (Novella River) of Northern Italy are reported. The watershed of Novella River is extensively used for the cultivation of apple orchards, and only narrow buffer zones are present between the river and the orchards; runoff events are then likely to cause significant loads of pesticides in the river. The final goal is to present a detailed site-specific risk assessment, with adequate temporal resolution, focusing on PPP loads due to surface runoff events. To do so, we adopt a combined strategy of modelling and event-triggered monitoring data to characterise the exposure, while effects on macrobenthic community were evaluated through regular monitoring, taking into account the relative abundance of vulnerable taxa (i.e. SPEAR<sup>pesticides</sup> index) compared to that of a reference system. Results highlight the presence of a risk for the invertebrate community, causing decreases of vulnerable taxa in terms of relative abundance. Nevertheless, the establishment of a clear causal relationship between time-variable exposure and effects was hardly detected. The ecological system proved to have good resilience, with a complete recovery achieved within one year. The fast resilience of the community can be probably linked to high input of organisms drifting from non-polluted areas that are present upstream.

**WE269 Seasonal Dynamics of Algae Communities Detected with Delayed Fluorescence Spectroscopy in a Small Stream in Central Germany** P. Ebke, MESOCOSM GmbH; f. breuer, Aquatic Ecotoxicology; L. Doeren, Institut für Gewässerschutz Mesocosm GmbH; E. Farrelly, Syngenta / Ecological Sciences. Algae play a key role in aquatic ecosystems. As primary producers they are at the bottom of the food web and take central functions in ecosystem processes. For lentic systems temporal and spatial distribution of algae is well understood but for lotic systems, especially small streams, very little is known. This study is a first attempt to provide data for effect based environmental modelling for small streams regarding algae and their seasonal and spatial dynamics. In this study the phytoplankton populations of a small stream in the centre part of Germany were monitored over a two year period in order to understand stream specific dynamics of algae. The catchment area of the stream is characterized by a large percentage of arable land and pasture. Algae sampling was carried out every second week and analysed with delayed fluorescence (DF) spectroscopy. The DF measurements provide data concerning the quantity of chlorophyll equivalents and their affiliation to four different colour classes of algae (Chlorophyta, Bacillariophyta, Cryptophyta and Cyanophyta). In order to validate the sampling technique, phytoplankton samples along a longitudinal and a cross transect were compared concerning potential differences. Additional taxonomic analyses were performed to gather information about the diversity of algae in streams. The phytoplankton distribution of the stream showed a distinct seasonal pattern, which was similar between the years. The population was dominated by Bacillariophyta and Chlorophyta, with significant population fluctuations measured throughout the year. The analysis along the transects revealed no significant difference in Chlorophyll equivalents and composition of algae colour classes. Thus it can be concluded that a representative depiction of the phytoplankton population is given by the

chosen method. Further analysis will be done concerning the influence of climatic events (e.g. rainfall and run-off) on the phytoplankton communities over the year.

**WE270 Simulated influence of the sulfonamide antibiotic sulfadiazine on nitrification and denitrification in soil.** S. Hotopp, Institute of Environmental Systems Research; J. Klasmeyer, University of Osnabrueck / Institute of Environmental Systems Research; A. Focks, Wageningen UR / Mathematics/Computer Science. Veterinary pharmaceuticals such as sulfadiazine used in animal husbandry enter the environment through manuring and have been shown to exert effects on nitrification processes in soil. Given the complex interdependencies in the nitrogen transformation network in soils, indirect effects of the antibiotic on soil functions are possible. Our objective was to gain insight into the impact of realistic sulfadiazine (SDZ) concentrations in soil on nitrification and denitrification processes using a modeling approach. The mathematical model describes the dynamics of nitrification, denitrification and nitrite respiration in soil in dependence of available nitrogen compounds (NH<sup>+</sup>, NO<sup>-</sup>, ...) explicitly considering the different activity of nitrifier and denitrifier biomass under varying carbon concentrations and differing oxygen conditions (aerobic, anaerobic). The model equations are defined in a way that aerobic conditions clearly favor nitrification processes, while they are inhibited under anaerobic conditions. Denitrification and nitrite respiration are inhibited by available oxygen and occur only under anaerobic conditions. The model assumes oxygen respiration of denitrifying microorganisms as long as possible switching to nitrate, nitrite, and nitrous oxide respiration once it is depleted. The growth of the nitrite respiring microorganisms is inhibited by oxygen. The inhibitory effect of SDZ on biomass is assumed to follow a logistic dose-response relationship. Inhibition is calculated in dependence of concentrations over time typically observed after amendment of SDZ-containing manure to soil. Different inhibition constants for nitrifying, denitrifying and nitrite respiring microorganisms have been assumed to investigate the relative influence of SDZ on the nitrifying biomass. Scenario simulations with the coupled nitrification-denitrification dynamics under intermediate oxygen conditions showed direct and indirect effects of SDZ. Direct effects of SDZ are visible for the nitrate dynamics, as under SDZ pressure nitrate is produced slower and to lower levels. The growth of denitrifying and nitrite respiring organisms is increased when SDZ is added because the competition for the carbon source with the SDZ-weakened nitrifying microorganisms is decreased.

**WE271 Impacts in bacterial richness in a phosphogypsum polluted area (Sfax, Tunisia)** S. Bouguerra, Laboratory of Water, Energy and Environment (3E), Engineering School of Sfax, University of Sfax / Department of Biology, Faculty of Sciences of the University of Porto; C.R. Marques, University of Aveiro / Department of Biology and CESAM, Center of Environmental and Marine Studies; N.C. Gomes, University of Aveiro / Department of Biology & CESAM, Center of Environmental and Marine Studies; J.P. Sousa, University of Coimbra / IMAR-CMA, Department of Zoology; D.E. Cleary, V.I. Nogueira, University of Aveiro / Department of Biology & CESAM, Center of Environmental and Marine Studies; O. Hentati, Engineering School of Sfax, University of Sfax / 1Laboratory of Water, Energy and Environment (3Es); S. Mendo, University of Aveiro / Department of Biology; F. Gocalves, University of Aveiro / Department of Biology & CESAM, Center of Environmental and Marine Studies; J. Roembke, ECT Oekotoxikologie GmbH; M. Ksibi, Engineering School of Sfax, University of Sfax / Laboratory of Water, Energy and Environment (3Es); A. Haddioui, University of Sultan Moulay Slimane / Faculty of Science and Techniques; R. Pereira, Department of Biology, Faculty of Sciences, University of Porto / CESAM, Center of Environmental and Marine Studies, University of Aveiro. Phosphate industry and phosphogypsum stockpiles in Sfax (Tunisia) have multiple adverse effects on soil health in the surrounding area. This area is a dynamic ecosystem with a great variability in terms of water level, nutrient availability, salinity and metals content, with different levels of ecological risk depending mainly from metal pollution of soil samples. It

was previously observed with DGGE and BIOLOG analysis that this area contains also diverse and distinct structural and functional microbial profiles. Studies of the influence of the site-specific contamination on the soil microbial community are critical for our understanding of the impacts in the ecosystem functioning and to delineate remediation strategies. A barcoded pyrosequencing approach for 16S rRNA gene was used to assess bacterial diversity. Here we present the potential functional association of bacterial guilds with the estimating risk degree of soil samples. The cluster analyses of the Ribosomal Database Project (RDB) pyrosequencing pipeline was used to estimate operational taxonomic unit (OTU's) richness and compare composition among risk degree and soil physicochemical characteristics. The species rarefaction curve of each sample revealed marked difference among samples and showed important OTU's in soils with high level of risk (0.6 - 0.85). In order to visualise variation in composition with distance we used a Principal coordinates analysis (PCO) using the Hellinger distance of OTU composition. The results proved that samples having a higher level of salinity were separated from other samples and this was confirmed by taxonomic analysis which proves a dominance of halophilic bacteria in these samples. The remaining samples were also grouped together showing several dominant OTU's in common. Further in-depth analyses are being conducted to perceive the role of soil contamination and of the dominant environmental variables at the site in structuring the soil microbial community.

**WE272 Effects of binary mixtures of cyanotoxins and anthropogenic contaminants on the growth rate of the freshwater algae *Chlorella vulgaris*** C. Pinheiro, Department of Biology; J. Passo, A. Campos, CIIMAR; V. Vasconcelos, Faculty of Sciences and CIIMAR Porto University; S. Loureiro, Universidade de Aveiro / Biology. Aquatic organisms may be exposed to several natural and anthropogenic contaminants such as cyanotoxins, metals and pesticides originating from harmful cyanobacterial blooms and human activities. However, the ecotoxicological risk of combined exposures to cyanotoxins and anthropogenic contaminants in aquatic environment is still little known. The purpose of the present work was to study the combined effects of the cyclic peptide microcystin-LR (MC-LR) and the alkaloid cylindrospermopsin (CYN) with the metal cadmium (Cd) and the s-triazine herbicide terbuthylazine (TBA) on the growth of the freshwater alga *Chlorella vulgaris*. The reference conceptual model for mixture evaluation based on the effect of individual compounds, independent action (IA), was applied to the growth data. Deviations from the IA model such as synergism/antagonism, dose-level (DL) and dose-ratio (DR) dependency were also assessed. Experiments were carried out in 96-well polystyrene microplate and the combined effects of the binary mixtures on growth of *C. vulgaris* were evaluated after 4 and 7 days of exposure. The results demonstrated that several patterns of response were obtained depending on the binary mixture. In the mixture of MC-LR and TBA, a DL deviation was observed for the two exposure periods indicating antagonism at low dose levels and synergism at high dose levels. In the MC-LR and Cd mixture, deviations for antagonism were found for a 4-day exposure period while a DL deviation was observed for a 7-day exposure period showing synergism at low dose levels and antagonism at high dose levels. A DR deviation was observed in the CYN and TBA mixture, with a pattern for antagonism when CYN was the dominant component, while deviations for antagonism were observed in the CYN and Cd mixture for a 4-day exposure period. Similar patterns of response were obtained for both mixtures involving CYN after 7 days of exposure, namely DL deviation indicating synergism at low dose levels and antagonism at high dose levels. Due to the diversity of patterns of response observed at the tested binary mixtures, this work shows the importance of evaluating the combined effects of cyanotoxins and anthropogenic contaminants in aquatic organisms.

**WE273 Does triclosan exposure alter sensitivity to ciprofloxacin and sulfamethoxazole in marine biofilm communities?** H. Johansson, University of Gothenburg / Department of Biological and Environmental Sciences; M.K. Eriksson, Chalmers Technical University

/ Department of Shipping and Marine Technology; A. Arrhenius, University of Gothenburg / Department of Biological and Environmental Sciences; H. Blanck, Goteborg University / Department of Biological and Environmental Sciences; V. Fihlman, University of Gothenburg / Department of Biological and Environmental Sciences; A. Grehn, University of Gothenburg / Department of Biological and Environmental Sciences; K. Sanli, T. Sircar, University of Gothenburg / Department of Biological and Environmental Sciences; T. Backhaus, University of Gothenburg / Dep. of Biological and Environmental Sciences. Antibacterial agents are commonly used substances that are found in the environment. As the usage pattern differs over the year, environmental concentrations are likely to fluctuate in dynamic complex mixtures. Traditionally, scenarios where compounds occur in mixtures or sequences have not been given much attention in risk assessment. Therefore an experiment was performed to investigate to what extent presence of the antibacterial substance triclosan change the tolerance of microbial communities toward the antibiotic substances sulfamethoxazole and ciprofloxacin. The experiment was performed using a microcosm system at Sven Lovén Centre – Kristineberg on the Swedish west coast. Sea water and toxicant solutions was continuously pumped into aquaria, resulting in concentrations of either 0nmol/L or 100nmol/L of triclosan. The microorganisms naturally occurring in the sea water were allowed to colonize circular glass discs in the aquaria over 10 days. Thereafter these discs were transferred to test vessels in climate chambers. Over 4 days the biofilms were exposed to Triclosan (5-10000nmol/L), Sulfamethoxazole (30-9000nmol/L) or Ciprofloxacin (30-3000nmol/L) in the semi-static SWIFT periphyton test. Toxicant-induced effects on algae and bacteria in the communities were thereafter analyzed through pigment and carbon substrate profiling. Pigment composition was used to estimate structural changes on the algal part of the communities. Bacterial efficiency to utilize different carbon sources were used as a measure on community function using the so called Biolog Ecoplates® methodology.

**WE274 Linking community tolerance and structure with low metallic contamination: a field study on biofilms sampled from 13 sites** L.C. Fechner, CEMAGREF / UR HBAN; C. Gourlay, Irstea / UR HBAN; M. Tusseau-Vuillemin, IFREMER; J.D. Lebrun, Irstea. The study was designed as an attempt to link biofilm metal tolerance, measured using a PICT (Pollution-Induced Community Tolerance) approach, and metallic contamination in a field survey covering 13 different sampling sites in the Seine river basin (north of France), an area characterized by diffuse, urban, multi-contamination. Cd and Zn tolerance of heterotrophic communities was assessed using a short-term toxicity test based on  $\beta$ -glucosidase activity. Metal tolerance levels varied between sites (5.9 and 8.0 factors were found between the lowest and highest tolerance levels for Cd and Zn respectively) but there was no obvious correlation between Cd/Zn tolerance and Cd/Zn contamination levels. Indeed, metallic contamination remained subtle when compared to environmental quality standards (for instance EQS of the Water Framework Directive) at all sampling sites. However, multivariate analysis of the data using Partial Least Squares Regression revealed that both metallic and environmental parameters were important variables explaining the variability of metal tolerance levels. Automated Ribosomal Intergenic Spacer Analysis (ARISA) was also performed on both heterotrophic and eukaryotic biofilm communities from the 13 sampling sites. Multivariate analysis of ARISA fingerprints (non metric multidimensional scaling) revealed that biofilms with similar tolerance levels had similar ARISA profiles. Those results confirm that river biofilms have a great potential as indicators of low, diffuse contamination levels of aquatic systems.

**WE275 Pesticide Interactions with N source and Tillage: Effects on soil biota and ecosystem services.** J. Jensen, Aarhus University DMU / Department of Bioscience; S.O. Petersen, Aarhus University / Department of Agroecology; L. Elsgaard, Aarhus University / Department of Agroecology; P.H. Krogh, Aarhus University / Department of Bioscience. Pesticide effects on soil biota must be interpreted in the context of the specific management practice, including

crop rotation, fertilization, tillage, and pest control. While pesticides can impose stress on soil ecosystems, the long-term significance of such effects in view of the known importance of other elements of agricultural management, such as tillage and manure/residue handling. The presentation will highlight results from a factorial design used to quantify effects and interactions of two contrasting pesticides in two field campaigns conducted during spring and fall 2012. The field experiment are organised by sampling in 48 subplots, i.e. 2 tillage treatments X 2 N fertilizer treatments X 3 pesticide treatments X 4 blocks. The endpoint of sampling are earthworms and microarthropod abundance and diversity as well as several microbial endpoints

**WE276 Metal(oid)s investigation in an active mine site: Panasqueira Mining District, Portugal** R. Pastorinho, Universidade de Aveiro / Biology; A.J. Nogueira, University of Aveiro / Department of Biology & CESAM; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; S. Takahashi, T. Itai, S. Tanabe, Ehime University. The *Panasqueira* Mining District, located in Central Portugal, has been in production, at an industrial scale, for over 100 years. Besides the immediate degradation of local aquatic ecosystems, potential impacts on human health exist. Agriculture - orchards, vegetable gardens, wine and spirits production, and cattle rearing - is a household tradition relying on surface waters. Simultaneously, these streams are tributaries to *Zêzere* River, the main water source to Lisbon and other municipalities. In order to assess impacts generated by the mining activities, nine metal(oid)s (Cr, Ni, Cu, Zn, As, Se, Ag, Cd, Hg, and Pb) were analyzed in water, sediment and biota from two creeks: *Casinhas* (receiving Acid Mine Drainage-AMD) and *Porsim* (not directly affected by AMD). Despite *Porsim* registering high values, metal variation between the two sites was very large, with variations according to each metal considered. For instance, Cd increased 1000 fold in water ( $0.1 - 103 \text{ ng.L}^{-1}$ ) and 10 fold ( $0.6 - 5.8 \text{ } \mu\text{g.g}^{-1}$ ) in sediment. The only biota common to the two sites - the bryophyte *Eurhynchium riparioides* - increased 4 fold ( $8 - 31 \text{ } \mu\text{g.g}^{-1}$ ) for the same metal. A decrease in dissolved metals, with a corresponding increase in sediment concentrations (and pH) is registered along *Casinhas* from the point source to the junction with *Zêzere*. Orchardgrass (*Dactylis glomerata*) and other non-perennial graminoids collected near the mouth of the stream denoted metal accumulation in aerial sections potentiating transference to grazing livestock. A simplified food chain collected from *Porsim* denoted biodilution with exception to Hg. Selenium/mercury Molar ratios calculation (after molar As:Se correction) demonstrated the possibility of Hg toxicity.

**WE277 Linking plant species- and arthropod population distributions using PRC- and co-correspondence analysis.** Aldershof, BioResearch Promotion; F.M. Bakker, Mitox Consultants. Terrestrial off-crop-studies designed to derive regulatory endpoints such as NOER/NOEAER etc. are performed in realistic spatially explicit structures such as grassy meadows. In these test sites the vegetation varies from site to site and from sometimes from plot to plot. Aldershof and Bakker (2012) developed a method to characterize grassland vegetation, which enabled: - a detailed characterization of the plant community prevailing in the experimental field - analysis of the homogeneity of plant distributions and abundance within different areas of the experimental field - analysis of relationships between plant species distributions and arthropod species distributions. Vegetation structure and in relation to that arthropod population distributions are expected to be less homogeneous in off-crop habitats than in in-field situations, which may result in reduced discriminatory power. The power of inferential tests of studies performed in heterogeneous habitats can be increased by taking plant species information as a co-variable in actual comparisons of arthropod community data in test item treatments with a control. Various analytical techniques, viz. conventional Principal Response Curves (PRC) analysis, adapted PRC analysis with plant species data included as co-variable and co-correspondence analysis are used to illustrate this with two case studies.

**WE278 Do community level ecotoxicological effects on arthropods differ between geographic or between regulatory zones?** S.

Aldershof, Bioresearch Promotion; F.M. Bakker, Mitox Consultants. In earlier work we analysed NTA field studies performed with the same active substance but in different cropping systems and different regions to provide a first insight into the importance of geographical gradient for the response of non-target arthropod communities to insecticide exposure. Using a variety of statistical techniques we investigated phenomena related to effects on community structure. PRC responses of North and South studies expressed by the first ordination axis were similar in all crop types. Both magnitude and duration of responses were similar, except the magnitude of the two studies performed in Spain were lower. It was concluded that treatment effects were similar in studies performed in N- and S-Europe. Only a slight trend was observed that more and longer lasting adverse effects were detected in studies performed in the North. However, effects were clearly lower in orchard studies performed in Spain and it was concluded that analysis of more Mediterranean studies was needed to examine whether this was related to geographical location or to other factors.

Here we present an extended meta-analysis that includes studies with additional active substances and additional locations. With this investigation we also assessed whether traits related to population response could be linked to latitude. For each study we calculated direct impact on populations sufficiently represented in the samples as the ratio of population densities in treatment and control groups at the first sampling moment after treatment. Duration of effects was categorized using the time to reach densities as in conditions of non-exposure. Analysis at the community level was performed using Principal Response Curves analyses (PRC). This analysis involved combination of PRC-axes. Additional multivariate techniques were used to investigate which autecological traits correlate to population level phenomena as initial impact and recovery potential. Furthermore, we investigated the prevalence of traits in communities at different latitudes.

**WE279 Does fly ash-aided phytostabilisation of Pb, Cd and Zn highly contaminated soils improve soil fauna communities?** C. Pernin, LGCgE EA; F. Grumiaux, S. Demuyne, S. Lemièrre, A. Lepretre, LGCgE - EA 4515. Soil contamination by trace elements is of major concern in Northern France. To contend with this problem, sustainable management of these highly polluted soils is crucial. Among remediation techniques, phytoremediation has attracted attention as a low-cost and ecologically sustainable alternative to physicochemical methods, applicable to large areas and accepted by local populations. A large scale pilot study in Northern France was set up around a former smelter 12 years ago. Before being planted with a tree mix, the study site was divided into three plots: a reference plot with no amendment (R), the second amended with silico-aluminous fly ashes (F1) and the third with sulfo-calcic fly ashes (F2). Although this aided phytostabilisation (trees+ashes) is efficacious in reducing metal bioavailability to plant species, no studies were performed to determine the effect of this remediation on soil fauna communities. Thus, the aim of our study was to determine whether fly ash-aided phytostabilisation of highly metal contaminated soils influence *in situ* earthworms, carabids and collembolans communities. In order to assess the effects of both phytostabilisation and aided-phytostabilisation, surrounding plots with similar metal levels but differing by use and cover were taken into account as control. Data obtained by trapping for carabids, chemical extraction and hand-sorting for earthworms and soil extraction for collembolans were analyzed through specific richness, abundance or density and specific functional traits. Results obtained were quite similar for the three. Indeed, the afforestation with a tree mix of these metal contaminated soils was shown to increase both abundance and species compared to soil with monospecific tree plots or unplanted plots. In addition, fly-ash aided-phytostabilisation appeared to be more benefit than single phytostabilisation. At last, among the two types of ashes used, sulfo-calcic ones appeared to be more powerful in restoring good soil conditions for soil fauna. Concerning collembolan, mainly epigeic species, less in contact with ashes and MTE, were found on the 3 plots, especially in F2. Concerning earthworm species in afforested plots, fly-ash amendments decrease density of the epigeic worms *Lumbricus castaneus*, more in contact with the soil/ashes mix, and increase density

of anecic worm *L. terrestris*. These results may explain the greater abundance of the large carnivorous carab species *Pterostichus madidus*.

**WE280 Seasonal variability of *Corbicula fluminea* populations health status from two estuaries of the NW Iberian Peninsula (Minho and Lima rivers)** C. Oliveira, ICBAS - Institute of Biomedical Sciences of Abel Salazar; P. Vilares, CIIMAR - Interdisciplinary Centre of Marine and Environmental Research / Laboratory of Ecotoxicology and Ecology; L. Guilhermino, Instituto de Ciências Biomédicas de Abel Salazar / Laboratory of Ecotoxicology. In the present study, the health status of two wild populations of *Corbicula fluminea*, an exotic invasive species in Europe, was compared to go further on the environmental factors influencing the invasive behaviour of this species. A seasonal monitoring study based on biomarkers and physico-chemical parameters of water and sediments was carried out in the freshwater tidal areas of the estuaries of Minho and Lima Rivers that have different levels of pollution. Biomarkers included condition indexes, and parameters indicative of neurotoxicity, alterations in the pathways of energy production, biotransformation and oxidative stress. Important seasonal variability was found in both populations for the most part of the biomarkers highlighting the importance of monitoring covering different seasons. Significant influence of both pollution and abiotic factors variation on the health condition was found within the Minho river estuary and among estuaries. The results suggest that pollution and other environmental factors have been influencing the invasive behaviour of *C. fluminea* in the studied estuaries.

**WE281 Two stressors and a community – Effects of hydrological disturbance and a toxicant on freshwater zooplankton** N.C. Stampfli, UFZ-Helmholtz Centre for Environmental Research / Dept. system ecotoxicology; S. Knillmann, UFZ-Helmholtz Centre for Environmental Research; M. Liess, UFZ Center for Environmental Research / Department of System Ecotoxicology; Y.A. Noskov, Institute of Systematics and Ecology of Animals; R.B. Schafer, University of Koblenz-Landau / Quantitative Landscape Ecology, Institute for Environmental Sciences; M.A. Beketov, UFZ - Helmholtz Centre for Environmental Research / Department of System Ecotoxicology. Climate change models predict an increase in the frequency and intensity of extreme fluctuations in water level in aquatic habitats. Therefore, it is necessary to understand the combined effects of hydrological fluctuations and toxicants on aquatic biological communities. We investigated the individual and combined effects of the insecticide esfenvalerate and recurring fluctuations in water level on zooplankton communities in a system of 55 outdoor pond microcosms. The communities were exposed to esfenvalerate contamination as a single pulse (at 0.03, 0.3, or 3 µg/L). Additionally they were exposed to gradual removal of water and its subsequent replacement over three cycles and monitored until 84 days after contamination. The results showed that the sensitivities of the community and its constituent populations to the toxicant were increased by the hydrological stress. Specifically, for both the community structure and abundance of *Daphnia* spp. the lowest-observed-effect concentrations (LOEC) were 0.03 and 0.3 µg/L for the series with fluctuating and constant water levels, respectively. Despite these differences in sensitivity, the interactive effects of the two stressors were found to be additive for both the community structure and the abundance of the most affected species. Presumably, it was not possible to detect synergism due to the strong individual effects of the water level fluctuations. Recovery times in the series exposed to the highest pesticide concentration were 64 and 55 days under fluctuating and constant water level regimes, respectively. Competition and water quality are suggested to be the major factors that underlie the observed effects of fluctuations in the water level. For the ecological risk assessment of toxicants, the present results suggest that (i) community sensitivity may vary substantially, depending on the environmental context, and (ii) this variability can be assessed experimentally to derive safety factors (coefficients used to avoid unexpected effects and define safe concentrations of toxicants). This contrasts with the current approach where such factors are usually

defined arbitrarily. For details see: Stampfli N.C., Knillmann S., Liess M., Noskov Yu.A., Schäfer R.B., Beketov M.A., 2012. Two stressors and a community – Effects of hydrological disturbance and a toxicant on freshwater zooplankton. *Aquatic Toxicology*, in press.

**WE282 Combining the effect of parasites and cadmium exposure in the estuarine isopod *Cyathura carinata*: a laboratory assay**

Martinez-Haro, IMARCA Marine and Environmental Research Centre / Department of Life Sciences; A.M. Goncalves, J.C. Marques, IMAR-Institute of Marine Research, Department of Life Sciences, University of Coimbra / Department of Life Sciences. Many aquatic crustaceans play an important role as intermediate hosts for parasites of fishes, birds and mammals. The ability of parasites to manipulate a large range of host traits (i.e. behaviour, morphology and/or physiology) have been widely recognized from the mid 20<sup>th</sup> century. These abilities allow to increase the probability of parasite transmission, and also the probability of parasite survival within a given host. For hosts, the presence of parasites has been suggested to have a similar cost to that invested in mechanism to combat predation. These costs could involve those related with antiparasite defences as well as those related to phenotypic or behaviour changes induced by parasite host manipulation. Although few studies have been done about the effect of parasites in polluted or stressed environments, pollution has been proposed as an uninvestigated source of variation of host manipulation by parasite. It seems that pollution could influence the host capacity to oppose manipulation as well as the manipulative capacities of parasites, but also reduced the lifespan of free-living infective stages of parasites and affect the transmission success. In this sense, pollution may be playing a trade-off in energy investment in parasitized hosts. This work aims to study the combined effect of parasitism and cadmium exposure of *Cyathura carinata*, one of the most abundant species in the Mondego estuary (Portugal). This isopod is the second intermediate host of different microphallid trematodes species, which decreases individuals' survival, growth and reproductive rates. Lethal effect (through the calculation of median lethal concentrations) and sublethal effect (through the calculation of half maximal effective concentration of feeding impairment) were studied under control conditions and a set of cadmium concentrations in individuals unparasitized, without internal cyst, and parasitized with different numbers of cysts. Our results show individuals parasitized with 2 or 3 cysts respond differently to stressors than those unparasitized, with one cyst or more than 3 cysts. The study of different environmental stressors as a more robust approach of the real environmental/biological conditions to which populations are exposed contributes to a further knowledge of organisms' responses, allowing to forecast how parasitism could be a benefit or harmful to populations.

**WE283 Effects of crowding on the reproductive behavior of *Daphnia magna*** I. Nerland, Ecotoxicology; H. Heiaas, A. Ailbe Macken, A. Lillicrap, Norwegian Institute for Water Research. The freshwater crustacean, *Daphnia magna* is widely used for assessing the toxicity of chemicals, in addition to being an indicator species for effects on freshwater environments. *D. magna* are easy to maintain, produce large numbers of offspring, reproduce parthenogenetically and are highly important for freshwater ecosystems. In nature *D. magna* produce females when conditions are favorable and males when less favorable (autumn and winter) reverting to sexual reproduction and overwintering ephippia spores. Sexual reproduction introduces genetic variability and maintaining a clonally reproducing culture of females is therefore necessary to have standardised and reproducible results. In order to measure the effect of chemicals, and not the effects of culture conditions, it is crucial that the cultures are kept in good condition prior to conducting reproduction tests. Ephippial egg production requires more energy than parthenogenesis and therefore, sexually reproducing cultures will produce significantly less offspring than a clonal culture. Number of offspring, male production and ephippia production are therefore good health indicators. Crowding has been suggested to be a stressor that affects these parameters. We assessed this by performing a 21 day reproduction study with a density gradient of *D. magna* individuals as well as different food concentrations. The endpoints were;

number of offspring produced per day per adult, male/female sex ratio, ephippial production, and body size of the adults at the end of the study. The results confirm that crowding is an important factor when food is limited, however when food is sufficient *D. magna* can be held in quite high densities. In addition, there were effects on the amount of offspring released and ephippial production as well as individual size differences at different food and culturing densities.

**WE285 Population age structure and recovery** F.M. Bakker, Mitox Consultants; J. Roig, MITOX Field Operations Southern Europe; F. Soudijn, University of Amsterdam / Institute for Biodiversity and Ecosystem Dynamics. With this combined modeling and empirical approach we relate recovery time to population age structure. Predatory mites serve as paradigm. Predatory mites provide for an important component of plant protection services in various agro-ecosystems. Depending on species, crop and region typical phenologies differ. Predatory mites overwinter as adult females and the winter generation dies in spring after oviposition has been resumed. Consequently, population development in spring starts from a cohort of eggs. Eggs of predatory mites are often placed in particular microhabitats (acarodomatia) and may be considered well protected to adverse conditions, including exposure to insecticide spray. In the course of the season population age structure stabilizes, but initially it changes rapidly. Using a modeling approach we show that in combination with the presence of a protected life stage (eggs) population age structure might be paramount to recovery time. Depending on population age structure 100% mortality of mobile stages may be followed by full recovery either within days or within months. We have tested this hypothesis experimentally by exposing predatory mite populations in an apple orchard and in a vineyard to insecticide sprays at regular intervals (10 days) throughout a growing season and by monitoring population development of each exposed cohort for 20 days. We found that for the same species in the same season, effects of insecticide sprays may indeed range from no effect after 3 days to a strong effect even after 20 days of recovery time. Such contrasting effects are not predictable from life history traits alone. We conclude that life history parameters have no predictive value for recovery time without knowing population age structure in combination with the vulnerability of the various life stages.

**WE286 The impact of nutrients and pollutants on biomagnification processes in food webs for the Yangtze and Daning River using the ecosystem model AQUATOX** U. Rings, T. Floehr, RWTH Aachen University / Institute for Environmental Research; A. Holbach, Karlsruhe Institute of Technology / Institute for Mineralogy and Geochemistry; H. Hollert, R. Ottermanns, M. Ross-Nickoll, A. Schaeffer, RWTH Aachen University / Institute for Environmental Research; W. Hu, Karlsruhe Institute of Technology / Institute for Mineralogy and Geochemistry; B. Scholz-Starke, RWTH Aachen University / Institute for Environmental Research. China passes through a rapid growth of its economy that is reflected by major infrastructural projects like the construction of the Three Gorges Dam at the Yangtze River. The dam and its newly created reservoir address the high demand for energy, prevent destructive flood events and safeguard the shipping in the region by elevating the water to a navigable level. The resulting impacts on the ecosystem in this area have been subjected by a joint research program on the water and sediment quality (Bergmann et al. 2011). We describe the impacts of nutrients and pollutants by comparing environmental scenarios adapted to the area of the inflowing Daning River into the Yangtze River by means of the complex model AQUATOX. The AQUATOX model is a general, mechanistic model that can be used to predict the fate, behavior and effects of various stressors such as toxic chemicals, nutrients or environmental variables in an environmental risk assessment context for aquatic ecosystems (Park et al. 2008). The chosen area is marked by a water level fluctuation zone of 30 meters and a special hydrological situation at the confluence of Daning and Yangtze River, forming a whirlpool with high residence times of the water in the Wushan Lake. For a realistic scenario, water flow data have been implemented into AQUATOX. The data have been generated by the one-dimensional hydrodynamic model HEC-RAS (US

Army Corps of Engineers 2001). Additionally, measurements of the structure and physico-chemical parameters of the water body have been provided by the MINIBAT project. Based on these data two food webs were modeled, one for a river scenario and one for a reservoir scenario. The food webs have been derived from the literature and confirmed by Chinese limnologists. As model substance the rice herbicide Propanil was chosen, including the two metabolites 3,4-dichloraniline and its dimer 3,3',4,4'-Tetrachlorobenzene (TCAB). 3,4-DCA is the major metabolite of Propanil. TCAB is lipophilic and can thus accumulate in the food web. Our simulations show that different stressors have clear impacts on the structure and the bioaccumulation patterns of the TGR-food webs. On the one hand there are high inflows of nutrients, which cause algal blooms, and on the other hand there are inflows of toxic chemicals. i.e. pesticides used in agriculture, which can accumulate in organisms and cause physiological disorder.

**WE287 Effects of potassium dichromate on predation behaviour, biomarkers and mortality of *Pomatoschistus microps* from two different estuaries** L.G. Luis, M. Oliveira, University of Porto, ICBAS - Institute of Biomedical Sciences of Abel Salazar; CIIMAR - Interdisciplinary Centre of Marine and Environmental Research / Laboratory of Ecotoxicology and Ecology; L. Guilhermino, Instituto de Ciências Biomédicas de Abel Salazar / Laboratory of Ecotoxicology. In this study, the effects of potassium dichromate on the predation behaviour and selected biomarkers of the common goby *Pomatoschistus microps* from wild populations of two estuaries of the NW Iberian Peninsula (Minho and Lima Rivers) with different levels of pollution were investigated. Juveniles (0+) were collected in the two estuaries and were maintained in laboratory conditions for acclimatization. Then, in independent bioassays, groups of fish from the two populations were exposed for 96h to several concentrations of the reference compound potassium dichromate (that is also an environmental contaminant of both estuaries) through artificial sea water, and control groups were exposed to artificial sea water only. Mortality was recorded at 24h intervals. At the end of the exposure period, a post-exposure feeding behaviour bioassay was carried out using *Artemia* as prey. After a resting period in the original test solutions, selected tissues/organs were collected and biomarkers of neurotoxicity, oxidative stress and damage, and biotransformation were determined. Potassium dichromate caused dose-dependent mortality, impairment of the predation behaviour and alterations in some biomarkers in fish from both populations, with some differences between them. The results are discussed in relation to historical exposure of the original populations to environmental contaminants present in the estuaries. Acknowledgements: This work done in the scope of the project *SIGNAL – Effects of pollution on estuarine ecological interactions zooplankton-zooplanktivorous fish in relation to climate changes* (PTDC/AAC-AMB/110331/2009), funded by the COMPETE programme (FCOMP-01-24-FEDER-01387601) and the Portuguese Foundation for the Science and Technology (FCT) through the OE component. M. Oliveira had a post-doc grant of FCT and L. G. Luís had a grant in the scope of the project.

**WE288 POPs fluxes from Tillet, a tributary of Lake Bourget, and their effects on aquatic biota through chemical, biological and ecotoxicological studies** C. BERNARD, ENTPELEHNAIPE / UMR CNRS 5023 LEHNA; E. NAFFRECHOUX, N. COTTIN, Université de Savoie / LCME (EA 1651); B.J. FERRARI, Irstea / MALY Laboratoire d'Ecotoxicologie; V. VERNEAUX, Université de Franche Comté / UMR 6249 UFC- CNRS Chrono-Environnement; S. CLAPPE, Université de Lyon ENTPE / UMR CNRS 5023 LEHNA; E. LYAUTEY, Université de Savoie INRA / UMR 042 CARRTEL; S. CACHERA, CISALB. The sediments of river Tillet, a tributary of Lake Bourget (France, Savoie) are contaminated by PCBs and PAHs. Restoration works are planned in 2013. The research program BIOSSED aims at assessing POPs fluxes to the lake littoral zone before and after restoration, as well as the contamination of - and the ecotoxicological effects on - the biota. The study is focused on three stations of the river, distributed on the section to be restored, and several lake stations (Tillet mouth and reference sites out of the plume of river Tillet). A first

campaign was launched in summer 2012. Chemical analyses (PCBs, PAHs) were carried out on water, suspended matters, in place sediments, and autochthonous fishes (PCBs only). The assessment of bioavailability and ambient toxicity was performed through exposure of caged organisms to suspended matters and sediments at each station and *in vitro* bioassays on the sediments. The macrobenthic biodiversity and the occurrence of morphological deformities among chironomids were studied. Microbial communities involved in the degradation of POPs and their spatio-temporal variability in the sediments and the epilithic biofilms were characterised using biomolecular approaches. The following first conclusions were drawn : high fluxes of PCBs and PAHs are observed towards the lake littoral zone, the impact on the quality of settling particles is noticeable for locations relatively far from the Tillet mouth, PCBs sorbed to the particles are bioavailable for aquatic invertebrates and fishes in river Tillet as well as in the lake, some ecotoxicological effects are noticeable during *in vitro* exposure of organisms, the macroinvertebrate communities of river Tillet are highly impaired by chemical and physical (substrate clogging) damages, PCBs and PAHs quantities lead to different bacterial community structures and abundances in the sediments and the epilithic biofilms. The study will continue after restoration of the river, including the assessment of the effects of an *in situ* treatment of sediments using granular activated carbon applied on a particular section of river Tillet in spring 2013. These effects will concern the fluxes of PCBs and PAHs towards the lake, the bioavailability of PCBs, the microbial communities and the recovery of biodiversity following restoration of river Tillet.

**WE289 Replicability of marine outdoor mesocosm to assess toxicity at ecosystem level** G. Park, Anyang University / Marine Biotechnology; S. Yoon, K. Kim, J. Shin, Anyang University. Mesocosms have been used in aquatic ecotoxicology over 30 years and were sometimes claimed to be essential tools, especially for regulatory purposes. The use of mesocosms refines the classical methods of ecotoxicological risk assessment because mesocosms provide conditions for a better understanding of environmentally relevant effects of chemicals. Ecological realism, representativity, and replicability of mesocosms are critical for evaluating their usefulness. Considering the objectives of most studies carried out in mesocosms, replicability is the most important point. Each natural ecosystem is unique because its structure and function mainly depend on local factors. Therefore, there is a conceptual opposition between realism and replicability when applied to mesocosms. Considering the objectives of most mesocosm studies, replicability should be preferred to realism. Replicability of outdoor marine closed mesocosm (MCM) was assessed to validate an ecotoxicological research at ecosystem level for one year. Chemical and biological variability was investigated between mesocosm sizes and light availability. Microcosm was designed by four size groups (200, 400, 600, 1000L) using cylindrical plastic water tanks (opaque or semitransparent) with five replicates. Each tank was filled with 5cm gravel layer on the bottom and another 5cm natural sediment layer with overlying natural seawater collected in Ganghwa tidal flat in west coast of Korea. Seawater in mesocosm was circulated vertically through outside vertical pipe by air injection from bottom to top. Water quality was measured daily (salinity, water temperature, pH and dissolved oxygen) and sediment quality by weekly. Phytoplankton, zooplankton and meiobenthic community were also analyzed by 10 day interval to identify the difference in community structure and variation within replicates. The results were analyzed by 2-way ANOVA. Important issues related to using mesocosms include: (1) the need to establish/maintain realistic communities; (2) need to establish replicate ecosystem; (3) variability that increases directly with realism; (4) problems associated with down scaling of physical systems; and (5) the cost of construction and operation. All of the above issues were discussed and evaluated through this preliminary microcosm experiment. This work was supported by Ministry of Land, Transport and Maritime Affairs of Korea (MLTM).

**WE290 INCLUSION OF TROPHIC NETWORK VARIABILITY IN REGULATORY ENVIRONMENTAL RISK ASSESSMENT**

N. Pucheux, M. Guiot, S. Andres, INERIS. Ecosystems can be defined as complex networks implying trophic relationships between species but also as functions to maintain ecosystem equilibrium. The basic paradigm in REACH or Biocides regulations or in the Water framework Directive assumed that the protection of the most sensitive species protect the structure and thus the function of the ecosystem. As the concept of the most sensitive does not take into account bioaccumulation mechanisms, this has to be completed (for bioaccumulative substances only) by the study of the predator compartment, which is assumed to reach the maximum concentration. Classical method described in the ECHA guidelines targets an undefined bird or mammal as predator feeding from their usual prey: fishes and/or worms. This unspecific scenario can be perceived as very simple and the exposure of the top predator in complex ecosystem may insufficiently be captured in case of biomagnification of chemical substances. In the plant protection product area, the assessment is slightly different when considering the initial hypotheses since the risk is basically evaluated independently for each trophic levels, and the assessment of secondary poisoning is linked to the crop under consideration: including type and age of crops to identify specific targets: bird, small mammalian, of various trophic regimen. Yet, industrial chemical following sludge applications (from Sewage Treatment Plant) ends up the very same agricultural field receiving plant protection products. The present work includes a case study comparing the two assessment frameworks for a selection of substances with different bioaccumulation potential. Nevertheless, the approaches used in these regulations remain overall linked to a generic environment. They do not consider the surrounding habitats and the impact of anthropic activities on landscape modeling. In other contexts, such exposure tools that could be adapted to specific or local trophic networks are developed, including a selection of target species, its habitats and diet. These models such as Terrasys™ or BERISP™ have the option to supply information on the top predators or modify the presence of specific habitat and thus the trophic network. We propose to confront the classical non specific exposure assessment with a much more elaborated scenario, including human intervention and management measures on ecosystem exposition in a fictional case study involving terrestrial compartment.

#### **WE291 A Spatial Eco-epidemiological Approach for Identifying and Ranking Potential Stressors in Aquatic Ecosystems of England and Wales**

K.E. Kapo, Waterborne Environmental Inc; M.J. Whelan, Cranfield University / Natural Resources; C.M. Holmes, Waterborne Environmental Inc; R. Williams, Centre for Ecology and Hydrology; V. Keller, A. Young, Centre for Ecology & Hydrology; D. De Zwart, RIVM / Centre for Sustainability, Environment and Health; L. Posthuma, RIVM / Lab. for Ecological Risk Assessment; S. Marshall, Unilever; G. Burton, University of Michigan / School of Natural Resources Environment; J. Murray-Bligh, Environment Agency. Spatial analysis of existing monitoring data and other environmental data resources provides a cost-effective means of enhancing more traditional approaches to ecological risk assessment. In this study, a probabilistic spatial data modeling approach employing weights-of-evidence and logistic regression was used to attempt to explain riverine macrofaunal community condition in terms of a range of potential stressors such as water chemistry, metal and pesticide toxicity, waste water effluent, flow regime and land use variables (as surrogates of diffuse pollution). Various monitoring and modeled data resources were used in a study area comprising 307 catchments in England and Wales. The analysis generated quantitative screening-level hypotheses related to ecological risk, based on relating spatial patterns of environmental data to delineate significant spatial associations between biological response (macrofaunal community condition) and environmental variables. Associations between biological response and environmental variables were compared between environmental stressors associated with land use, measured water chemistry and modeled wastewater effluent contributions, as well as modeled seasonal pesticide exposure and associated mixture toxicity. Analysis performed using seasonal data (spring and autumn) allowed for the delineation of other seasonal differences in stressor-response relationships, such as increased

influence of biological oxygen demand and nitrate in the autumn season. This study demonstrated the potential of this type of spatial eco-epidemiological analysis for screening-level assessments, which can be used for river basin management or to help identify further diagnostic assessment needs. It has also shown that improvements in model explanatory power can be achieved as new data sources and methods become available.

#### **WE293 Effects of glyphosate-based herbicides on the skin and oxygen uptake of bullfrog tadpoles**

M.J. Costa, Federal University of Sao Carlos UFSCar / Soroca / Department of Biology; R. Rissoli, Federal University of Sao Carlos (UFSCar) / Department of Physiology; E. Abdalla, UFSCar / Biology; R. Salla, F. Gamero, Federal University of Sao Carlos (UFSCar); F.T. Rantin, Federal University of Sao Carlos (UFSCar) / Department of Physiology; A.L. Kalinin, Universidade Federal de São Carlos / Department of Physiology. Glyphosate based herbicides are widely used in agriculture and fishery to control weeds, being found commonly in water bodies. Pre-metamorphic amphibians are very sensitive to environmental contamination due to the presence of a thin, permeable and widely vascularized integument, which acts as the main respiratory structure in many species. The aim of this study was to determinate if the sublethal exposition to glyphosate and two Roundup® formulations could cause alterations on the skin morphology and oxygen uptake of bullfrog tadpoles (*Lithobates catesbeianus*). Tadpoles were

randomly divided into 4 replicated experimental groups: Control (CT; n = 10), Glyphosate-exposed (GLI; n = 10), Roundup Original®-exposed (360 g/L glyphosate; RO; n = 10), and Roundup Transorb R®-exposed (480 g/L glyphosate; RTR; n = 10) at the sublethal concentration of 1 mg/L of acid-equivalent (a/e) of the commercial formulation for 96 h. The oxygen uptake ( $\dot{V}O_2$  - mL O<sub>2</sub> · kg<sup>-1</sup> · h<sup>-1</sup>) was measured by respirometry. After exposure, animals were euthanized and skin samples (n = 5) of each group were collected and prepared to conventional optic microscopy. Despite there was no differences in  $\dot{V}O_2$  values obtained for any treatment in normoxia in relation to the control, during hypoxia  $\dot{V}O_2$  of GLI increased, while  $\dot{V}O_2$  of RTR increased. In contrast, in RO-group  $\dot{V}O_2$  remained unaltered. These results are directly correlated to different levels of skin alteration observed to each xenobiotic. Altogether, the results demonstrate that GLY-group presented decreased values of when compared to CT, which may be related to changes caused by this compound in the skin of these animals, increasing diffusional distances and thereby reducing the O<sub>2</sub> uptake. RTR presented increased values of when compared to CT, suggesting the pronounced effect of the surfactant and/or of the “inert” components present on this parameter. The RTR group presented slight skin damage when compared to GLI and RO. The lack of difference between CT and RO oxygen uptake may suggest that an increase in metabolic rate was prevented by the thickening of the epidermal layer, making it difficult to increase the O<sub>2</sub> uptake and decreasing the . The negative effects of the exposure to an environmental relevant concentration of glyphosate or two of its commercial formulations (resulting in skin damage) become more severe when the animal faces an environmental hypoxia, which is very common in the species’ natural habitat.

#### **TH001 Effects of a mixture of herbicides on non-target organism**

M.A. Moura, Instituto Biológico / Laboratório da Ciência das Plantas Daninhas; M.J. Ranzani-Paiva, Instituto de Pesca, APTA, SAA; E.A. Oliveira, Embrapa - Brazilian Agricultural Research Corporation / Embrapa Environment; Z. Clemente, Embrapa Meio Ambiente / Embrapa Environment; J.H. Valim, C.M. Jonsson, Embrapa - Brazilian Agricultural Research Corporation / Embrapa Environment. Herbicides are widely used in agriculture and are known as a diffuse source of water pollution. The impact of herbicides use on non-target organisms is not well known although negative effects have already been observed. Sugarcane is the predominant culture of São Paulo State, Brazil, with around 6 million hectares of land cultivated. Acute and chronic toxicity tests with juvenile tilapia (*Oreochromis niloticus*) were undertaken to determine if the combination of herbicides commonly applied on sugarcane crops (ametryn, tebuthiuron and a commercial mixture of diuron and hexazinone) affect hematological and enzymatic parameters.



The LC<sub>50</sub> determined to fish exposed to herbicides was: ametryn 4.41 (3.63<sup>50.96h</sup> - 5.26) mg L<sup>-1</sup>; mixture of diuron+hexazinone 18.62 (14.79 - 24.45) mg L<sup>-1</sup> and tebuthiuron 223.04 (199 - 250) mg L<sup>-1</sup>. When fish were exposed to the mixture of these herbicides the LC<sub>50</sub> was 11.09 (9.25 - 15.85) mg L<sup>-1</sup>, showing antagonism between components of the mixture (Additive Index = -0.129). During the prolonged toxicity (14 days) test, fish were exposed to the following concentrations of herbicides mixture: 0 (control), 0.119 e 1.190 mg L<sup>-1</sup> a.i.; corresponding to 1/100 e 1/10 of LC<sub>50</sub>. Fish (Ls = 9.90 ± 0.125 cm; Wt = 32.35 ± 1.33 g) (n=36) were fed *ad libitum* and maintained in tanks of 115L with aeration, in a density around 3.8 g L<sup>-1</sup>, in room with temperature (26.0 ± 2 °C) and photoperiod (16:8h, light: dark) control. At seven and 14 days of exposition, fish were sacrificed (benzocaine solution 8%) and blood and liver were collected. Blood was drawn by caudal puncture, with the help of a needle and syringe previously heparinized. The blood specimens were assayed for: number of erythrocytes (RBC); hematocrit (Ht) and hemoglobin level (Hb). After these procedures, the following RBC indices were calculated: MCV (mean corpuscular volume) and MCHC (mean corpuscular hemoglobin concentration). Assays were performed to analyze enzymatic activities (GST, CAT, GPx and SOD) in fish liver. These samples were stored at - 80 °C until enzymatic analysis. With regard to hematological analyses, it did not show any significant differences between control and other treatments (P > 0.05). This study presented complementary tools available for pesticide risk assessment in tropical ecosystems, helping to establish maximum environmental concentrations of herbicides mixtures in aquatic ecosystems.

**TH002 Innovation of 3Rs in ecotoxicology** G.H. Panter, Brixham Environmental Lab; S.F. Owen, G.D. Readman, M.J. Winter, AstraZeneca. For over 10 years Brixham Environmental Laboratory has had a strong input into the development and validation of alternative assessment methodologies and the 3Rs (reduction, replacement and refinement), in ecotoxicology. In this poster we describe some of the key achievements and various initiatives that have been undertaken to address this issue. For example: REDUCTION: Fish full life-cycle tests are considered the “gold standard” for assessing chronic effects of chemicals and use hundreds of animals. However, by utilizing all available information (e.g. preclinical data), it is possible to focus testing on sensitive life-stages and endpoints. This strategy was employed to design a ‘targeted’ chronic study, for an endocrine disrupting chemical, that used fewer fish than the standard test<sup>1</sup>. REFINEMENT: There is pressure to improve environmental enrichment for fish, although, most current approaches are based on mammalian research. Consequently, we aimed to identify enrichments that are suitable for fish and compatible with prescriptive regulatory protocols<sup>2</sup>. In addition, little information is available on pain alleviation in fish and alternative procedures are being developed that are both scientifically and ethically preferable to existing approaches to anaesthesia and analgesia<sup>3</sup>. REPLACEMENT: Cell culture is a promising alternative to fish use, although questions remain around *in vivo* translation. The use of fish hepatocytes and spheroids have been assessed to understand metabolic functionality and to develop alternatives to *in vivo* screens for assessing bioconcentration<sup>4</sup>. We have also evaluated algae and crustaceans, as surrogates for fish genotoxicity assessment, with the data generated suggesting effective metabolic activation and measurable induction of DNA damage<sup>5</sup>. <sup>1</sup>Panter et al., 2012. *Aquatic Toxicol.* 114-115, 31-38. <sup>2</sup>Wilkes et al., 2012. *Appl Anim Behav Sci.* 139: 143-150. <sup>3</sup>Readman, 2012. Platform presentation at BJZH, UCL, 17<sup>th</sup> May. <sup>4</sup>Baron et al., 2012. *Ecotoxicology*, 21, 2419-2429. <sup>5</sup>David et al., 2012. *Chemosphere*, 88, 912-927.

**TH003 Consensus Model for Predicting the Acute Fish Toxicity of Organic Compounds** G. Schuurmann, R. Ebert, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry; N. Ost, Helmholtz centre for environmental research - UFZ; R. Kühne, Helmholtz Centre for Environmental Research UFZ / Department of Ecological Chemistry. According to REACH, in-te-grated testing strategies (ITS) are recommended to reduc-ing animal test-ing as far as possible. Since different non-animal methods may differ substantially in

their reliability, a way forward is to combine the out-comes of several methods through a consensus model approach. This holds in particular for in silico ITS components such as QSARs, struc-tural alert models, and read-across approaches, because all of them can be applied with-out undertaking laboratory experiments. A consensus model approach is presented for combining QSAR, structural alert and read-across predictions of the acute fish toxicity into a consensus outcome, taking into account both the ap-pli-ca-tion domains of the individual models and their variation in scope (quan-ti-ta-tive vs. categorical prediction). To this end, 692 96-h LC<sub>50</sub> values (acute toxicity to-ward fat-head minnow) have been taken<sup>50</sup> from lite-rature. The new approach is organised in a hierarchical manner, and yields either a quan-ti-tative or a categorical (narcosis level vs. excess toxicity) estimate for the acute fish to-xicity of the com-pound of interest. The latter is triggered by a computerized as-sess-ment of the individual model domains through appli-cation of the ACF (atom-cen-tered frag-ment) approach.<sup>1</sup> In addition to an extended version of the recently intro-duced quan-ti-ta-tive read-across,<sup>2</sup> the consensus model includes a classification of the compound of in-ter-est as exerting narcosis-level vs. ex-cess toxicity, and ECOSAR-model<sup>3</sup> predictions that in turn are based on class-spe-ci-fic linear regression equa-tions employing log K (octanol/water partition coefficient) as only molecular des-crip-tor. The fully automatized model ap-proach has been implemented in the OSIRIS<sup>4</sup> edition of the ChemProp software<sup>5</sup> that is available free of charge due to a license agreement. [1] Kühne R, Ebert R-U, Schüürmann G 2009. *J. Chem. Inf. Model.* 49: 2660-2669. [2] Schüürmann G, Ebert R-U, Kühne R 2011. *Environ. Sci. Technol.* 45: 4616-4622. [3] U.S. Environmental Protection Agency 2012. ECOSAR v.1.11. [4] OSIRIS. EU Project, contract no. GOCE-CT-2007-037017, 2007-2011. <http://www.osiris-reach.eu/>. Web-tool: <http://osiris.simppl.com/OSIRIS-ITS>. [5] ChemProp 5.2.8, 2012. UFZ Dep. Ecological Chemistry, <http://www.ufz.de/index.php?en=6738>.

**TH004 Evaluation of CADASTER QSAR models for aquatic toxicity of (benzo-)triazoles and prioritization by consensus** S. Cassani, University of Insubria / QSAR Research Unit / Department of Theoretical and Applied Sciences; S. Kovarich, University of Insubria; E. Papa, QSAR Res. Unit Environ. Chem/Dep. Structural Functional Biology; P. Roy, University of Insubria; M. Rahmberg; S. Nilsson, IVL Swedish Environmental Research Institute; U. Sahlin, School of Natural Sciences, Linnaeus University; N. Jeliakova, IdeaConsult Ltd; N. Kochev, O. Pukalov, University of Plodiv / Department of Analytical Chemistry and Computer Chemistry; I. Tetko, S. Brandmaier, Helmholtz Zentrum Munchen, German Research Center for Environmental Health; M.K. Durjava, Public Health Institute; B. Kolar, Public Health Institute Maribor; W.J. Peijnenburg, RIVM / Laboratory for Ecological Risk Assessment; P. Gramatica, University of Insubria / QSAR Res. Unit Environ. Chem. Ecotox./Dep.Structural & Functional Biology. The REACH regulation encourages the use of alternative *in vitro* and *in silico* methods in order to minimize animal testing, costs and time. Among these, Quantitative Structure-Activity Relationships (QSARs) represent a useful tool to predict unknown activities/properties for existing or even not yet synthesized chemicals. Triazoles and benzo-triazoles ((B)TAZs), which are a class of synthetic molecules studied in the European project FP7 CADASTER (CAse studies on the Development and Application of in-Silico Techniques for Environmental hazard and Risk assessment), have different industrial and pharmaceutical uses, i.e pesticides, antimycotic and antidepressants medicines, UV-light stabilizers for plastics, anti-corrosives, dishwashing additives, and components of liquid aircraft de-icing agents and for airport runways. (B)TAZs have been found distributed throughout the environment, mainly in water compartments, and are cause of concern due to their possible effects mainly on aquatic organisms. Several QSAR models for toxicity of triazoles and benzo-triazoles to algae (*Pseudokirchneriella subcapitata*), *Daphnia magna* and fish (*Onchorhynchus mykiss*), the three key species which are usually considered to perform risk assessment of chemicals in water, were developed by five partners in the CADASTER project. The models were developed by different methods (Ordinary Least Squares (OLS), Partial

Least Squares (PLS), Bayesian regularized regression and ASsociative Neural Network (ASNN)), using various molecular descriptors (calculated from DRAGON, PaDEL-Descriptor and QSPR-THESAURUS online platform), and different procedures for variable selection, validation and Applicability Domain inspection. The predictions of the developed models, as well as those obtained in a consensus approach by averaging the data predicted from each model, were compared with the results of experimental tests that were performed within the CADASTER partners. The individual and the consensus models are able to correctly predict the chemicals tested in CADASTER according to their toxicity class, confirming the utility of the QSAR approach. The models were also applied to predict the aquatic toxicity for over 300 (B)TAZs without experimental data, many of which are included in the REACH pre-registration list. This work highlights the importance of QSAR for screening and prioritization of untested chemicals, in order to reduce and focus the experimental tests.

#### **TH005 Read-across Approach to Predict Acute Daphnid Toxicity**

**R. Kühne**, R. Ebert, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry; P.C. von der Ohe, N. Ulrich, W. Brack, Helmholtz centre for environmental research - UFZ / Department of Effect-Directed Analysis; G. Schuurmann, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry. REACH requires the assessment of daphnid toxicity for industrial chemicals with market volumes of at least 1 t/a. A hierarchical read-across approach allows predicting the 48-h LC<sub>50</sub> toward the *Daphnia magna* from experimental data [1]. Dependent on the similarity of the actual compound to the training set chemicals, the method yields quantitative, qualitative, or screening level qualitative predictions. An identification of the applicability domain is integrated. The model alternatively exploits the octanol/water partition coefficient  $K_{ow}$  or an Abraham type LSER equation to calculate the baseline toxicity. Since the latter is the more promising approach for further refinements, the current study focuses on the LSER version. The reliability of this version with regard to particular compound classes is examined, and the dependence of the performance on different sources of LSER parameters is investigated. The presented model can be applied in a fully automated manner in the OSIRIS edition of the software system ChemProp [2]. It is publicly available free of charge due to a bilateral license agreement. [1] Kühne R, Ebert R-U, von der Ohe PC, Ulrich N, Brack W, Schuurmann G 2013. Read-across prediction of the acute toxicity of organic compounds toward the water flea *Daphnia magna*. *Mol. Inf.*, accepted November 2012. [2] Chemical Proper-ties Estimation Software System (ChemProp) 5.2.8, 2012. UFZ Department of Ecological Chemistry, <http://www.ufz.de/index.php?en=6738>.

#### **TH006 Why the NOEC is not a NOEC but a function of power:**

**Finding alternatives** **M. Wang**, WSC Scientific GmbH / Dept. Eface & Modelling. In toxicity tests and in ecotoxicological risk assessments the NOEC is one standard measure to address the risk of pesticides or other substances. It is used routinely for example to address the chronic risk in aquatic organisms or in birds and mammals. However, the concept of the NOEC has been discussed for several decades and criticism has often been raised, since the NOEC depends very much on the selected dose or exposure levels and the sample size. In the present study we systematically conducted a series of statistical evaluations to determine the NOEC based on varying datasets. We show that the NOEC is not a constant, reliable value, but that it depends very much on the study design and sample size. We show that the estimation of alternative methods, including ECx calculations and benchmark doses result in much more robust and reliable estimates.

#### **TH007 Applying mechanistic TKTD models to evaluate acute-toxicity tests: An approach to phase out the NOECs and regression-based LCx**

**B. Daniels**, RWTH Aachen; D. Kulkarni, RWTH Aachen University / Institute for Environmental Research; H. Ratte, Institute for Environmental Research; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research. One of the persisting teething troubles in ecotoxicology is the determination of an appropriate

statistical evaluation for acute-toxicity tests. 15 years after the OECD recommendation against using the NOEC and related concepts to evaluate toxicity tests (OECD 1998), these practices have still not been banned in ecotoxicology. Nowadays, even the recommended alternative approach, the application of two-parameter regression models to calculate effect levels (LCx) is increasingly being criticized. The detection of LCx values is still a poor and imprecise description of toxicity, mainly because it does not include a statement about the time course of a toxic effect. Considering the required cost, effort and number of test organisms, the information obtained from an acute-toxicity test is quite negligible. Therefore, the objective of our study was to focus on how to improve the quality of the output of acute-toxicity tests. We analyzed and compared the frequently used two-parameter regression models Probit, Logit and Weibull. Alternatively, the toxicokinetic-toxicodynamic (TKTD) model GUTS (General Unified Threshold Model of Survival) was used to evaluate acute-toxicity tests by simulating survival with various exposure concentrations at a defined time point. The study was based on laboratory experiments with the cyclopoid copepod *Mesocyclops leuckarti* exposed to triphenyltin (TPT). The GUTS can contribute to a more powerful test evaluation by interpreting mortality as a response of a dynamic system (the organism). This mechanistic approach provides appropriate simulation results with a good description of the dose-response relationships. Furthermore, due to the integrated threshold of survival in the model, the TKTD model generates comparatively small but meaningful confidence intervals at low concentration ranges, which are of particular importance in the environmental risk assessment of chemicals. In addition, GUTS also provides the opportunity to differentiate quantitatively between toxicokinetic and toxicodynamic influences on toxicity and to extrapolate to more realistic exposure scenarios. Therefore, in comparison to NOECs and regression-based LCx, GUTS results are not purely specific and descriptive anymore. The study demonstrates another potential use of TKTD models and presents an alternative to the recommended regression models to assess toxicity tests.

#### **TH008 Are fish early lifestage data protective of reproductive effects in fish?**

**J.R. Wheeler**, S.K. Maynard, Syngenta Ltd; B. Marks, M. Gross, WCA Environment; C. Merrington, WCA Environment Limited. Currently, fish chronic toxicity is assessed by using the fish early lifestage test (OECD 210). Fish full lifecycle tests are only required when toxicity, bioaccumulation and persistence triggers are met, or when there is suspicion of potential endocrine disrupting properties. This regulatory approach is based on a relationship between the results of fish early lifestage (ELS) and full lifecycle (FLC) studies that is now 35 years old (McKim 1977). However, a recent EFSA opinion has challenged whether more FLC testing should be undertaken. In addition, a project proposal has been submitted to the OECD to develop a fish partial lifestage test. The aim of this OECD test proposal is to develop a fish reproduction test that could be used to detect potential reproductive toxicants. Both fish full lifecycle and partial lifecycle tests are animal intensive, technically challenging and resource intensive (cost and capacity) and should only be undertaken if there is clear evidence that they are necessary when coming to a regulatory decision. We constructed a database of matching fish ELS and FLC endpoints for plant protection product active substances from EU Draft Registration Reports and the US-EPA OPP Pesticide Ecotoxicity Database. Analysis of this database shows a strong correlation ( $r > 0.9$ ,  $p < 0.001$ ) between ELS and FLC responses, within approximately two-fold when the most sensitive ELS NOEC is compared with the most sensitive FLC NOEC. This shows that fish ELS tests are generally sufficient to estimate the long term toxicity of plant protection product active substances to fish, and indicates that current testing strategies and guidelines are fit for purpose.

#### **TH009 A Multi-Criteria Decision Analysis methodology for quantitatively scoring the relevance and reliability of ecotoxicological data**

P. Isigonis; A. Zabeo, Venice Research Consortium; P. Ciffroy, EDF / LNHE Department - I 894; E. Semenzin, CVR Consorzio Venezia Ricerche / C/o Vegapark; A. Critto, University

Ca Foscari of Venice; **A. Marcomini**, University of Venice / Department of Environmental Sciences. The current European legislation (e.g. REACH regulation) and advances in the field of ecotoxicology strongly suggest the reduction, refinement or replacement of animal tests, as well as exploring the advances in the current methods for the evaluation of toxicity. As a result there is clear necessity for researching new ways of applying the existing methods, as well as identifying ways to make more efficient use of the existing ecotoxicological datasets. In this scope and context, the poster presents a new methodology which intends to assist the optimization of existing methods, by providing a tool for assessing the relevance and reliability of ecotoxicological data for the definition of Species Sensitivity Distributions (SSDs), within the framework of ecological risk assessments (ERA). In order to estimate a single aggregated reliability score for a given ecotoxicological datum, a 'Multi-Criteria Decision Analysis (MCDA)-based' Weight of Evidence (WoE) methodology has been developed, including a hierarchical structure of 57 evaluation criteria, which was created based on the review of the state of the art frameworks for the assessment of ecotoxicological data. The methodology is able to integrate different types of inputs and incorporates the use of Fuzzy Logic operators for handling the inherent uncertainty, which appears in the form of unreported information as well as possible lack of knowledge of the experts. A panel of scientific experts on ecotoxicology was involved throughout the development of the methodology for identifying, if any, the relations between criteria and evaluating the hierarchical structure to be used in the aggregation process. The methodology is planned to be used and tested in case studies as part of the evaluation and follow up process of the research project "AMORE" (Multi-Criteria Analysis for the Development of a Decision Support Tool for the prevention of Environmental Risks), which is funded by the National French Research Academy (ANR).

**TH010 Reactive oxygen species and cytotoxicity in rainbow trout hepatocytes: effects of medium and incubation time** **M. Yazdani**, University of Oslo / Dept of Biology; R.E. Paulsen, T. Gjoen, University of Oslo / Schools of Pharmacy; K. Hylland, University of Oslo / Department of Biology. Primary hepatocyte cultures from fish are widely used to clarify mechanisms of toxicity. There is however a need to understand how exposure conditions may affect results, particularly for measures of oxidative stress. The aim of this study was to evaluate the influence of (1) exposure medium and (2) culture age, on measurements of ROS in primary hepatocytes from rainbow trout (*Oncorhynchus mykiss*). Intracellular ROS was quantified using two fluorescent probes, DHR 123 and CM-H DCFDA. In addition, cytotoxicity was assessed using CFDA-AM and the concentration of cellular glutathione quantified by mBCl. Responses of hepatocytes to Cu (1 -100 µM) and H<sub>2</sub>O<sub>2</sub> (1 - 125 µM) exposure was quantified in Leibovitz' medium (L-15) or Tris-buffered saline (TBS). Effects of culture age was investigated for hepatocytes maintained for 1, 2 and 4 days in L-15, then exposed to Cu or H<sub>2</sub>O<sub>2</sub> in L-15 or TBS. There was a significantly higher response in hepatocytes exposed to Cu in L-15 compared to TBS using CM-H DCFDA, but not using DHR 123. No such difference was observed for H<sub>2</sub>O<sub>2</sub> exposure. The age of the primary hepatocyte culture significantly affected the development of ROS measured by DHR 123, but not by CM-H DCFDA. The exposures did not cause increased cytotoxicity or change the glutathione content in cells for any exposure. The results from this study shows that both the choice of exposure medium and the age of a primary hepatocyte culture may affect responses to stressors. Key words: reactive oxygen species; rainbow trout (*Oncorhynchus mykiss*); primary hepatocyte culture; incubation time

**TH011 Ability of a gill, a liver and an intestinal cell line of rainbow trout (*Oncorhynchus mykiss*) to take up and biotransform benzo(a)pyrene** **F. Weiss**, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; K. Tanneberger, Ecosens AG / Environmental Toxicology; D.J. Madureira, N. Bramaz, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; K. Schirmer, Eawag. One determinant of bioaccumulation of hazardous chemicals is the ability of animal tissues

or organs to transform and excrete such chemicals on exposure. Whole organism studies, while providing an overall measure of chemical uptake and elimination, cannot provide information on tissue-specific biotransformation. Therefore this study aims to investigate the uptake and elimination of benzo(a)pyrene (BaP) in rainbow trout cell lines established from gill (RTgill-W1), liver (RTL-W1) and intestine (RTgutGC). BaP was selected as model chemical because it is a well-known representative of the ubiquitous polycyclic aromatic hydrocarbon group and because its biotransformation, at least in mammalian liver cells, is rather well studied. A major pathway of BaP biotransformation is thought to be via monooxygenation by cytochrome Cyp1A, which is induced by binding of BaP to the aryl hydrocarbon receptor. Indeed, BaP induced Cyp1A enzyme activity, which we measured as 7-ethoxy-resorufin-*O*-deethylase activity, in liver and intestinal but not in gill cells. Time-resolved analysis of radiolabelled BaP revealed fast and comparable uptake by all three cell lines with maximal cell-internal concentrations being recorded after 6 h of exposure. All three cell lines were found to eliminate almost all of the BaP between 48 to 72 h after addition of BaP; this was also true for the gill cell line despite its lack of BaP-inducible Cyp1A. Assessment of cell viability over 11 days revealed little to no impact on exposure up to 10 µM BaP in the RTgill-W1 cell lines whereas severe cell damage was observed in the RTL-W1 and RTgutGC cells as early as 48 h of exposure, indicating different mechanisms underlying biotransformation and/or excretion. Elucidation of the potentially different mechanisms of BaP biotransformation is currently under investigation through the use of inhibitors of Cyp1A induction and activity as well as of the activity of other oxygenase enzymes. Results obtained thus far show that (i) multiple biotransformation pathways are active in all cell lines and that (ii) RTgill-W1 cells respond with reduced biotransformation in particular by inhibition of cyclooxygenase/lipoxygenase. Our study indicates that tissues other than liver might play important roles in the overall potential of fish to eliminate hazardous chemicals, thereby diminishing bioaccumulation. As for BaP, lack of Cyp1A inducibility does not necessarily result in an inability of cells to biotransform BaP.

**TH012 Dose Metric Considerations in In Vitro Assays to Improve Quantitative In Vitro-In Vivo Dose Extrapolations** **F.A. Groothuis**, Institute for Risk Assessment Sciences; M. Heringa, Utrecht University / Institute for Risk Assessment Sciences; J.L. Hermens, Utrecht University; B. Blaauw, Utrecht University / Institute for Risk Assessment Sciences; N. Kramer, Utrecht University. Ethical, legal as well as scientific drivers, like the visionary strategy for toxicity testing in the 21<sup>st</sup> century published by the US National Research Council, have accelerated the development of non-animal based methods for toxicological risk assessment in recent years. Unfortunately, uncertainties remain surrounding the power of alternative methods, such as *in vitro* cell assays, to predict *in vivo* dose-response relationships, which impedes their use in regulatory toxicology. An important issue is the lack of a well-defined dose metric for use in concentration-effect relationships obtained from *in vitro* cell assays. Traditionally, the nominal concentration has been used to define *in vitro* concentration-effect relationships. However, studies have shown that several processes like evaporation, degradation or e.g. serum binding may reduce the bioavailable and biologically effective dose of test chemicals in *in vitro* assays to levels far below their nominal concentration. The aim of this work was to review the scientific literature considering the bioavailability of test chemicals in *in vitro* assays and to develop a set of guidelines to help the researcher in choosing the most appropriate dose metrics for different chemicals and with varying *in vitro* assay setups. Major dose metrics available for both animal and *in vitro* toxicity assays, 'loss' pathways of chemicals in *in vitro* assays, as well as chemical properties affecting their bioavailability *in vitro* were considered. Moreover, techniques to determine and model the various dose metrics in *in vitro* assays were also assessed. The findings indicate that chemical properties, such as the potency, lipophilicity, volatility, (metabolic) stability, ionization state, and (the reversibility of) the mechanism of action, as well as assay setup, such as serum levels, microtiter plate plastic, headspace, pH, the metabolic competency of

cells, cell concentration, and exposure time, may affect the bioavailability and the measured effect concentration of a test chemical. These findings have been incorporated into a flow chart that should help the reader in choosing the most appropriate dose metric for an *in vitro* assay.

**TH013 Xenobiotic uptake by fish: an *in vitro* gill model to better understand bioaccumulation in ecotoxicology** L.C. Stott, Kings College London / Division of Diabetes and Nutritional Sciences; S. Schnell, C. Hogstrand, King's College, London / Division of Diabetes and Nutritional Sciences; S.F. Owen, AstraZeneca Safety / Health and Environment; N. Bury, King's College, London / Division of Diabetes and Nutritional Sciences. The gills of fish are the principle site of Xenobiotic uptake and elimination as they are constantly and continuously bathed in water. Both passive and active transport of Xenobiotics across the gill are likely principle drivers in determining the rate of uptake of these waterborne compounds, and accumulation is the result of a net influx. We are investigating whether an *in vitro* gill cell system can accurately predict the uptake of Xenobiotics, using pharmaceuticals as an example, from aquatic environments and into fish. If successful then there is potential for it to be used as a means of Replacing, Refining and Reducing (3Rs) the large numbers of fish used in regulatory bio-accumulation tests. There are currently over 30,000 compounds that require assessment for their bio-accumulative properties as part of the EU Registration, Evaluation, Authorisation & Restriction of Chemicals (REACH). Typically these *in vivo* assessments require large numbers of fish and extensive studies. An effective *in vitro* screen is required to help prioritise these compounds, and a gill model that can determine uptake from water and efflux rates in a manner similar to live fish would have the potential to replace many fish studies and promote the principles of 3Rs. Studies show that our Rainbow trout primary gill cell culture grown on porous membranes form a polarised epithelium that can tolerate water exposure, producing a transepithelial resistance (TER) of >20,000 ohms/cm<sup>2</sup> with low permeability. Initial results presented here show the uptake and efflux of seven pharmaceuticals across the gill membrane *in vitro*. These rates appear to support findings from *in vivo* data, suggesting that this gill model may work as an appropriate substitute for these types of compound. We have also conducted passive and active transport assays in the absence and presence of specific membrane based transporter inhibitors to further characterise the movement of these pharmaceuticals. We believe that this model could further be used for assessing parameters that influence uptake of compounds (water chemistry, unstirred layers, microclimate pH and apical and basolateral transporters) and help to develop Physiologically Based Structural Activity Relationship models (PBSARs) that could better predict uptake of Xenobiotics in fish. Keywords: 3Rs, bioaccumulation, gills, PBSARs

**TH014 The gill cell line RTgill-W1: Differentiation into epithelial cells using epidermal growth factor and cortisol -back to the roots?** S. Schnell; L. Stott, N. Bury, C. Hogstrand, King's College London. The demand of developing alternative methods for *in vivo* experimentation is very high. A great number of ecotoxicity tests using live fish has to be carried out *inter alia* due to the REACH legislation, which came into force in 2007. One model for the replacement of fish in ecotoxicity testing we are currently using is the Fish *In-vitro* Gill Cell System (FIGCS), a primary gill cell culture method, which allows the exposure of gill cells to waterborne chemicals, mimicking one of the most critical sites of toxicity of fish, the gill. Going a step further, we are trying to minimize or replace the use of fish completely by utilising the rainbow trout (*Oncorhynchus mykiss*) gill cell line RTgill-W1. An ability to form a polarised epithelium capable of tolerating water when applied to the apical surface would be a desirable characteristic of the RTgill-W1 if it were to be used as a replacement for whole fish assays. To assess the ability of RTgill-W1 cells to grow on inserts a number of different culture scenarios have been performed: control (cultured in L15); addition of cortisol (CORT) 100ng/ml + epithelial growth factor (EGF) (50 or 100 ng/ml); culture in flasks with CORT and/or EGF for up to 6 months prior to seeding on inserts at different densities; culture

in flask with or without CORT + EGF for differing periods (up to 1 month) seeded on inserts and with primary gill cells seeded on top. For each scenario the trans epithelial electrical resistance (TEER) was monitored in symmetrical conditions (L15:L15) for up to 2 weeks or asymmetrical conditions (water:L15) for up to 48 hrs. The epithelial permeability of RTgill-W1 cells grown on inserts was monitored using <sup>14</sup>C-mannitol and ability to form cellular tight junction formation assessed by staining with ZO-1 antibody. The addition of CORT and EGF to RTgill-W1 cells during culture on flasks prior to seeding on inserts increased TEER for 160 ? to between 240 – 270 ?. The clearer results were obtained when primary gill cells were combined with the RTgill-W1 cells with TEER rising to above 1000? on the addition of water to the apical surface. Additional growth factors that are predicted to stimulate tight junction formation in mammalian cells are currently being investigated. The scenario of combining cell line with primary cells would allow reducing the number of fish in animal experimentation and therefore supporting the 3R's principles.

**TH015 In-vitro biotransformation of hydrophobic chemicals by fish liver enzyme fractions: a dosing approach using molecular carriers for substrate delivery** D. Gilbert, NERI Aarhus University / Department of Environmental Sciences; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; P.N. Fitzsimmons, U.S. Environmental Protection Agency / Mid-Continent Ecology Division; J.W. Nichols, US EPA / Mid-Continent Ecology Division. The intrinsic metabolism of chemicals is a crucial parameter in the evaluation of their toxicity and bioaccumulation potential. Recently, *in-vitro* assays using fish liver S9 fractions have been developed as an alternative to whole animal tests. However, it is challenging to provide well-defined substrate supply when working with hydrophobic chemicals because of sorptive losses and their low aqueous solubility which often requires the use of a cosolvent for dosing. To avoid the use of cosolvent and to ensure sufficiently fast delivery of hydrophobic organic chemicals, several molecular carriers were considered, and the non-ionic surfactant Tween 80 (polysorbate) and ?-hydroxypropyl cyclodextrin chosen for the *in-vitro* biotransformation assay with S9 fractions from rainbow trout (*Oncorhynchus mykiss*). Seven PAHs covering a wide range in hydrophobicities were selected as model compounds, and their intrinsic clearance rates were determined. Biotransformation rates in carrier treatments were similar to or lower than those in treatments with solvent-spiking. These differences may have been due to differences in the free chemical fraction in solution. Ongoing binding studies will provide a basis for normalizing measured activities to unbound chemical concentrations. Interestingly, both solvent and carrier treatments showed a consistent trend of increasing biotransformation rate with increasing hydrophobicity of the chemical.

**TH016 Assessing the dioxin-like activity of sediments and soils – Bioassays as an alternative for chemical analysis?** K. Eichbaum, T. Seiler, RTWH Aachen University / Institute for Environmental Research; K. Winkens, M. Brinkmann, RWTH Aachen University / Institute for Environmental Research; G. Umlauf, European Commission - Joint Research Centre / Institute for Environment and Sustainability; B. Stachel, Ministry of Urban Development and Environment Hamburg / Dep. of Water Management; G. Reifferscheid, S. Buchinger, German Federal Institute of Hydrology; H. Hollert, RWTH Aachen University / Institute for Environmental Research. The aim of this study was to determine, if *in vitro* bioassays are useful screening tools to investigate the dioxin-like potential of sediment and soil samples, polluted by polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F), dioxin-like polychlorinated biphenyls (DL-PCB) and polycyclic aromatic hydrocarbons (PAH). The bioassays were compared regarding their sensitivity, reproducibility as well as their possibility to possess an alternative to chemical investigations. The bioassays included the EROD assay with the permanent fish cell line RTL-W1, the Micro EROD with the rat hepatoma cell line H4IIE, as well as the H4IIE-Luc assay with the eponymous cell line, stably transfected with a firefly luciferase reporter gene. Bioassay results were expressed as Bio-TEQs (toxicity equivalent quotients) and compared

with their respective Chem-TEQs, which accounted for the sum of PCDD/F and DL-PCB TEFs (toxicity equivalent factors) and bioassay specific TEFs for PAHs. All bioassays showed sediments and soils to exhibit dioxin-like activity. The Micro EROD Bio-TEQs were generally lower than those of the remaining assays. The H4IIE Luc and the Micro EROD both classified the same sample to exhibit the highest toxicity. Whilst the EROD was the most sensitive assay, the Micro EROD offered the highest reproducibility with a remarkable coefficient of variation of 15 % (EROD = 35 %; H4IIE Luc = 40 %). The H4IIE Luc showed the highest comparability with chemical analysis with Chem-TEQs accounting for up to 51 % of the respective Bio-TEQs (EROD = 18 %). On the contrary, Chem-TEQs in the Micro EROD assay were even higher (up to 26-fold) than their respective Bio-TEQs. In summary, all of the three bioassays are suitable for a biochemical investigation of the dioxin-like potential of soil and sediment samples. Due to their various advantages, a combination of three assays is advisable and can be seen as an addition to chemical investigation.

**TH017 Exploring A New Screening Technique For Assessing the Bioconcentration Potential of Pharmaceuticals in Fish** L.A. Constantine, Pfizer Inc / Pharmacokinetics, Dynamics and Metabolism. Understanding whether an active pharmaceutical ingredient is likely to be taken up from the aquatic environment by fish and bioaccumulate has triggered the need to conduct bioconcentration studies in fish. As per EMEA Guidelines, a bioconcentration study is required in the PBT assessment for pharmaceuticals having a  $\log K_{ow} > 4.5$  and in the Phase II, Tier A assessment for pharmaceuticals having  $\log K_{ow}$  values  $> 3$ . The standard OECD Guideline 305 includes an exposure (uptake) phase followed by a post-exposure (depuration) phase typically equal to  $\frac{1}{2}$  the duration of the uptake phase. Based on the sampling schedule outlined in the guideline,  $>100$  fish per study may be required to determine a kinetic BCF value. Based on the resources required to conduct the full bioconcentration study and the potential for fish to metabolize xenobiotics, alternatives to the current bioconcentration tests as a first tier have been explored and employed for the last several years. An abbreviated protocol including up to 7 days of uptake and 7 days of depuration has been conducted in the zebrafish with several pharmaceuticals. The BCF values obtained from these studies will be compared to the full OECD 305 BCF values to support use of the 14 day study as a screening tool to assess the bioconcentration potential in fish.

**TH018 The *Xenopus* oocyte, a reliable tool in ecotoxicology?** S. Lemiere, University of Lille / Nord; G. Marchand, PRES Univ Lille Nord de France / Univ Lille1; S. Demuyne, PRES Univ Lille Nord de France / LGCgE Univ Lille1; M. Marin, PRES Univ Lille Nord de France / Laboratoire de regulation des signaux de division EA 4479, IFR 147, Université Lille1, Sciences et Technologies. Among lab animal models, amphibians take up a privileged position, in particular *Xenopus laevis*. This well-known frog, coming from South Africa, has proved – and is still – useful in many lines of researches. In one hand, in cellular biology, *Xenopus laevis* oocytes are considered as one of the best models to investigate cellular events, such as meiosis and mitosis, and associated pathways. Indeed, *Xenopus* oocytes are numerous, and giant vertebrate cells easily amenable to biochemical, cytological, histological and electrophysiological studies. In addition, these cells are naturally arrested at prophase I of meiosis, that is to say they are synchronous and stimulation with progesterone *in vitro* initiates a non-transcriptional signaling pathway, which presents a paradigm for the G2/M transition of the cell cycle. In other hand, as many amphibians are bathed in both water and air, their skin but also their egg-laying and larvae are constantly in contact and exposed to the substances in their surroundings. In this context, amphibians, *a fortiori* *Xenopus laevis*, could represent good indicators of habitat diversity, biological diversity and local stressors on the environment. These are the reasons why *Xenopus laevis* larvae and adults are suitable tools in environmental toxicology and developmental sciences (FETAX assay for instance). Nevertheless, in these areas of research, few studies were conducted using *Xenopus* oocytes. The aim of this work was to highlight the effect of various environmental pollutants on *Xenopus* oocytes. Drug

(ibuprofen), metal (cadmium) and pesticide (atrazine) were chosen. We first focused on cell survival with phenotypical (morphology, pigmentation) and electrophysiological (resting potential) methods. Then, maturation was assessed *in vitro*, again with morphological (migration and breakdown of the germinal vesicle, also called GVBD or white spot appearance) and electrophysiological (recordings of calcium activated-chloride currents associated to maturation process) aspects. Taken together, our results showed that oocytes could represent an important – the first – link in amphibian's ecotoxicology studies.

**TH019 Toxicity testing with Zebrafish (*Danio rerio*)- Malformations as an indicator for pollutant effects** S. Schubert, N. Keddig, Institute of Fisheries Ecology; U. Kammann, Thünen Institute / Institute of Fisheries Ecology; R. Hanel, Thünen Institute / Institute of Fisheries Ecology. The zebrafish (*Danio rerio*) is a common model in toxicological studies. Among others, zebrafish eggs were frequently used in Zebrafish Embryo Toxicity Tests to determine dose-response relationships for chemicals mentioned as relevant hazardous substances by OSPAR and HELCOM Committees. Besides death events, many contaminants cause varying malformations with different ecological effects for the developing fish. Indeed only death events are used, so far, to determine threshold values to “conserve the good environmental state” as postulated by the Descriptor 8, European Marine Strategy Framework Directive (EU MSFD). Generally, malformations may result in a reduction of fitness of the individuals affected, which subsequently may result in e.g. a loss of offspring. This may have an effect on the whole fish population. Therefore, in our opinion, also the appearance of malformation needs to be documented and used for the determination of threshold values for the EU MSFD. Hence, toxicological studies were determined with eggs of the zebrafish. Chemicals were applied via waterborne exposure (Zebrafish Embryo Toxicity Test) and microinjection to determine effect (malformations) and lethal concentrations (death events) of certain chemicals.

**TH020 The Application of Passive Dosing for Determining Chronic Early Lifestage Toxicity of an Aromatic Hydrocarbon Mixture to Embryo-Larval Zebrafish** J. Butler, T. Parkerton, D. Letinski, G.E. Bragin, ExxonMobil Biomedical Sciences Inc; M. Lampi, ExxonMobil Petroleum Chemical; A.D. Redman, Exxon Mobil Biomedical Sciences; K.R. Cooper, RUTGERS UNIVERSITY. The target lipid model (TLM) has been used to derive water quality objectives (HC-5's) that are intended to be protective of chronic effects posed by hydrocarbons. However, reliable experimental early life stage chronic toxicity data for fish are limited and further data are needed to confirm model predictions. Recent efforts are underway to develop a zebrafish embryo toxicity test guideline to reduce, refine and replace the use of vertebrates in animal testing. An adaptation of this method which includes embryo lethal and sub-lethal developmental endpoints after a 4 d exposure as well as larval survival and growth endpoints during a subsequent 26-day test period is described. Zebrafish embryo/larval lifestages were exposed to a defined mixture of 10 Aromatic Hydrocarbons (AHs) with a Kow range of ca. 4 - 7.5 at 3 treatment levels with different concentrations that corresponded to a toxic unit range above and below unity assuming concentration addition. To deliver well controlled exposure concentrations, a passive dosing system consisting of silicone o-rings was employed. Results indicated that effects on 30-day embryo-larval survival and growth endpoints were similar to embryo effects after 4-day. Coagulation, heart-beat and immobilization were lethal endpoints observed. Observed sub-lethal effects included pericardial edema, yolk sac edema and tail curvature. Acute and chronic effects were consistent with the assumption of additive toxicity. Furthermore, results confirm that TLM-derived HC-5 was protective of these early life stage effects to zebrafish for this AH mixture. Further work is needed to determine the relative sensitivity of embryo versus larval chronic endpoints for additional hydrocarbons and fish species.

**TH021 Observations enrich the zebrafish embryo toxicity test** H. R. Zodeczko; A. Swierkot, P. Fochtman, Institute of Industrial Organic Chemistry Branch Pszczyna. Zebrafish embryo toxicity test (draft

OECD Guideline on FET Test, July 2012) is designed to determine acute toxicity based on apical observations of egg coagulation, lack of somite formation, lack of tail detachment and lack of heartbeat. Other observations are possible and can bring insights into developmental retardation, morphology changes and possible mode of action. Embryos were exposed since 3 hpf for 96 hrs in semi-static system with daily renewals of solutions spiked with 4,6-dinitro-o-cresol, triethyleneglycol, prochloraz, propiconazole, 3,4-dichloroaniline, carbamazepine, isoproturon and 3,5-dichlorophenol. Delay in hatching and yolk-sac edema followed by irregular heartbeat were observed among survivors exposed to 4,6-dinitro-o-cresol. Triethyleneglycol caused deformations of head followed by coagulation. Yolk-sac edema was common to survivors exposed to prochloraz, propiconazole and 3,4-dichloroaniline. Pericardium deformations in embryos exposed to carbamazepine indicate cardiotoxicity. Although not required by the draft OECD Guideline, observations of physiology and morphology changes during exposure of embryos may be performed and used in designing further detailed studies on toxicity.

**TH022 Back to basics - Zebrafish Embryo Toxicity Test comes under scrutiny** N. Keddig, Institute of Fisheries Ecology; W. Wosniok, University of Bremen / Institute of Statistics; S. Schubert, Institute of Fisheries Ecology; U. Kammann, Thünen Institute / Institute of Fisheries Ecology; M. Haarich, Thünen Institute / Institute of Fisheries Ecology. The *Zebrafish Embryo Toxicity Test* is widely employed in accordance with the *OECD* or *DIN* guidelines. For a long time it was usual to use 10 eggs per concentration and for the negative control. Since 2011 20 eggs are recommended for every concentration and negative control by the *OECD*. Additionally a lethality of 10% was accepted for a long time until the *OECD* reduced it to 5%. But do these recommendations guarantee a reasonably safe conclusion? What kind and size of error must be expected when following this recommendation? In which way are the number of eggs and tolerated lethality related? When considering two different concentrations (like negative control and a concentration > 0) then the associated observed effects come from two distributions of random values around the concentration-specific mean values. Usually these two distributions show a certain overlap, and an effect in the overlapping area can be assigned to the wrong distribution. A type I error (or error of the first kind) occurs, if an effect is assigned to a concentration effect although it is an effect of the negative control. On the other hand we have a type II error (or error of the second kind) if a value of a concentration effect will assigned to the negative control. How likely these errors are depends on the distance between the two distributions, on the position of the negative control and most notably on the quantity of the eggs tested. It is possible and necessary to calculate the probabilities of these two types of errors and on that basis an adequate amount of eggs to get reliable results. These amounts make sure that type I and type II errors both occur with probabilities below specified (small) limits. The research project “MERIT-MSFD” uses results of the *Zebrafish Embryo Toxicity Test* to calculate reliable threshold values for environmentally relevant contaminants. These thresholds indicate concentrations with defined small effects in the marine environment. They are also useful for assessing the condition of the marine environment, as required by Descriptor 8 of the *European Marine Strategy Framework Directive*.

**TH023 Fish Mucosa Test, a non destructive, non invasive method to detect xeno-estrogens via vitellogenin induction** B. Allner, GOBIO GmbH; M. Hennies, Tecomedical Development; G. Hoerstgen-Schwark, L. Luehmann, University of Goettingen; P. Stahl Schmidt-Allner, Gobio-GmbH. Serological determination of the induction of yolk protein vitellogenin is the central endpoint in endocrine disruptor testing. Due to the instability of yolk protein in blood and the difficulties of taking suitable blood samples from small sized laboratory test fish species the standardisation of test procedures is still insufficient. For the large group of percid indicator species no reliable method for the detection of exogenous induction of vitellogenin is available. We developed an animal friendly method to detect vitellogenin in epidermal mucosa. The method fulfils the requirements of OECD Guidelines to measure

estrogenic activity in fish (OECD 229, OECD 230) and can be combined with standard OECD procedures of chemical testing (OECD 215). Mucosa samples are taken with flocked swabs with braking point and transferred to caps containing sample dilution buffer. Competitive EIA determination can be conducted without further sample preparation. The test principle is applicable to cyprinids (zebrafish, carp, goldfish, golden ide) perciform species (cichlid fish, perch) and salmonids. Sensitivity of the percid assay for natural estrogens seems to be lower than in blood and body burden assays. Mucosal vitellogenin concentration rises significantly after 5 days of exposure of juvenile *Oreochromis niloticus* all male strains to 10 ng estradiol. Water born synthetic estrogens and estrogenic active industrial chemicals cause responses in the same range as assays with focus on activation of the hepatic estradiol receptor.

**TH024 Evaluation of treated waste waters by combination of estrogenic in vitro assay and in vivo zebrafish embryo test** A. Viteckova Wunschova, Masaryk University / Research Centre for Toxic Compounds in the Environment (RECETOX); B. Jarosova, Masaryk University / Faculty of Science, RECETOX; B. Gawlik, EC Joint Research Centre (JRC); K. Hilscherova, Masaryk University Faculty of Science RECETOX / Faculty of Science, RECETOX; L. Blaha, Masaryk University, Faculty of Science, RECETOX / Research Centre for Toxic Compounds in the Environment (RECETOX). *In vitro* bioassays can serve as a rapid and relatively cheap screening method to estimate total activity or toxicity of all compounds that act through the same mode of action (e.g. binding to estrogenic receptor). However, their relevance towards *in vivo* situations and use for environmental risk assessment remains arguable. In contrary, highly relevant *in vivo* assays require more time, labour and money and their use is also questionable with respect to the animal welfare issues. Therefore alternative tests such as embryo assays complemented with molecular methods have been recently developed. In this study, estrogenic reporter gene assay and *in vivo* 5-day embryo zebrafish test employing molecular endpoints were used to study potential risks and negative effects of estrogenic chemicals present in effluents of European waste water treatment plants (WWTP). First, 75 European WWTP effluents were screened by MVLN *in vitro* reporter gene bioassay, and the bioassay showed significant estrogenic activity at 27 out of 75 samples (E2 equivalent ranging 0.53 to 17.9 ng/L). In the next step, effects of samples were studied with zebrafish embryos using molecular biology tools as qPCR for expression of genes responding to estrogenic activity (aromatase B, vitellogenin 1). Detailed outcomes of *in vitro* vs. *in vivo* comparisons will be presented. Key words: zebrafish embryo test, *in vitro*, Waste Water Treatment Plant effluents, qPCR

**TH025 Optimizing laboratory toxicity test methods for bumblebees (*Bombus terrestris* L.)** N. Hanewald, M. Bergtold, F. Sattler, N. Mumbach, D. Petrik-Steisslinger, S. Niederdrenk, A. Ufer, BASF SE, Ecotoxicology. EFSA has evaluated the current risk assessment of pesticides for bees resulting in an EFSA Scientific Opinion and Draft Guidance document. Gaps in the current tiered testing systems concerning non-*Apis* bees have been identified. Thus, the development of reliable, scientifically sound and efficient test methods for non-*Apis* bees has gained a high level of importance. Several methods for toxicity testing with bumblebees (*Bombus terrestris*) have been described (van der Steen et al., 1996). The principles for testing bumblebees in the laboratory are based on established methods for honey bee testing. A major challenge during the oral toxicity test is to ensure that the individual animals feed enough sugar solution to ensure exposure. Given that bumblebees do not practice trophallaxis, large variation in the amount of consumed test item occurs. Moreover bumblebees often have difficulties to locate the food source. This leads to a limited number of individuals that can be used in the observation period. An improved test method has therefore been developed. Two set ups for testing oral toxicity to bumblebees were compared. Handling efficiency, feeding behavior and percentage of consumed sucrose solution were used to evaluate the results. Starved bumblebees, taken from young colonies, were put individually in hatching cages at 25°C and 70% RH, in the dark and exposed to a range of test concentrations in 50% sucrose solution.

The improved method is based on individual housing of bumblebees (Nicot honeybee queen rearing system). In these hatching cages bumblebees are able to locate the test solution much easier because the shape of the cage leads them directly to the food source. 48 h after adding the bumblebees to the hatching cages, the feeding behavior was investigated for 24 h. The efficiency was compared by assessing the amount of consumed sucrose solution. In parallel the test method by van der Steen et al. (1996) was applied. A significantly higher food uptake was observed with the improved method. Therefore the likelihood to achieve the quality standard of required food consumption in all animals is significantly higher. At the same time variability of consumption was reduced. Our results suggest that the proposed adaptations to the method of van der Steen et al. will lead to increased reliability and reproducibility of toxicity test results for bumblebees.

#### **TH026 Biomarkers of pollution in tigerfish (*Hydrocynus vittatus*) from two different localities**

**J.H. van Vuren**, University of Johannesburg / Department of Zoology, Auckland Park Kingsway Campus; V. Wepener, University of Johannesburg / Zoology; N. Smit, North West University / School for Biological Sciences; W. Vlok, W. Vlok, I. Wagenaar, R. Tate, University of Johannesburg / Zoology. Tigerfish has a high ecological, economical and social value to South Africans. They are lost through habitat changes caused by water extraction, pollution and obstructions like impoundments and weirs. Tigerfish is therefore a protected species in South Africa and the potential adverse effects of different contaminants present in the aquatic environment through industrial, agricultural and urban activities have to be determined to predict maximum tolerable levels for conservation management. Carefully selected biomarkers are recognised indicators of the levels of pollution that could compromise the health of aquatic organisms. Effects of known concentrations of contaminants on fish physiology and organ function in specific impacted areas can be determined in laboratory and field studies. Tigerfish is a sensitive species and therefore difficult to keep under controlled laboratory conditions for exposure experiments. Effects of water quality on tigerfish can currently be determined from field samples only. Recently completed studies in game reserves included carefully selected biomarkers of pollution to establish the value thereof in the assessment of toxicant effects on tigerfish physiology. Biomarker values obtained for tigerfish sampled in the Luvuvhu and Olifants Rivers in the Kruger National Park, and the Phongolo River, KwaZulu-Natal provided information on the effects of pollutants e.g. DDE and DDD in the sampling localities on this species' physiology. Two groups of biomarkers were analysed. The first group is biomarkers of exposure that consists of Metallothioneins (MT) that responds to metal exposure, acetylcholine esterase (AChE) an indicator of organophosphate and carbamate pesticide exposure and ethoxyresorufin-O-deethylase (EROD) a biomarker of organochlorine. Malondialdehyde (MDA) and protein carbonyl (PC) used to determine antioxidant stress responses as well as cellular energy allocation (CEA) to show changes in energy reserves are biomarkers of effect. Standard techniques were employed for all analyses. The results obtained on biomarker responses are discussed in view of the importance of the findings to assist in the assessment of tigerfish health. The validity of biomarker responses in toxicity testing as a component of water quality monitoring programmes is considered. Responses of biomarkers in fish sampled from the two localities are interpreted to identify similarity in reaction to the contaminants present.

#### **TH027 Hsp70 Expression in Fish Gill Used as an Ecotoxicological Biomarker: A Rapid, Sensitive, Reproducible and Simple Molecular Approach**

**H.H. Agus**, S. Sumer, Hacettepe University / Department of Biology; B. Erkmen, Aksaray University / Department of Biology; F. Erkoc, Gazi University / Department of Biology Education. Global concern in exposure to estrogens, metals, organics, plasticizers, pesticides, industrial wastes, nanomaterials and persistent organic pollutants have led to increased efforts and strengthening of biomonitoring programs, prevention of environmental impacts and taking legislative action. Rapid, reliable, sensitive methods/approaches to aquatic monitoring of threatened freshwater and marine habitats and

biota have recently been recruited with emphasis on molecular biology and organismal toxicology methodologies. The gills are the major target of toxicants and have immediate contact with environment; being the first organ to react to environmental factors. Alterations in some stress proteins and morphology of gills in response to xenobiotic contamination are considered as primary indicators in environmental monitoring. Determination of highly conserved Hsp70 expression levels, inducible stress protein present in all cells in all life forms, as a specific biomarker of in vivo ecotoxicological stress is studied in the present study. Carp (*Cyprinus carpio*) obtained from State Hydraulic Works General Directorate (DSI, Turkey) were exposed to sub-lethal concentration of di-n-butyl phthalate (DBP; a highly abundant organic pollutant) (1 mg/L) for 24 and 96 h. A control group was included. After sacrifice and macroscopic examination, necropsies were performed and gills were evaluated histopathologically. Gill tissue RNA quantification for inducible Hsp70 stress protein levels were carried out using a two step real-time RT-PCR with a special primer pair designed for carp hsp70 mRNA. Results showed a dramatic increase in hsp70 mRNA levels up-regulating transcription in response to DBP exposure. Within 24 h transcription levels increased upto 2-10 fold when compared to controls. Histopathological findings support up-regulated stress protein transcription results as histological alterations. Real-time PCR methodology presently serves as a rapid, simple and sensitive alternative to conventional analytical and biochemical methods, including the time consuming western blotting. In PCR, total tissue RNA can be prepared and results available only within 1-1.5 hours. In addition, sensitivity is very high. In conclusion Hsp70 up-regulation data can be used as a rapid, sensitive and a good ecotoxicology biomarker and all members of this protein family can further be used for standardization of pollution biomarkers.

#### **TH028 Effect of pesticide exposure on neopterin levels in blood plasma of common carp**

**P. Marsalek**, I. Mikulikova, H. Modra, Z. Svobodova, L. Zelnickova, University of Veterinary and Pharmaceutical Sciences Brno / Department of Veterinary Public Health and Toxicology. Neopterin belongs to a group of unconjugated pterins, derived from guanosine triphosphate (GTP) by guanosine triphosphate cyclohydrolase. Neopterin is synthesized mainly by activated monocytes/macrophages following stimulation by interferon-gamma cytokine (IFN- $\gamma$ ), which is released by NK cells and T-lymphocytes. Because the neopterin level in body fluid reflects immune responses in vivo, neopterin is a useful biomarker of the activation of the cellular immune system. A number of studies have found that environmental pollutants, such as pesticides, significantly affect fish immune systems. The aim of the present study was to investigate the effect of subchronic exposure to prochloraz and propiconazole pesticides on blood plasma concentrations of neopterin in common carp. A total of 60 specimens of juvenile common carp were placed into 4 groups and exposed to prochloraz at concentrations of 0, 50, 150 and 380  $\mu$ g/L and another 60 specimens of juvenile common carp were placed into 4 groups and exposed to propiconazole at concentrations of 0, 70, 180 and 580  $\mu$ g/L. The total length of exposure was 28 days. Neopterin concentrations were determined by reverse phase high performance liquid chromatography with fluorescence detection. The concentrations of prochloraz and propiconazole in the glass aquaria were tested by gas chromatography coupled with ion trap tandem mass spectrometry. Our results identified different trends relating to the neopterin. Plasma neopterin concentrations in common carp were higher in groups exposed to prochloraz compared with the control group. On the other hand, plasma neopterin concentrations in carp from the control group and groups exposed to 70 and 180  $\mu$ g/L of propiconazole were higher compared with the group exposed to 580  $\mu$ g/L of propiconazole. The results of our study showed that subchronic exposure to prochloraz and propiconazole pesticides influenced plasma neopterin concentrations. However, more research involving other xenobiotics and measurements of pro-inflammatory cytokines is needed to fully characterize the effect of xenobiotics on neopterin concentrations in fish.

#### **TH029 The dioRAMA joint project – Methods for the detection of**



**dioxin-like chemicals in risk assessment and management of contaminated sediments** K. Eichbaum, RTWH Aachen University / Institute for Environmental Research; M. Brinkmann, RWTH Aachen University / Institute for Environmental Research; S. Buchinger, German Federal Institute of Hydrology (BfG); M. Hecker, University of Saskatchewan / Toxicology Centre & School of the Environment and Sustainability; M. Engwall, University of Örebro / Man-Technology-Environment Research Centre (MTM), Department of Natural Science; G. Reifferscheid, German Federal Institute of Hydrology (BfG); H. Hollert, RWTH Aachen University / Institute for Environmental Research. Given the complex interactions of re-suspension processes and bioavailability of sediment-bound pollutants, such as dioxin-like chemicals (DLCs), there is need for a better integrative understanding of the cause-effect-relationship of these pollutants. Currently, most studies investigating the role of DLCs focus either on (a) chemical analyses or (b) on the characterization of sediment extracts via *in vitro* bioassays. These bioassays are rapid and inexpensive and the resulting biological 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (Bio-TEQs) are a simple and integrative measure of contamination with DLCs. However, since a range of different compounds with varying physical and chemical properties is described with just one value, it is not possible to forecast the distribution of TEQs between environmental compartments, e.g. in bioaccumulation studies. The fact that, e.g. bioavailability, uptake and elimination rates *in vivo* are not considered, limits the use of bioassays and resulting Bio-TEQs in risk assessment and management of contaminated sediments. To determine to which extent TEQs determined *in vitro* can be predictive of possible adverse effects *in vivo*, the joint project dioRAMA of the Institute for Environmental Research of RWTH Aachen University and the Department Biochemistry / Ecotoxicology of the German Federal Institute of Hydrology (BfG) was established. Common roach (*Rutilus rutilus*) will be exposed to selected DLC-contaminated sediments from the two major German rivers Rhine and Elbe. Exposure will be performed in an exposure system that allows concurrent monitoring of environmental parameters. Kinetic *in vitro* studies will be conducted to determine TEQs in sediment and fish extracts from the exposure experiments using different cell lines with varying endpoints. In combination with *in vivo* biomarker analyses, resulting data will give insights in how TEQs of different compound classes in sediment relate to TEQs in fish and how these relate to adverse effects. Moreover, extract fractionation procedures, using the strategy of effect directed analysis, will enable the detection of specific groups of DLCs responsible for the biological activity observed. A closer interconnection between applied ecotoxicological science and regulatory needs will facilitate the establishment of tools for the assessment of DLCs in sediment and biota for their application in sediment management, which is the central goal of the dioRAMA project.

**TH030 QAAR Modeling Using Covariance Structure Analysis and its Application to Calculating NOEL Values and Relative Toxicity Values of Chemical Substances** J. Takeshita, AIST / RISS; M. GAMO, National Institute of Advanced Industrial Science and Technology (AIST) / Research Institute of Science for Safety and Sustainability (RISS); K. Kanefuji, H. Tsubaki, The Institute of Statistical Mathematics. We developed a Quantitative Activity–Activity Relationship (QAAR) model by using covariance structure analysis to estimate the missing No-Observable-Effect Level (NOEL) values in animal testing data from repeated dose toxicity studies. In general, the availability of animal testing data on existing chemical substances is insufficient. Additionally, in recent years, the ability to obtain such data on new chemical substances has been limited because of increased emphasis on the need for animal protection. Nevertheless, there is increasing demand for these data globally. Considering the present conditions, the Organisation for Economic Co-operation and Development (OECD) and some countries have been developing Quantitative Structure–Activity Relationship (QSAR) models and discussing their possible application. Simply stated, the QSAR is a method for estimating the properties of a chemical from its molecular structure by using regression analysis. However, because of the

availability of *in vivo* data, we adopted the QAAR approach for estimating missing NOEL values. Although the QAAR approach was introduced to estimate the relationships between different toxicity endpoints, few practical examples of its application have been systematically investigated. Therefore, we created a QAAR model based on a statistical method (namely, a covariance structure analysis). We then estimated the missing NOEL values and relative toxicity values of chemical substances by using confidence intervals. Our model's characteristics enable the estimation of NOEL values for all endpoints if values for at least are available. In our QAAR model, the training set was built from the Initial Risk Assessment Report published by NITE (the National Institute of Technology and Evaluation). The report includes animal testing data for about 150 chemical substances listed on the Japan Pollutant Release and Transfer Register as Class 1 Designated Chemical Substances. Our covariance structure model was based on correlations among the endpoints that were included in the training set. We discuss the validation of the model in conformity with OECD Principles for QSAR models.

**TH031 Cytotoxicity assessment approach in short-term bacterial genotoxicity assays** A. Skrzypczak, Environmental Health Sciences; G. Nalecz-Jawecki, Medical University of Warsaw / Environmental Health Sciences. Cytotoxicity determination is an important aspect of the bacterial assays procedures dedicated to evaluate the genotoxicity. Such an approach allows to determine the whole activity profile of the sample to the decomposers trophic level. On the other hand toxicity assessment is essential to interpret the genotoxic data properly and to receive reliable test results. While the cytotoxic sample can inhibit the bacterial growth, lack of the cytotoxicity assessment can lead to the false non-genotoxic response in the test. *Umu*-test with *S. typhimurium* TA1535 is an ISO standard (13829) to evaluate the genotoxicity of water and wastewater. To indicate the cytotoxicity of the samples for the tested strain, the growth inhibition factor (G) in comparison to the negative control is being calculated. The test is considered to be valid if the negative controls reach the minimum growth (140FNU). Additionally the results obtained for the tested samples cannot be evaluated if G factor is lower than 0.5. However the cytotoxic potential of the sample can be revealed always when G level is lower than 1. Therefore we have established an additional factor Minimal Toxic Concentration (MTC) which indicates more than 20% growth inhibition in comparison to the negative control. In SOS-Chromotest two factors show toxic properties of the sample for *E. coli* PQ47. Measurement of the optical density (OD600) of the bacteria suspension as well as the measurement of the alkaline phosphatase activity (constitutive enzyme) are being executed. These parameters are being compared to the negative control. However, there is no official scale to classify toxic and non-toxic samples. According to OECD 471 there is no cytotoxic evaluation step in classic Ames test (Bacterial Reverse Mutation Test) with a number of *S. typhimurium* and *E. coli* strains. There is available the microplate format (MPF) of Ames test produced by Xenometrix (Switzerland). We have improved the procedure of the Ames MPF with the additional step to estimate the cytotoxic influence of the sample for the bacteria. After the exposition of the particular strain (to determine the mutagenicity of the sample), subsequent 3h incubation with fresh growth medium is being carried out to determine the cytotoxicity. Then measurement of the optical density of the bacteria suspension is being made. Likewise in the *umu*-test the threshold of growth inhibition has been determined at the level of 50% and 20% in comparison to the negative control.

**TH032 Adverse effects of the artificial sweetener sucralose** A. Wiklund; M. Adolfsson-Erici. Sucralose is an intensively sweet food additive that has become a popular substitute for sugar. It has been approved for human use in more than 70 countries. Sucralose is frequently found in recipient water, but also further out in the oceans. Due to its exceptional stability in combination with high water solubility and widespread use, it has been suggested as an ideal tracer for human activities in the aquatic environment. Animal and human studies have concluded that sucralose is safe for human use and earlier studies in

aquatic organisms indicate low bioaccumulation potential and negligible acute/chronic toxicity. The close structural resemblance with sucrose in combination with the importance of sugar in nature implies that other assessment techniques than traditional methods might be of interest. In our previous studies, both physiology and locomotive behaviour were affected by exposure to sucralose. The aim of this investigation was therefore to combine traditional methods with behavioral studies in crustaceans and algae. Our results show that no effects were obtained by traditional toxicological tests, but in *Daphnia magna*, the behavioural response was manifested as altered swimming height and increased swimming speed. In gammarids, effects on the time to reach food and shelter as well as activity responses measured by a Multispecies Freshwater Biomonitor™ were observed. It is unclear whether these behavioural responses were initiated via traditional toxic mechanisms or stimulatory effects. To increase the understanding behind the mechanisms we also performed biochemical analyses of the daphnids. Combining the results of the cellular responses with the results from the behavioral experiments we will be able to enhance the understanding of the observed effects.

**TH033 Read Across of Sucralose Mammalian Toxicological Data For Environmental Decision Making** D.B. Huggett, University of North Texas / Department of Biological Sciences. The intense artificial sweetener sucralose has been detected in municipal wastewater effluent and surface waters at concentrations ranging from ng/L to low ug/L. Mammalian toxicology data have been shown to be useful in prioritizing environmental safety issues in fish and other aquatic vertebrates. The premise of mammalian to environmental read-across is based on the conservation of pharmacological and toxicological pathways across species. A wide array of mammalian data (e.g. acute, sub-chronic and chronic rodent studies) suggest that sucralose is unlikely to produce acute or chronic toxicity in aquatic vertebrates. Specifically, no changes in growth or reproduction (e.g. fertility) have been reported in the mammalian literature, suggesting a low probability of growth or reproduction changes in aquatic vertebrates (e.g. fish). Further, sucralose does not accumulate in mammalian tissues following exposure. Acute and chronic fish studies with trout, bluegill and fathead minnow support the mammalian read-across. No lethality or growth changes were observed in fish up to 100 mg/L, which is well above concentrations detected in aqueous matrices. In addition, bioconcentration factors in fish are < 3, indicating that sucralose is not bioaccumulative. Collectively, the mammalian and environmental data suggest that sucralose poses a negligible risk to aquatic vertebrates.

**TH034 Teratogenic and histological effects of microcystins on early life stages of zebra fish (Danio rerio)** M. Lopez-Varga, F. Martinez-Jeronimo, Escuela Nacional de Ciencias Biológicas IPN / Zoología; R. Lopez-Santiago, Escuela Nacional de Ciencias Biológicas, I. P. N. / Departamento de Inmunología. Ecotoxicology is aimed to identify the toxic effects of chemical pollutants in the different organization levels in an ecosystem. *Danio rerio* is a fish model worldwide used as test organism in aquatic toxicology, enabling the assessment of teratogenic, mutagenic and immunotoxic effects in early life stages. Among the most important effects of pollution is eutrophication in freshwater ecosystems; this is manifested as the massive growth (blooms) of phytoplankton organisms, mainly cyanobacteria and microalgae. Particularly cyanobacteria are of environmental concern because they are able to produce toxic metabolites, such as the microcystins (MC's), which are cyclic heptapeptides, with neurotoxic, hepatotoxic, and dermatotoxic effects in mammals. During toxigenic blooms, adult fish can avoid in some extent the toxic effects of toxins and chemical contaminants by moving away; on the other hand, the fish egg chorion could be a barrier against some pollutants, but some other compounds can penetrate the eggs and injure the embryo. In the present study 2 hpf eggs of *Danio rerio* were exposed to crude extracts of two local strains of *Microcystis aeruginosa* (CH-10 and VU-5) responsible of toxigenic blooms in Mexico City; these two strains produce microcystins. Test procedures were carried out in accordance to the OECD early life stages of fish (ELS) guideline, in 24-microwell plates; determined LC<sub>50</sub> were

2.17 and 0.82 mg L<sup>-1</sup> (for strains CH-10 and VU-5, respectively). Components of both extracts were able to trespass the chorion (observed with confocal microscopy), producing teratogenic effects in embryos; for strain VU-5 significant concentration-related effects were determined. On the other hand, 72 hpf larvae were also exposed to crude extracts for histological effects assessment; exposed larvae were processed at different times using Hematoxylin and Eosin, and Sudan Black stains. Damages in larvae tissues were observed since the first hour of exposure, evinced as leukocyte infiltration and inflammation. This study revealed teratogenic effects in embryos, and histological damages in fish larvae exposed to microcystins, that could be added to other toxic effects elicited by cyanotoxins. Observed effects had not been reported previously so it could be the first evidence of this kind of damages in ELS of *D. rerio*; it is relevant information from the ecotoxicological point of view.

**TH035 Biochemical biomarkers for freshwater monitoring in areas contaminated with phenanthrene, atrazine and copper** J.D. Simonato, University of Londrina / Physiological Sciences; M.N. Fernandes, Universidade Federal de Sao Carlos / Ciencias Fisiologicas; A. Bianchini, Universidade Federal do Rio Grande FURG / Instituto de Ciências Biológicas; C.B. Martinez, Universidade Estadual de Londrina / Ciencias Fisiologicas. The aquatic environment represents a final sink for many effluents from agriculture and industries, domestic sewage and urban waste, which affect water use and promote serious problems for organisms. Among these contaminants we can highlight three main groups: petroleum products, pesticides and metals. Thus, the present study aimed to identify the more suitable, practical and economical biochemical biomarkers in a freshwater fish exposed to one of the following contaminant: the polycyclic aromatic hydrocarbon phenanthrene, the herbicide atrazine and the metal copper. The Neotropical fish *Prochilodus lineatus* were submitted to semi-static toxicity tests, during 96 h, and exposed to environmentally relevant concentrations of copper (5, 9 and 20 µg L<sup>-1</sup>), atrazine (2, 10 and 100 µg L<sup>-1</sup>) or phenanthrene (10, 20 and 200 µg L<sup>-1</sup>). After exposure, the animals were anesthetized and killed by medullary section. The liver was immediately removed for determination of the antioxidant enzymes catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and superoxide dismutase (SOD), in addition to the concentrations of reduced glutathione (GSH) and metallothionein (MT) and the occurrence of lipid peroxidation (LPO). According to the results, MT concentration increased significantly in animals exposed to copper, in all three concentrations tested, being elected as a biomarker specific to copper. The animals exposed to atrazine showed a significant decrease in CAT and GSH, which are important biomarkers of oxidative stress. As both parameters are relatively easy to measure and of low cost they were elected as possible biomarkers to be applied in areas impacted with this herbicide. However, it is important to note that these biomarkers are not specific and may show similar results for other contaminants. Fish exposed to phenanthrene showed no significant differences in the antioxidant defense parameters analyzed. The occurrence of LPO was chosen as a biomarker for a general analysis of impacted environments with different types of contaminants, since it increased significantly in fish liver exposed to all the concentrations for the three pollutants.

**TH036 Comet assay in fish erythrocytes for freshwater monitoring in areas contaminated with phenanthrene, atrazine and copper** J.D. Simonato, University of Londrina / Physiological Sciences; M.N. Fernandes, Universidade Federal de Sao Carlos / Ciencias Fisiologicas; A. Bianchini, Universidade Federal do Rio Grande FURG / Instituto de Ciências Biológicas; C.B. Martinez, Universidade Estadual de Londrina / Ciencias Fisiologicas. In recent decades, advances in molecular biology have led to the development of methods based on the analysis of genetic material that enable the detection of damage caused by the so called genotoxic substances. Thus, it is very important to know the genotoxic effects of pollutants on organisms, since this information can be used in the monitoring of contaminated areas. The present study aimed to evaluate genotoxic damage by using the comet assay in

freshwater fish exposed to polycyclic aromatic hydrocarbon phenanthrene, the herbicide atrazine and the metal copper. The Neotropical fish *Prochilodus lineatus* were submitted to semi-static toxicity tests, during 96 h, and exposed to environmentally relevant concentrations (in  $\mu\text{g L}^{-1}$ ) of copper: 5 (Cu5), 9 (Cu9) and 20 (Cu20), atrazine: 2 (AT2), 10 (AT10) and 100 (AT100) or phenanthrene: 10 (Phe10), 20 (Phe20) and 200 (Phe200). After exposure, the animals were anesthetized and an aliquot of blood was withdrawn from the caudal vein to run the comet assay, alkaline version. DNA damage was classified in four classes (0 to 3) according to the length of DNA migration and 100 nucleoids were evaluated per each fish. The damage was rated from 0 to 3, according to the migration of the DNA fragments, in order to calculate the score of damage for each treatment (mean  $\pm$  EP). The results of DNA damage scores, obtained after 96 hours of exposure to different concentrations of atrazine (AT2:  $96.25 \pm 19.88$ ; AT10:  $78.5 \pm 18.69$ ; AT100:  $109.8 \pm 17.4$ ), copper (Cu5:  $161.0 \pm 28.66$ ; Cu9:  $108.67 \pm 9.96$ ; Cu20:  $194.0 \pm 28.66$ ) and phenanthrene (Phe10:  $81.0 \pm 11.68$ ; Phe20:  $93.50 \pm 11.56$ ; Phe200:  $74.57 \pm 6.84$ ), showed a significant increase at all concentrations tested, when compared to respective control groups (AT CTR:  $54.83 \pm 7.45$ ; Cu CTR:  $65.0 \pm 6.77$ ; Phe CTR:  $25.6 \pm 4.08$ ), except for the intermediate concentration of copper ( $9 \mu\text{g L}^{-1}$ ). It can be concluded that all three contaminants tested are genotoxic and that the comet assay was effective for the analysis of freshwater with different types of contaminant.

**TH037 Detection of Ah receptor agonists in sediments from the Three Gorges Reservoir and its feeder rivers using the H4IIE-luc Cell Line** J.T. Koch, RWTH Aachen / Institute for Environmental Research; T. Floehr, B. Scholz-Starke, RWTH Aachen University / Institute for Environmental Research; L. Wu, Institute of Environmental Science and Engineering Shanghai; J. Hou, East China Sea Fisheries Research Institute; X. Yuan, Chongqing University / College of Resources and Environmental Science; M. Ross-Nickoll, A. Schaffer, RWTH Aachen University / Institute for Environmental Research; M. Hecker, University of Saskatchewan / Toxicology Centre; H. Hollert, RWTH Aachen University / Institute for Environmental Research. Sediments play a major role as a sink for many kinds of environmental pollutants. Potentially contaminated sediments in the regions of the Yangtze Three Gorges Reservoir (TGR) in China pose a risk to aquatic organisms. Additionally, annual changes in water level cause flooding events which lead to a relocation of potentially contaminated particulate matter onto agricultural areas along the river bank. For the investigation of the sediments a German-Chinese team took samples at multiple locations along the TGR and its feeder rivers near the towns of Chongqing, Fengdu, Yunyang and Wushan in September 2011. To examine Ah receptor activity in the sediments, 19 of these samples were tested in the H4IIE-luc bioassay. H4IIE rat hepatoma cells were stably-transfected with an inducible reporter plasmid which contains the firefly luciferase gene under control of four dioxin response elements. Exposure of these H4IIE cells to Ah receptor agonists results in induction of luciferase activation in a time- and dose-dependent manner. All samples were extracted (Acetone:n-Hexane, 1:1) for examination and then tested in different sediment equivalent concentrations to generate dose-response curves. 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) was used as standard. All curves were linearized. Effective concentrations  $\text{EC}_{-}$ ,  $\text{EC}_{-}$ , and  $\text{EC}_{-}$  of TCDD were divided through  $\text{EC}_{-}$ ,  $\text{EC}_{-}$ , and  $\text{EC}_{-}$  of the samples<sup>80</sup> to calculate relative potency values<sup>20</sup> ( $\text{RP}^{50}$ ,  $\text{RP}^{50}$ ,  $\text{RP}^{80}$ ).  $\text{RP}^{50}$ -values up to  $1.72^{9}$  were calculated for sediments<sup>20</sup> near the city of<sup>50</sup> Yunyang. Results shall be compared to EROD data and chemical toxic equivalence factors (chem TEQs). In a recent gas chromatography-mass spectrometry-analysis polycyclic aromatic hydrocarbons (PAHs) could be detected as the only hazardous group of substances in the sampled TGR sediments. Therefore PAHs are considered to be responsible for the observed effects. Multiple sources of pollution such as flooded urban, agricultural and industrial areas as well as the release of sewage from adjacent cities into the TGR make it difficult to identify the exact source of hazardous compounds. Additionally complex flow behavior, especially during flooding events, seems to play an important role in the relocation of contaminated

sediments. The authors acknowledge financial support by the German Federal Ministry of Education and Research and the UROP Abroad program of the German Excellence Initiative. Keywords: Yangtze River - sediment toxicity - H4IIE-luc- Ah receptor - ?dioxin response

**TH038 Implementation of a bioavailability approach for nickel in Australian Surface Waters** A. Peters, WCA Environment G. Merrington, Environment Agency; C. Schlekot, Nickel Producers Environmental Research Association (NiPERA); J. Stauber, CSIRO Land and Water; R. van Dam, Environmental Research Institute of the Supervising Scientist, Department of Sustainability, Environment, Water, Population and Communities; G. Batley, CSIRO Land and Water; R. Smith, Hydrobiology; A. Harford, Environmental Research Institute of the Supervising Scientist, Department of Sustainability, Environment, Water, Population and Communities; J. Chapman, Independent Environmental Consultant; M. Warne, Qld Department of Environment and Resource Management (DERM); C. Hickey, National Institute of Water and Atmospheric Research (NIWA); P. Glazebrook, Rio Tinto. A bioavailability-based approach is now being implemented across the EU for metals for which chronic biotic ligand models (BLMs) are available. The recent EU Proposal for Environmental Quality Standards under the Water Framework Directive to apply EU-wide includes a bioavailability-based EQS for nickel. However, the BLMs have been developed and validated using North American and European temperate species and waters, but how applicable are these models to ecosystems elsewhere, including the tropics? The applicability of the nickel BLMs to Australian freshwaters is currently being assessed through an extensive validation exercise consisting of two key steps; determination if the ranges of physico-chemical parameters for which the BLMs were developed in EU/US are similar to the ranges in Australia, and hence determine if the models are applicable; and verification that the physiological relationships developed for the three trophic levels for EU/US standard species are valid when applied to temperate and tropical species from Australia. The key hypothesis being tested through this work is that the physico-chemical relationships and the physiological relationships upon which these models are based are universal, not locale specific. Therefore, the competitive binding of major cations and nickel for the biotic ligand are constant across organisms, only the intrinsic sensitivity is different. We will present the findings of this project, including the geochemical survey data and the results from the comparison of chronic nickel BLM predictions developed for EU/US species versus chronic ecotoxicity results for five Australian-specific test species performed in five field waters. The field waters are representative of the range of physico-chemical freshwater conditions across Australia, including the tropics. This validation exercise will establish the level of applicability of the nickel BLM developed from laboratory data on EU/US species to test species and waters from a different ecoregion.

**TH039 Behavioral ecotoxicology modeling of freshwater clam valve rhythm in response to waterborne copper** L. Jou, National Ilan University. The purpose of this study is to use an improved clam-based on-line behavioral response monitoring system to approach valve movement response of freshwater clam *Corbicula fluminea* following exposure to waterborne copper. In this work, a probabilistic-based approach describing the valve behavioral response of *C. fluminea* exposed to unpolluted environment and Cu was developed. A strict laboratory procedure associated with an improved valvometric technique was performed in the bioassay experiment to respectively obtain the magnitudes of shell gape as the determining thresholds of the valve closing (VC)/valve opening (VO) and siphon extension (SE)/ siphon withdrawal (SW) status to digitalize the valve movements in bivalves. The observed data of valve closure response was analyzed to construct the time-varying dose-response profiles ( $R^{VC}$  and  $R^{SW}$ ) based on an empirical three-parameter Hill model. It allowed the estimation of the integration time-specific  $\text{EC50}^{VC}$  and  $\text{EC50}^{SW}$  values as a bioassay approach. The results revealed that the  $R^{SW}$ -based bivalve behavioral observation has a better sensitive response for detecting a lower waterborne Cu concentration than that of  $R^{VC}$  within one hour. The

daily valve opening and closing rhythm was characterized by a three-parameter lognormal function. The results also demonstrated that the response sensitivity of clams exposed to Cu depended on the initial valve opening and closing state of *C. fluminea* after Cu occasional addition, indicating that clams in response to different Cu exposure concentrations resulted in various valve closing rates in clams. In the future, the related response characteristics of valve activities can promote the predictive capabilities of this bioassay approach as building a basis of metal toxicity detection mechanism in a bivalve-based early warning system under various site-specific field water quality conditions.

**TH040 Histopathological maladies and biological accumulation of nickel in the periwinkle *Tympanotonus fuscatus* var. *radula*** A. Enuneku, L.I. Ezemonye, University of Benin / Animal and Environmental Biology. The periwinkle *Tympanotonus fuscatus* var. *radula* was exposed to sub lethal concentrations (0.5, 1, 2, 4 and 8mg/l) of nickel in the laboratory for 28 days. The test was conducted using the Organization for Economic Cooperation and Development (OECD) protocol #218 in a sediment medium. There were three replicates per treatment and 6 periwinkles per tank of nickel concentration including control groups. There was high bioaccumulation of nickel in the test organism. It was observed that bioaccumulation of nickel in *T. fuscatus* var. *radula* increased ( $p < 0.05$ ) with increase in concentration of the heavy metal relative to control groups. Histopathological alterations were studied in the Kidney and Muscular foot of the *T. fuscatus* var. *radula*. It was found that periwinkle exposed to lower concentrations of toxicant in the kidney showed hyperchromatic nuclei (0.5 and 1mg/l). Excess fatty changes were observed in the 2, 4 and 8mg/l nickel. Necrosis and refractile bodies occurred in the 2, 4 and 8mg/l nickel concentrations. In the muscular foot, fatty changes occurred in all concentrations which increased in severity (excess) with increase in concentration of nickel. Brown deposits occurred in the 1 and 2 mg/l Ni concentrations. Hyperchromatic nuclei were observed in the 4 and 8mg/l nickel. The observed alterations due to nickel toxicity have effect on the health of the organism. This study shows that nickel could inflict histopathological maladies on *T. fuscatus* var. *radula*. The discharge of effluents containing heavy metals especially nickel into aquatic habitats where periwinkles live should be discouraged as this could affect the health of the organism and exacerbate the problem of global biodiversity loss.

**TH041 A calibration of metal disturbance in Luxembourgish rivers using DYMBAM model** S. Massarin, CRP Henri Tudor / Resource Centre for Environmental Technologies (CRTE); r. carafa, TUODOR / CRTE; T. Galle, CRP Henri Tudor / CRTE. The contamination of freshwater ecosystems with metals remains a serious problem resulting in bioaccumulation in resident species that may affect species diversity and ecosystem function. The link between metal exposure and adverse effects in aquatic organisms remains poorly understood due to complex biological responses. We took the opportunity of recent development on the dynamic multipathway bioaccumulation model DYMBAM to explain the variability of bioaccumulation in nature using laboratory and field data from Luxembourgish rivers. To determine the species sensitivity and bioaccumulation constants, laboratory tests were conducted with a set of insect larvae species, whose nutrition is essentially based on grazing and collection, allowing for a link with the monitored field subsidies. Hydropsychid caddisflies was chosen as metal tolerant species whereas 5 mayflies within the Heptageniidae, Ephemerellidae, Baetidae and Leptophlebiidae families are selected as more sensitive species to metals. The Zinc and Copper exposures confirmed that Baetidae and Leptophlebiidae families were the most sensitive taxonomic groups for both metals studied, whereas *Hydropsyche angustipennis* was the most tolerant species. Water, Suspended Particulate Matter and macro-invertebrate communities monitoring was conducted in 9 different metal contamination sites in Luxembourgish rivers. In the highest contaminated site (1860 mg/kg Zn and 110 mg/kg Cu during spring 2012 period) there were no mayfly species whereas the benthic structure community in the uncontaminated site was varied widely and the mayflies, with Baetidae and

Ephemerellidae, were the most abundant species. Metal concentrations measured in water and Suspended Particulate Matter, associated with bioaccumulation constants from laboratory tests will allow to calibrate the DYMBAM model. Validation of predicted internal concentrations will be realized with the internal metal concentration and carbon and nitrogen isotopes will be measured to check the assumptions on preferential feeding. Finally, the presence of *Hydropsyche*, assumed strong bioaccumulator of metals, in almost sites and tissue residues of all species will allow a relative calibration of sensitivity and accumulation rates for the freshwater communities in these rivers.

**TH042 Nickel toxicity in sediments: biological species, bioavailability and toxicity** L.T. Nguyen, Ghent University / Applied Ecology and Environmental Biology; M.B. Vandeghechuchte, Ghent University / Applied Ecology & Environment Bio; J. Garrevoot, Ghent University / X-Ray Microspectroscopy and Imaging group; E.R. Garman, NiPERA / Ecotoxicologist; C.E. Schlegat, NiPERA; L. Vincze, Ghent University / X-Ray Microspectroscopy and Imaging group; M. van Gheluwe, ARCHE; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology. Sediment toxicity is known to be affected by both abiotic factors such as the concentration of total organic carbon (TOC) and acid volatile sulphides (AVS) in the sediment and biotic factors such as the intrinsic sensitivity and the behaviour of (epi)benthic test species. The goal of this research was to evaluate the relative importance of species sensitivity and bioavailability for the toxicity of nickel (Ni) in freshwater sediments. In order to achieve this, Ni toxicity and uptake are being evaluated with several species representing different taxonomic groups and life styles. Three uncontaminated natural sediments covering a range of different binding capacities (AVS and TOC) were used. The first results demonstrate that, in sediment with low concentrations of AVS ( $1.4\text{--}2.1 \mu\text{mol.g}^{-1}$ ) and TOC (1.45%), a concentration-effect relation could be observed for the biomass of juvenile *Tubifex tubifex* (Oligochaetae) after 28 days exposure. The EC50 and EC10 for juvenile biomass are, respectively, 554 and 159 mg Ni/kg dry sediment with 95% confidence intervals being [344,764] and [-13,332] mg Ni/kg dry sediment. The biomass of adult worms of this species was only significantly reduced at the highest tested concentration of 1713 mg Ni/kg dry sediment. For the midge *Chironomus riparius*, body mass of 14 day old larvae after 12 days exposure was affected at 1061 mg Ni/kg dry sediment. At this concentration, the average emergence of adult midges was also reduced to 56% of the initial number of larvae. Moreover, the emergence was delayed compared to the lower Ni concentrations. These results indicate that, for the sediment tested, *T. tubifex* is more sensitive to Ni toxicity than *C. riparius*. In this low AVS sediment, the difference between the molar concentrations of simultaneously extracted metals (SEM) and AVS was greater than zero for all tested concentrations. Ni tissue concentrations increased as the difference between SEM Ni and AVS increased. This supports the basis of the SEM-AVS concept, as it is reflective of the presence of Ni in exchangeable sediment phases, as opposed to sulphide phases. In on-going and future experiments, additional species and sediments will be evaluated. By means of micro-X-ray fluorescence (XRF) analysis and based on previous research, we will test the hypothesis that Ni accumulation and the internal distribution of Ni over different tissues is affected by bioavailability modifying factors such as TOC, AVS and/or diet.

**TH043 Oxidative stress and histopathological alterations are early biomarkers of Cu-induced damage in european seabass, *Dicentrarchus labrax*** M. Diaz de Alba, University of Cadiz; A. Canalejo, University of Huelva; B. El Mai, University of Cadiz; F. Cordoba, University of Huelva; E. Espada, M. Oliva, University of Cadiz; J. Palazon, CSIC-Cadiz; R. Torronteras, University of Huelva; d. galindo. The aim of the present study was to assess the potential induction of oxidative and histopathological damage after a waterborne Cu-exposure in gills, muscle, liver and brain of european seabass, *Dicentrarchus labrax*, a very important commercial fish. Juvenile fish were exposed under laboratory conditions for 24 or 96 hours to nominal Cu concentrations of 0 (controls), 0.01-10 ppm by adding

CuSO<sub>4</sub> · 5H<sub>2</sub>O. Cu concentrations were analyzed in water by ICP-AES or DPASV<sup>2</sup> and in fish tissues by ICP-MS. A set of biomarkers including lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and the presence of histopathological alterations, were evaluated. A full mortality was observed for Cu 10 ppm after 24 and 96 h and for 1 ppm after 96 h. Cu concentrations (mg/Kg) in tissue samples ranged from 62.9-121.4 (liver), 0.68-1.14 (muscle), 3.04-11.53 (gills) and 3.09-5.17 (brain). Liver showed the higher Cu content, followed by similar levels in gills and brain, while the lowest was recorded in muscle. Regarding the time of exposure, Cu concentration increased significantly in gills and slightly in liver, and decreased in muscle; brain was not affected. LPO content was increased in all the studied organs. The higher LPO levels were observed in gills and liver. Similar levels were observed for 24 and 96 h. Accordingly, as a rule, the higher levels of antioxidant activities were also recorded in gills and liver. SOD were increased by Cu in descending order gills > liver > muscle, but it was decreased in brain. For the 24 h, CAT was increased in all the organs but, for 96 h, it was increased only in liver. GPx resulted elevated by Cu in all tissues and exposure times; gills showing a dramatic stimulation. Lesions as steatosis, blood cells accumulation in vascular system and cellular atrophy leading to parenchyma disorganization were detected in liver. Gills showed alterations as oedemas, aneurysms, hypertrophy of lamellar epithelia and epithelial desquamation. Thus, Cu exposure caused an accumulation of the metal that correlated with the induction of oxidative stress and organ specific antioxidant response and histopathological damage in the fish tissues, serving as early biomarkers of Cu toxicity in fish. **Keywords:** Copper, fish, oxidative stress, biomarker *Supported by the Projects RNM-6641 and CTM2010-17474*

**TH044 Speciation of cerium and its bioavailability to *Chlamydomonas reinhardtii*** P. El-Akl, Department of Chemistry. Interest in the Rare Earth Elements (REEs) has been growing rapidly in the past decade, with uses in such applications as glass polishing, catalysers, reusable batteries and fluorescence probes. Unfortunately, little is known about the environmental impact of these elements. This research focuses on the bioavailability of cerium, one of the most widely used lanthanides, by studying its speciation and bio-uptake by the unicellular alga *Chlamydomonas reinhardtii*. Fluorescence quenching titration and nanofiltration were used to give us information on complexation of cerium by natural organic matter (NOM) and the formation of colloidal forms of Ce(IV) at circumneutral pH. Results indicated that complexation with organic matter was important with a conditional stability constant of approximately  $7.9 \times 10^4 \text{ M}^{-1}$ . Furthermore, more than 75% of the metal appeared to be found in colloidal forms. Cerium bio-uptake was proportional to the concentration of free ion in the exposure medium obtained using WHAM7. Uptake fluxes followed a Michaelis-Menten kinetics ( $B_{\text{max}} = 6.8 \times 10^{-13} \text{ mol/cm}^2$  and  $K = 5.4 \times 10^{-9} \text{ M}$ ), which corresponds roughly to an uptake constant<sup>d</sup> of  $1.9 \times 10^8 \text{ M}^{-1}$  for biotic ligand modeling.

**TH045 Dietborne-metal toxicity to aquatic organisms: A literature review** D.K. DeForest, Windward Environmental LLC; J.S. Meyer, ARCADIS. Recently published studies of dietborne-metal toxicity to aquatic biota provide increased insight into the relative importance of dietborne-metal versus dissolved-metal exposure. To provide an updated synthesis of the literature, we reviewed the state of the science about dietborne-metal toxicity to aquatic biota, with a focus on 12 metals: Ag, Al, As, B, Cd, Co, Cu, Mo, Ni, Pb, V, and Zn. Although Hg and Se are also of concern for dietborne-metal toxicity, they have recently been reviewed extensively. Of the metals we reviewed, limited or no dietborne toxicity data were identified for B, Co, Mo, and V. Additionally, little or no data were available about the toxicity of dietborne Al and Pb to aquatic invertebrates; however, available data suggest toxicity of dietborne Al and Pb to fish is unlikely to be of concern. In contrast, Ag, As, Cd, Cu, Ni, and Zn have been demonstrated to cause dietborne toxicity to aquatic organisms in laboratory exposures. For Ag, Cd, and Zn, dietborne-metal toxicity occurred at potentially environmentally-relevant concentrations (i.e.,

some of the waterborne concentrations to which the food was exposed were at or near the U.S. Environmental Protection Agency's existing waterborne criteria for Ag, Cd, and Zn, sometimes resulting in dietborne concentrations that contributed to effects reported for the most sensitive species [usually filter-feeding herbivores like freshwater daphnids and saltwater copepods] beyond the toxicity caused by waterborne exposure alone). Additionally, although generally ignored in the past, dietborne As has recently been demonstrated to be toxic to fish. These results indicate that dietborne metal can be more toxic than waterborne metal under some exposure scenarios; however, these results might also simply indicate that some water quality criteria are outdated and do not adequately incorporate the same sensitive species or endpoints that are most sensitive to dietborne metals. To develop an understanding of the relationships between waterborne and dietborne exposures in natural systems, aqueous and algal concentrations of metals should be surveyed in a variety of real-world freshwater and saltwater systems to determine dietborne:waterborne metal ratios and the chemical forms in which the metals occur in various food items.

**TH046 Considering both dissolved and precipitated forms of aluminium for understanding Al bioavailability to aquatic organisms** R.C. Santore, HydroQual Inc; A.C. Ryan, HDR HydroQual; F. Kroglund, Norwegian Institute for Water Research (NIVA); H. Teien, Norwegian Univ of Life Sciences; P. Rodriguez, CIMM; B.A. Stubblefield, Oregon State University / Environmental and Molecular Toxicology; A.S. Cardwell, Oregon State University / Faculty Research Assistant; W. Adams, Rio Tinto; E. Nordheim. Aluminium (Al) toxicity to aquatic organisms is strongly affected by water chemistry. Bioavailability factors such as pH, dissolved organic carbon (DOC), and hardness can change the observed toxicity of Al to aquatic organisms resulting in wide variation in observed effects. The importance of water chemistry on the aquatic toxicity of Al suggests that interactions between Al and constituents of exposure waters influence bioavailability and toxicity. These types of interactions have typically been well-described by the biotic ligand model (BLM) framework that has previously been applied to cadmium, copper, cobalt, lead, nickel, silver, and zinc. The BLM framework for other metals has considered how chemical changes can impact the chemical speciation of dissolved metal and the interaction of metal ions with biological surfaces, thereby resulting in changes in metal bioavailability. A review of Al toxicity data, however, shows that concentrations sufficient to cause toxicity are frequently in excess of solubility limitations. Al solubility is strongly pH dependent, with a solubility minimum near pH 6 or 7, depending on temperature and other water chemistry characteristics (e.g. DOC concentrations). Conceptually, the BLM framework should be a valid descriptor of metal bioavailability when toxicity is a result of exposure to dissolved Al, but the mechanistic framework needs to be extended to allow Al toxicity resulting from a combination of dissolved and precipitated Al. We have developed a modified BLM for Al that considers Al solubility and combines effects due to dissolved and precipitated Al when water chemistry conditions indicate that both forms of Al would be required to reach a specified effect level. This model determines, for given chemistry conditions, if the solubility of Al is sufficient for Al to accumulate at the biotic ligand (BL) to a critical level associated with a specified toxic effect. In cases where solubility is limiting, a combination of dissolved and precipitated Al is considered. In these cases, a response-additivity calculation is performed to determine the predicted effect concentration that results from both dissolved and precipitated metal. This approach requires a modified BLM parameter list that includes the specification of concentration-response relationships based on Al bound to the BL and on the basis of precipitated Al.

**TH047 Evaluation of the effect of 3 metals on ostracod *Cypris* sp.** A.S. Sobrino-Figueroa, Universidad Autonoma Metropolitana Iztapalapa / Hidrobiologia, Laboratorio Alejandro Villalobos. Metals are highly persistent and toxic compounds to aquatic communities. In this work was conducted an evaluation of deleterious effect of the metals cadmium, chromium and lead on ostracod *Cypris* sp. which is an

organism that lives in the water-sediment interface, to compare their sensitivity with neonates of *Daphnia magna*, a species that is used for the evaluation of toxicity of effluents (NMX-AA-087-1995-SCFI). Static bioassays were performed with 48 hours duration were tested 5 metal concentrations plus a control. LC<sub>50</sub> was determined after 48 hours of exposure by means of Probit method.<sup>50</sup> The toxicity of metals based on the LC<sub>50</sub> was (high to low toxicity): Cd>Pb>Cr neonates *Cypris* sp. were more sensitive to cadmium metal compared with neonates of *Daphnia magna*. Because the values of LC<sub>50</sub> (48 hours) for metals Cd and Cr are lower than the limits of the NOM 001-SEMARNAT for discharges in aquatic systems, it is important to continue research and monitoring to detect responses that indicate the possible damage populations in *Cypris* sp. by the action of the discharge, to prevent irreversible deterioration of the populations in the medium and long term.

#### **TH048 Development of Reliable Chronic Algae and Ceriodaphnia Toxicity Data for 6 PAHs using Passive Dosing with Silicone O-Rings**

G.E. Bragin, ExxonMobil Biomedical Sciences Inc; D. Letinski, ExxonMobil Biomedical Sciences; M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences, T&ES Division; C. Sutherland, ExxonMobil Biomedical Sciences Inc / Toxicology and Environmental Science; J. Butler, ExxonMobil Biomedical Sciences Inc; T. Knarr, ExxonMobil Biomedical Sciences Inc / Toxicology & Environmental Science; B. Kelly, ExxonMobil Biomedical Sciences, Inc; A.D. Redman, Exxon Mobil Biomedical Sciences; T. Parkerton, ExxonMobil Biomedical Sciences Inc. The target lipid model (TLM) has been used to establish predicted no effect concentrations (PNECs) for hydrocarbons including polyaromatic hydrocarbons (PAHs). To derive PNECs, the TLM was first calibrated with acute toxicity test data to define the species sensitivity distribution (SSD) for short-term effects. An empirical distribution of acute to chronic ratios was then used to extrapolate the acute SSD to concentrations that are intended to be protective of chronic effects. To evaluate if TLM predictions are protective, comparisons to reliable chronic effects data are needed. However, such comparisons are often hindered by the poorly characterized or variable aqueous exposure concentrations that often characterize past chronic studies. Recent advances in passive dosing techniques enable highly controlled delivery of PAHs in chronic toxicity tests that can serve as a sound basis for critically evaluating TLM performance. To evaluate this new dosing paradigm, chronic effects independently posed by selected three, four and five ring PAHs (flourene, 1-methyl flourene, pyrene, 1-methyl pyrene, chrysene and benzo(a)pyrene) to the algae *Pseudokirchneriella subcapitata* and crustacean, *Ceriodaphnia dubia* were investigated in standard 72-hr growth and 7-d survival and reproduction laboratory guideline toxicity tests, respectively. Silicone o-rings were fortified with the test compounds by dosing via a methanol stock solution containing the test PAH. Spiked silicone o-rings were then placed in test chambers to deliver and carefully control exposure concentrations that were confirmed analytically using GC/MS. Chronic test results obtained from this study will be compared to earlier chronic studies reported for these same test compounds and species as well as TLM-derived PNEC predictions. Implications of study design and findings for establishing defensible water quality objectives for PAHs will be discussed.

#### **TH049 Chemical substance solubility in freshwater : not so simple**

J. Berlusconi, P. Thomas, CEHTRA SAS. Water solubility of substances is a key property for ecotoxicological testing as well as environmental risk assessment. For instance, if an ecotoxicological study shows effects above the solubility limit, one can suspect physical effects rather than toxicity effects. However, for regulatory use (e.g. REACH) the water solubility of substances is measured in pure water, while the solubility limit as measured in test medium is critical for the interpretation of aquatic toxicity tests. It is commonly assumed that the solubility in both media is equivalent because in freshwater no significant *salting out* effect is expected (Rene P. Schwarzenbach, 2003). However, the solubilities of some organic substances have been measured both in pure water and in standard medium used in OECD 202

study (acute daphnia), following the OECD 105 guideline with an adaptation taken from the slow-stir method in the OECD 123 guideline (logKow) (Poster No TH 288, Improved OECD 105 water solubility test design Paul Thomas & Virginie Burosse, 2012). Some of these tested substances were significantly less soluble in daphnia medium than in pure water (2 to 10 times) even for non-polar narcotics. The suitability of the aqueous solubility value determined in pure water for interpretation of ecotoxicological test results in such cases is questionable. Potential factors that may explain this difference of solubility between these two media will be presented, such as salting out effect at low solubility, physical state of substance, nature of 'pure water' used. We also explore reasons for the variation of the difference in solubility between non-polar substances in two media. Finally, the implications of this on regulatory aspects and environmental risk assessment will be discussed. Keywords: water solubility; salting out effect; non-polar narcotic; ecotoxicological interpretation

#### **TH050 Equilibrium sampling of marine sediment with Coated Glass Jars to estimate acute toxicity and fish bioaccumulation of PAHs.**

E. Rojo-Nieto, CactymarUniversity of Cadiz / Department of Environmental Technologies; P. Mayer, Aarhus University / Department of Environmental Science; J. Perales, CACYTMAR-University of Cadiz / Department of Environmental Technologies. In soils and sediments contaminated by hydrophobic organic compounds (HOCs), the total concentrations are less indicative of potential exposure and distribution than the associated freely dissolved concentrations (C<sub>free</sub>) and chemical activity. The C<sub>free</sub> and chemical activity of PAHs in marine sediments were measured by equilibrium sampling with PDMS coated jars of multiple coating thicknesses, and then used to predict baseline toxicity and bioaccumulation potential. The sediments were from a chronically polluted area and had similar total PAHs concentrations. Results indicate that chemical activities of the sediments differed up to one order of magnitude and were below the level at which lethal baseline toxicity is expected, but being of special concern due to the presence of other pollutants. The use of C<sub>free</sub> and site/species-specific Biota to Sediment Accumulation Factors (BSAFs), allowed estimating concentrations in different target organs of benthic flatfish, hypothetically exposed to these chronically polluted sediments.

#### **TH051 Equilibrium sampling of hydrophobic contaminants in sediments from the River Elbe**

S. Schaefer, Federal Institute of Hydrology; C. Antoni, Goethe University; C. Moehlenkamp, E. Claus, G. Reifferscheid, P. Heininger, Federal Institute of Hydrology; P. Mayer, Aarhus University. Since the early 1990s, sediments from the German part of the River Elbe have been regularly monitored for total concentrations of contaminants by the German Federal Institute of Hydrology. In contrast, freely dissolved concentrations (C<sub>free</sub>) of hydrophobic contaminants in these sediments are largely unknown though they are considered the effective concentrations. They are more indicative of potential exposure of aquatic organisms than total concentrations. Recently, the applicability of equilibrium sampling using polydimethylsiloxane (PDMS) coated glass jars has been shown in marine sediments with background contamination of polychlorinated biphenyls (PCBs) (Jahnke et al. 2012 ES&T 46 (18), pp 10114–10122). Advantages of equilibrium sampling with coated glass jars are that 1.) samples can be taken in parallel to regular sediment sampling in the field and 2.) the time-consuming and risky equilibration is done in the laboratory. The aims of the present study were to 1.) test the applicability of equilibrium sampling using coated glass jars to sediments from the River Elbe, 2.) assess freely dissolved sediment porewater concentrations of hydrophobic contaminants such as PCBs and Dichlordiphenyltrichlorethane (DDT) and its metabolites and 3.) determine site-specific sediment/water distribution ratios (K<sub>d</sub>). For this purpose, sediments were sampled at ten different locations within the German part of the River Elbe from the Czech border to Geesthacht. In the laboratory, sediments were incubated in PDMS-coated glass jars for two weeks. For validation of equilibrium sampling, sediment subsamples were incubated in coated glass jars with differing PDMS thicknesses of 2, 4 and 8 µm. After incubation, glass jars were cleaned,

extracted with heptane and extracts were analysed by gaschromatography with MS/MS detection. Freely dissolved porewater concentrations were calculated by dividing analyte concentrations in PDMS with analyte-specific partition coefficients. Furthermore, the usefulness of equilibrium sampler extracts in bioassays was tested and the results were compared to toxicity data obtained with conventional porewater extracts that were obtained after centrifugation of sediments.

**TH052 Passive sampling combined with chemical and biological analyses for monitoring spatial and temporal exposure profiles in two river catchments** E. Vermeirssen, Eawag / Dept. of Environmental Toxicology; N. Homazava, Swiss Centre for Applied Ecotoxicology Eawag/EPFL; C. Kienle; M. Scheurer, Eawag, Swiss Federal Institute of Aquatic Science and Technology; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy, Physiology and Cell Biology; C. Stamm, Eawag, Swiss Federal Institute of Aquatic Science and Technology. Within the Swiss National Research Programme on Sustainable Water Management (NRP61), a survey was conducted in two river catchments, the Gürbe and the Mönchaltorfer Aa. The aim of the survey was to examine key water quality parameters and use the data to generate a modelling tool for decision making purposes to predict the outcome of river management measures on water quality. Within the framework of this survey, we used passive sampling to establish toxicity profiles and evaluate the presence of individual pollutants in the catchments. In two sampling campaigns, Chemcatchers (Empore SDB-disks with PES membranes) were deployed for two week periods. One period in late spring, coincided with the main application period of herbicides. The other sampling campaign was conducted 4 months later. Samples were analysed by means of two LC-MS/MS methods and three bioassays. An algal assay was used to quantify inhibitors of photosystem II and effects on algal growth. With a yeast based assay we assessed the presence of compounds that can bind to the human estrogen receptor (YES, yeast estrogen screen). Using an acetylcholinesterase (AChE)-inhibition assay the presence of neurotoxic compounds such as organophosphate and carbamate pesticides was determined. Clear temporal and spatial differences emerged for different kinds of toxicity and for individual compounds. As expected, the presence of photosystem II inhibitors was much higher in the spring than in summer. Furthermore, the presence of photosystem II inhibitors was noticeably tied to inputs of treated sewage effluent into particularly the catchment of the Aa. The presence and quantity of AChE inhibitors tended to correlate with photosystem II inhibitors. Estrogenic substances occurred in slightly larger amounts in Chemcatchers placed in the more densely populated catchment (the Aa). The input of treated sewage effluent into the Aa was not clearly tied to elevated levels of estrogens, but estrogenicity correlated well with the concentrations of individual estrogens like estrone, but only in samples from the summer sampling period. Chemcatchers allowed for an effective exposure characterisation for a diverse range of effects and individual compounds. While the presence of estrogens and photosystem II inhibitors was detected both by means of chemical analysis and bioassays, the presence of AChE-inhibitors was mainly detected by bioassay alone.

**TH053 A novel SPME approach for measuring PAHs in biota** S. Lang, R. Ernst, L. Poenitzsch, G. Witt, Hamburg University of Applied Sciences. The monitoring of marine environmental contaminants such as persistent organic pollutants (POPs) in tissue of biota, e.g. fish, is standard way of proceeding in nowadays environmental risk assessment. A classical analytical procedure for the determination of organic contaminants in biota is the extraction of tissue with different solvents followed by a clean up step of the extract. This method may be resource consuming regarding time, expenditure and material utilisation. Therefore we would like to introduce a novel approach for measuring organic pollutants in the tissue of biota based on the principal of solid phase micro extraction (SPME), a passive sampling technique effectively applied for the measuring of freely dissolved concentrations ( $C_{free}$ ) in sediment pore water systems. As a test organism the worm *Lumbricus variegatus* was chosen, due to especially focusing on the uptake of contaminants of the benthic community. For method

development and initial experiments one lower molecular weight PAH was applied and subsequently the range of PAHs was extended to the US EPA PAHs. Commercial available polydimethylsiloxane (PDMS) coated glass fibers served as the passive sampling material. The first aim was to determine the respective partition coefficients of the PAHs between fiber coating and the worm tissue ( $K_{PDMS/TISSUE}$ ). Therefore a large amount of worms were collected and spiked with the test substances ranging from lower to higher PAH concentrations. An aliquot of the spiked worm-material was extracted to determine the total concentration in the tissue ( $C_{TISSUE}$ ) and another aliquot was taken for equilibrium passive sampling experiments.  $K_{PDMS/TISSUE}$  was then calculated by dividing the concentration in the PDMS ( $C_{PDMS}$ ) coating by the total concentration in the extract ( $C_{TISSUE}$ ). Separately a time series experiment was conducted to confirm that equilibrium had been achieved. Finally field contaminated worms were analysed for PAHs via the novel SPME method and the determined  $K_{PDMS/TISSUE}$  coefficients were used to calculate the total concentration in the worm. These results were compared with results found via the classical extraction method and with the tissue concentration calculated out of  $C_{TISSUE}$  and the bioconcentration factor (BCF) and the outcome is presented here. Although further research on this topic is relevant, our novel approach revealed an encouraging future prospective for the application of passive sampling applied on biological tissue.

**TH054 Kinetics of membrane dialysis extraction of pyrene, phenanthrene and chrysene from n-hexane and cow milk** C. Schuer, RWTH Aachen University / Institute for Environmental Research (Biology V); M. Brinkmann, RWTH Aachen University Institute for Environmental / Department of Ecosystem Analysis; A. Schaeffer, RWTH Aachen University / Chair of Environmental Biology and Chemodynamics; H. Hollert, T. Seiler, RWTH Aachen University / Institute for Environmental Research (Biology V). Membrane devices have gained importance in many different scientific fields. The applications in passive sampling and extraction are of particular interest for ecotoxicology and especially for the assessment of toxic potentials. Membrane dialysis extraction (MDE) uses semi-permeable membrane "layflat" tubing (low-density polyethylene) into which the sample is applied. The tubing is placed inside an acceptor solvent (hexane), hence utilizing a concentration gradient as the driving force for extraction. The compounds investigated were three polycyclic aromatic hydrocarbons (PAH): pyrene, chrysene and phenanthrene. The focus was to better understand the substance properties influencing the timely manner of diffusion. Extractions were performed on 2 different matrices, hexane and milk. Half-times and recoveries were derived from the resulting data. MDE achieved extraction efficiencies for PAH ranging between 83.9 % and 96.3 %. No distinct correlation was found between analyte molecular weight, molecular volume or  $\log(K_{ow})$  and the half-times. Molecular structure (aromatic ringsystems, sterical extensiveness) appeared to be the most influential property on extraction kinetics. Extractions with milk as a matrix gave increased recoveries, likely as the result of altered volume ratios between n-hexane inside/outside the tubing and therefore shifted equilibrium concentrations. The data pointed towards lipophilicity as a major influence besides the structure regarding the half-time-increasing effect of milk. MDE showed good results regarding close-to-exhaustive extraction for PAH. Results indicate that extraction kinetics likely depends on the analyte's molecular structure. Further investigations have gone into researching more different PAHs, environmental relevant concentrations and alterations in the extraction process. Data are expected to reveal a clearer pattern of the influential properties and therefore enable predictions of extraction behaviour. This might render MDE applicable for fractionated extraction. Other matrix materials (tissue, sediment) should be investigated to prove applicability in other areas of ecotoxicology.

**TH055 Passive Samplers for Phytoforensics Analytics: Delineating Solvent Contamination via Novel Plant Sampling and Analysis** J.G. Burken, Missouri University of Science and Technology / Civil Architectural and Environmental; M.A. Limmer, Missouri University of



Science and Technology / Civil, Architectural, Environmental Engineering; M. Shetty, Missouri University of Science and Technology / Environmental Engineering. Rapid, inexpensive detection of contaminants in our biosphere is important to protect human health from fugitive contaminants. Effective subsurface plume delineation is an ongoing challenge due to the traditionally cumbersome, time, money and labor intensive techniques. Vegetation growing on sites can be used as biosensors for detection and sampling of subsurface contamination as plants actively extract all water and nutrients needed from the subsurface and simultaneously accumulate numerous contaminants. The development of innovative methods in order to effectively use trees as sources of information leading to accurate delineation of the plume boundary and size is a worthy endeavor, to protect human health. This study developed innovative techniques using polymeric solid phase samplers (SPSs) and solid phase microextraction (SPME) fibers as *in planta* passive sampling devices. SPSs characteristics tested included a high material:air partitioning coefficient for chlorinated solvents PCE, TCE, cDCE and chloroform, rapid equilibration time once placed *in planta*, and a degree of reproducibility regarding field performance. Of the 6 materials tested, polydimethylsiloxane (PDMS) sampler appears to most rapidly reach equilibrium *in planta*, within 4 days of deployment. Linear low density polyethylene (LLDPE) sampler demonstrated the highest partitioning. The polyvinylchloride (PVC) sampler also shows favorable characteristics. All results were validated both in greenhouse experiments as well as multiple on-site field trials. SPME fibers were used in an alternative *in-planta* approach, looking for rapid, reproducible analysis in specially designed ports. Testing has shown that PDMS-SPME can be operated as an equilibrium passive sampling device or and that carboxene- and divinylbenzene-SPME can also be deployed for time weighted average (TWA) analysis with increased sensitivity. This approach opens an entirely new approach to analysing trees repeatedly. Long term monitoring can now allow assessment of contaminant removal rates by plants in phytoremediation systems and literally turns trees into long term monitoring (LTM) portals to the subsurface. The results obtained indicate that SPSs and SPME prove to be applicable passive sampling devices for *in planta* analysis for phytoforensics to supplement initial site investigations while simultaneously incorporating decreased costs, simple operations and minimal impact to the surrounding property and environment.

**TH056 Using flow-through samplers to measure pesticide vapour drift** T.S. Geoghegan, K.J. Hageman, University of Otago / Chemistry; A.J. Hewitt, Lincoln Ventures Limited. Pesticides play an important role in maintaining crop health; however, volatilisation and subsequent vapour drift reduce a pesticide's efficiency and contribute to environmental contamination. Previous studies to understand these processes have used high-volume air samplers, which are expensive to purchase and require network power, limiting the number and type of sites where they can be deployed. The flow-through air sampler (FTS) is a passive sampler that has recently been developed to quantify organic contaminants in remote ecosystems. FTS's differ from other passive samples in that they turn into the wind and use the Venturi effect to draw air through the sampling medium, producing comparable sampling rates to active samplers. The objective of this work was to test the FTS in a near-field pesticide vapour drift study by comparing the air concentrations of pyrimethanil measured by the FTS to those measured by a high-volume sampler. Pyrimethanil was sprayed onto a vineyard as part of normal pest management procedures. Air samples were collected every eight hours for 48 hours after spraying using one high-volume air sampler and three FTS. Both types of samplers contained polyurethane foam sampling media that was extracted using accelerated solvent extraction and analysed with gas chromatography-mass spectrometry (GC-MS). Solid phase microextraction (SPME) was used as an additional clean-up step with the FTS extracts. The volume of air sampled was calculated using the measured relationship between ambient wind speed and the wind speed inside the sampler as determined with a separate wind tunnel study. The FTS sampled 2.8 to 55 m<sup>3</sup> of air in each 8-hour sampling period, depending on wind speed. The total volume sampled was 214 m<sup>3</sup> in 48 hours; this was 27.7% of

the air sampled by the high-volume sampler. Pyrimethanil concentrations recorded with the FTS ranged from 0.35 to 2.38 µg m<sup>-3</sup> of air. The highest concentrations were recorded during the 8-16 time period during which a suspected temperature inversion occurred. This work shows that the FTS is suitable for near-field applications but that caution should be applied if used with very low wind speeds. With this criterion met, FTS technology could make multi-sampler experimental designs more feasible as they are comparatively inexpensive to build and operate and do not require network power.

**TH057 Calibration of a Passive Air Sampler for Volatile Methyl Siloxanes** L.S. Krogseth, Norwegian Institute for Air Research; X. Zhang, University of Toronto / Environmental Sciences; Y.D. Lei, University of Toronto at Scarborough; F. Wania, University of Toronto at Scarborough / Department of Physical & Environmental Sciences; K. Breivik, Norwegian Inst for Air Research. The atmosphere is a key compartment for understanding the environmental fate and behavior of volatile methyl siloxanes (VMS). Due to their fairly high volatility, VMS are mainly emitted to, and largely remain in, the atmosphere where they are relatively persistent and subject to long-range transport. However, also because of their high volatility, the applicability of standard air sampling methods to VMS cannot be assumed and must be proven. The purpose of this study was to evaluate the suitability and to calibrate an existing, polystyrene-divinylbenzene co-polymeric resin based passive air sampler (XAD-PAS) for VMS. The XAD-PAS has been tested and applied for different compounds and environmental conditions, but has not yet been calibrated for more volatile compounds like VMS. To achieve this, a number of samplers were deployed at a suburban site in Toronto, Canada, and collected after different time points. Simultaneously the VMS concentration in air at the sampling site was measured using an active air sampling method based on solid phase extraction cartridges, previously validated and applied for VMS. The uptake in the XAD-PAS of octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and three linear VMS was linear throughout the whole deployment period of 98 days. The sampling rate was approximately 0.5 m<sup>3</sup>/day for all congeners. For hexamethylcyclotrisiloxane (D3) and dodecamethylcyclohexasiloxane (D6) the blank levels were too high relative to the concentrations of D3 and D6 in air at the site to assess the uptake in the sampler. Subsequent to the calibration study, XAD-PAS samplers were deployed at 26 sites in Toronto and the surrounding area to evaluate the influence of both population density and proximity to sewage treatment plants (STPs) on urban atmospheric levels of VMS. The sites could mainly be binned into three groups; two STP sites with strongly elevated levels of VMS in air, the urban sites with intermediate levels; and three rural background sites with very low levels of VMS.

**TH058 Calibration of passive air samplers for monitoring halogenated flame-retardants and phthalates concentration in indoor air.** A. Saini, University of Toronto / Department of Physical and Environmental Sciences; S.R. Chaudhuri, E. Goosey, M.L. Diamond, University of Toronto; M. Shoeib, Environment Canada. Flame-retardants are halogenated or non-halogenated chemicals that are added to resins or polymers to reduce their flammability. Phthalate esters are one of the highest production chemicals in the world, primarily used as plasticizers. The indoor environment, with its limited air exchange rate and degradation pathways, holds a large inventory of products and materials that contain these compounds. Phthalate air concentrations have been determined via active sampling and, to the best of our knowledge, this is first use of passive samplers to measure indoor air concentrations of phthalate esters. Reasonable measurement of air concentration using passive samplers depends upon their proper calibration. We report on calibrating polyurethane foam (PUF) and sorbent impregnated PUF (SIP) passive samplers that were deployed in an indoor environment at the University of Toronto for up to 35 days along with a low volume pump. Sampling media from the low volume pump was exchanged at weekly intervals to obtain a time weighted air concentrations of the target compounds. To determine the sampling rates of each compound, the equivalent air volumes sampled by each PUF and

SIP disk was calculated over a given exposure period using mass of the compound sequestered by the sampling media within the deployment period and concentration of the target analytes. SIPs show higher concentration of analytes due to better sorbing capacity than PUFs (Shoeib et al, 2008). The study follows previous indoor air calibration studies done by Harrad and Abdallah (2008) and Hazarti and Harrad (2007).

#### **TH059 Enhancing polycyclic aromatic hydrocarbon (PAH) monitoring of Lake Superior using polyethylene passive samplers**

**Z. Ruge**, URI Graduate School of Oceanography; **R. Lohmann**, University of Rhode Island / Graduate School of Oceanography. Polyethylene passive samplers were deployed simultaneously in both near surface air and water at 19 coastal and mid-lake sites around Lake Superior from April-October, 2011. The deployments were separated into three time periods to determine distinctions in concentrations and flux rates at different times of the year. Overall, 22 polycyclic aromatic hydrocarbons (PAHs) were analyzed. Trends clearly followed populations centers, with the highest concentrations and diversity of PAHs found at Sault Saint Marie, Marquette, Duluth, and Ashland. Acenaphthene, fluorene, and phenanthrene dominated the air concentrations, while water samples also exhibited significant concentrations of methyl phenanthrenes, fluoranthene, and pyrene. Overall air-water flux trends appear to be from atmospheric deposition into the lake, but this was not the case for all compounds, especially at sites away from urban centers. Ashland and northern sites exhibited the highest concentrations of Retene, a product of forest fires, with the highest overall concentrations in the late summer months. Increased passive sampling coverage and frequency has the potential to significantly enhance our understanding of PAHs and other persistent organic pollutants in the Great Lakes region.

#### **TH060 Polyethylene as passive samplers of dissolved organic pollutants in air and water**

**R. Lohmann**, University of Rhode Island / Graduate School of Oceanography; **P. Luey**, **Z. Ruge**, **M. Haynes**, **M. Khairy**, URI. Passive sampling of trace organic compounds has become a widely accepted means of measuring concentrations of truly dissolved compounds in water and atmosphere. As compared to other matrices for passive sampling, polyethylene (PE) is the simplest (in its chemical make-up) and cheapest polymer available. This contribution focuses on PE sheets as simple samplers of hydrophobic trace organic contaminants both in atmosphere and water. PE-water equilibrium partitioning constants,  $K_{PEW}$ , were reviewed for trace hydrophobic organic contaminants (HOCs). Relative standard deviations were < 30% for phenanthrene, anthracene, fluoranthene and pyrene implying excellent reproducibility of  $K_{PEW}$  across laboratories and PE sources. Averaged  $K_{PEW}$  values of various HOCs were best correlated with aqueous solubility,  $\log C_{sat}(L)$ :  $\log K_{PEW} = -0.99(\pm 0.029)\log C_{sat}(L) + 2.39(\pm 0.096)$  ( $r^2=0.92$ ,  $SE=0.35$ ,  $n=100$ ). For 80% of analytes, this equation predicted  $\log K_{PEW}$  within a factor of 2. A first-order estimation of  $K_{PEW}$  can be obtained assuming constant solubility of the compounds in the PE, such that the variation in  $C_{sat}(L)$  determines the differences in  $K_{PEW}$ . For PE samplers,  $K_{PEW}$  values do not change with the thickness of the PE sampler. There seems to be no effect of pressure on  $K_{PEW}$  values, suggesting that it can be used at various depths in the oceans. The effect of salt is rather well understood, using a Schetschenow-style approach. The air-PE partitioning constant,  $K_{PEa}$ , can be approximated as the ratio of  $K_{PEW}/K_{aw}$  (the air-water partitioning constant). Recently published experimental  $K_{PEW}$  values were shown to correlate well with those predicted. Additional work shows that PEs look promising as samplers of a much wider range of gas-phase pollutants.

#### **TH061 Estimating freely dissolved concentrations of petroleum hydrocarbons using passive samplers and GC x GC**

**G. Tcaciacu**, Massachusetts Institute of Technology / Department of Civil and Environmental Engineering; **R.K. Nelson**, Woods Hole Oceanographic Institution / Department of Marine Chemistry and Geochemistry; **J.K. MacFarlane**, Massachusetts Institute of Technology / Department of

Civil and Environmental Engineering; **C.M. Reddy**, Woods Hole Oceanographic Institution / Department of Marine Chemistry & Geochemistry; **P. Gschwend**, Massachusetts Institute of Technology / Department of Civil and Environmental Engineering. Hydrophobic organic chemicals often occur in the environment as complex mixtures, for which identification and quantification of each component is not feasible. For example, unresolved complex mixtures (UCMs) are commonly found at petroleum contaminated sites; and such hydrocarbons are known to cause narcosis toxicity in an additive fashion. An accurate evaluation of the risk associated with such sites must take into account the cumulative effects of all the chemicals in the UCM, but identification and characterization of all the UCM compounds may not always be feasible. To that end, we developed a methodology for inferring pore water concentrations of various petroleum compounds in contaminated sediments by using comprehensive two-dimensional gas chromatography (GC x GC) and polyethylene (PE) passive sampling. In short, the passive sampler is thoroughly mixed with the contaminated sediment, and the sampler extract is analyzed by GC x GC coupled with a flame ionization detector (FID). Firstly, by using an FID detector, which has a relatively constant response factor for hydrocarbons, we can quantify various UCM components in the absence of a standard for each of them. Secondly, using literature data and additional equilibration experiments, we have developed a training set of compounds with known PE-water partition coefficients ( $K_{PEW}$ ), which we then used to develop a linear relationship between the retention time in the GC x GC chromatogram and the logarithm of  $K_{PEW}$ . Using this expression, we can estimate the  $K_{PEW}$  for all the compounds in the chromatogram of a passive sampler, and calculate the corresponding pore water concentration at equilibrium. Lastly, to validate this approach of assessing pore water concentrations, we have compared GC x GC chromatograms of passive sampler and pore water extracts, and we obtained good agreement in the areas where both chromatograms showed concentrations above the detection limit. This work presents a novel application of *ex situ* passive sampling and GC x GC which is useful for the characterization of the environmental hazards of petroleum mixtures.

#### **TH062 Mapping bioavailability onto GCxGC chromatograms of complex mixtures containing bioaccumulative analytes**

**D. Nabi**, EPFL / IIE; **J. Gros**, EPFL, Switzerland / IIE; **P. Dimitriou-Christidis**, S.J. Arey, EPFL, Switzerland. Environmental partitioning properties control bioavailability and transport of organic compounds in natural and engineered systems. However, reliable property data is often not available for the hundreds to thousands of potentially bioaccumulative contaminants found in complex mixture of nonpolar analytes. In the present study, we showed that these partitioning properties could be mapped onto the separation space offered by comprehensive two-dimensional gas chromatography (GCxGC). We used GCxGC retention indices to estimate environmental partitioning properties for a diverse set of non-polar compounds which undergo negligible hydrogen bonding interactions. Environmental properties such as vapor pressure, water solubility, air-water partition coefficient, octanol-air partition coefficient, and octanol-water partition coefficient were estimated with root mean square errors (RMSEs) ranging from 0.26 to 0.44 log units. Properties relevant to bioavailability such as air-hexadecane, air-passive sampling polymer, water-passive sampling polymer, water-biomembrane, water-dissolved organic matter, and water-sediment organic carbon partition coefficients were fitted with RMSEs ranging from 0.15 to 0.45 log units. Property maps overlaid onto GCxGC chromatograms can help in modeling the bioavailability and mass transfers of large number of nonpolar analytes found in complex mixtures. Thus, GCxGC can be used as a powerful tool for the risk assessment and fate modeling for complex mixtures of nonpolar organic compounds in environmental media. **Keywords:** GCxGC, environmental partitioning properties, fate modeling, bioavailability

#### **TH063 Silicone rod passive sampling – partition coefficient measurement and LSER modelling**

**R. Gunold**, Department of Ecological Chemistry; **R. Speer**, Martin Luther University of Halle-

Wittenberg / Institute for Chemistry - Food Chemistry and Environmental Chemistry; S. Naumann, Dresden University of Applied Sciences / Faculty of Mechanical Engineering/Process Engineering; A. Paschke, Helmholtz Centre for Environmental Research (UFZ); G. Schuurmann, Helmholtz Centre for Environmental Research UFZ / Department of Ecological Chemistry. Passive sampling gained increasing reception in the monitoring of aquatic ecosystems during the last decades [1]. By offering a cost-competitive alternative to traditional spot sampling approaches required by legal authorities, environmental monitoring can be accomplished satisfactorily. For hydrophobic compounds with a log  $K_{ow}$  larger than 4, silicone rubber passive sampling devices have proven useful as monitoring devices in recent years [2]. Nevertheless, they have not yet obtained widespread acceptance, also due to the need for a laborious and time-consuming pre-treatment and reprocessing of the silicone polymer [3]. Advances in chromatographic analytical techniques nowadays facilitated the development of silicone rubber receiving phases being directly analysed by obtaining the sample without time-consuming solvent extraction over a thermic desorption unit directly into the GC-MS analytical system [4]. For the calculation of equilibrium and time-weighted average concentrations with the bare silicone rod, silicone-water partition coefficients are necessary. Since the applied silicone differs from other silicone polymers described in literature, the relevant  $K_{ow}$  values were not available, and thus have been measured for a variety of priority pollutants. In addition, the newly determined  $K_{ow}$  values were used as basis for deriving a respective LSER (linear solvation-energy relationship) model following the Abraham approach [5], enabling the  $K_{ow}$  prediction for compounds not yet investigated that meet the model application domain. This poster explains the experimental setup, compares the newly derived  $K_{ow}$  values with their  $K_{ow}$  counterparts, and discusses the scope of the LSER-based  $K_{ow}$  prediction model.

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**TH064 Sorption of various polar chemicals to different sorbents and membrane materials applied in passive sampling** F. Smedes, DELTARES / RECETOX; H. Beeltje, TNO; M.T. Jonker, Utrecht University; M. Kotte, RWS. Several different passive samplers, including POCIS and Chemcatcher are applied for the sampling of polar chemicals in the environment. All of these samplers contain an adsorption material for the accumulation of the target compounds, which is separated from the water phase by a membrane or filter. Compounds adsorbing from the bulk water phase therefore need to pass the aqueous boundary layer as well as the membrane or filter in order to reach the adsorbent material. Furthermore, compounds adsorbed to the first layer of sorbent particles will need to diffuse to deeper layers. Hence, knowledge on adsorption to and diffusion through the different layers is a prerequisite for understanding the uptake of chemicals in polar passive samplers. In this study, we measured the adsorption of various polar compounds to 6 sorbent materials and 4 types of membranes/filters. Further, the diffusion rate of the compounds through the membranes was measured, providing an estimate for the transport resistance. For the adsorption materials, the sorbent

**TH065 Adsorption of polar (un)charged micropollutants – Effect of the molecular structure and the water matrix on the adsorption** Bauerlein, J. Mansell, KWR; S. Droge, University of Utrecht - IRAS; R. Hofman-Caris, T. ter Laak, P. de Voogt, KWR. Adsorption of polar (charged) organic solutes from the water phase onto solid matter is a crucial process in the environment, water purification and in analytical science. The ability to adsorb to and absorb into a material dictates if a compound can be concentrated on a SPE material. Many solutes are polar or even charged. Adsorption of these compounds to SPE materials can be a promising way to help detecting them, as concentrations are

often still low and preconcentration can overcome this problem. Many materials have been employed for this purpose, but little is still known about the exact sorption mechanism of these compounds. The role of functional groups of these solutes and the SPE materials is still ambiguous. How do they interact with each other and what is the part of water in the adsorption process? Apart from that the effects of other compounds, such as inorganic salts, in the aqueous phase on the sorption behaviour is not yet well-understood. This knowledge can also be used for the removal of these compounds from drinking water. Aim of the research is to get a better insight into the influence of various functional groups of selected chemicals and SPE materials on the sorption to be able to take a grounded decision which SPE material should be used. In case of charged organic compounds, additionally the impact of competing inorganic electrolytes was monitored. We decided to use OASIS polymers as SPE material. These polymers carry polar moieties, hydrophilic parts as well as charged groups, which should allow the adsorption of the target compounds. The results of the batch experiments were also put to the test in more complex samples to see if they hold true under more realistic conditions. The results of this research indicate that especially apolar functionalities have a great impact on the sorption, whether the compound is charged or not. The more pronounced the apolar moiety is, the better the compound can adsorb. However, the results show that there is no direct correlation between solubility and adsorption behaviour. Furthermore, it emerged that the conditions of the aqueous phase, such as salt concentration, influence the sorption behaviour of charged molecules dramatically. The higher the salt concentration is, the lower the adsorption of the charged compounds. Apart from concentration also the type of ion is important. Experiments using a more realistic water samples show that the results can be used to predict - at least qualitatively - if a compound can be adsorbed.

**TH066 Method development for extracting small cationic compounds (illicit drugs) using a new coating in solid-phase microextraction** H. Peltenburg; F. Groothuis, S. Droge, Institute for Risk Assessment Sciences, Utrecht University; I. Bosman, Netherlands Forensic Institute; J. Hermens, Institute for Risk Assessment Sciences, Utrecht University. Solid-phase microextraction is a passive sampling technique with several advantages over existing sampling methods. Whereas commonly used coatings such as polyacrylate (PA) are capable of extracting neutral compounds, charged compounds do not sorb well. Hence, sorption of these compounds requires a matrix modifying step. However, this is not recommended for all compounds or not possible in every matrix. We introduce a new coating (C18/propylsulfonic acid or C18/SCX) which possesses both cation exchange groups and hydrophobic components. This coating is biocompatible and can be applied *in vivo*. Our aim is to characterize sorption to the fiber for several cationic compounds. Here, we investigate the sorption of amphetamine as a model compound to establish a proof of principle for the C18/SCX fiber. The efficiency of the new C18/SCX fiber was compared to PA fibers. Sorption of amphetamine was determined in buffered solutions at physiological pH. Fibers were exposed at equilibrium, which was 2 hours for C18/SCX and 24 hours for PA. Amphetamine was desorbed from PA fibers with HPLC mobile phase (12% acetonitrile/88% phosphate buffer pH3). C18/SCX fibers were desorbed using 90% acetonitrile with 1% NH<sub>4</sub> in H<sub>2</sub>O which was acidified with 0.1 M HCl before injection on HPLC-UV. Over a pH range between 2 and 12, for PA a common S-curve is observed with very low sorption coefficients for charged amphetamine (log K<sub>fw</sub> -1.5) and high sorption coefficients for the neutral compound (log K<sub>fw</sub> 1.8). Sorption coefficients for the C18/SCX fiber are nearly constant over the whole pH range (log K<sub>fw</sub> 2.3-3.1). Comparison of the sorption at physiological pH shows much higher sorption coefficients, increased sensitivity and a shorter time interval to equilibrium for the C18/SCX compared to the PA fiber. Competition for sorption sites occurred between charged amphetamine and other cations and is correlated to the ionic strength and the ion's hydrated radius. The new C18/SCX fiber has several benefits over the conventional fibers such as PA, especially for small/polar cationic compounds at physiological pH. The fiber can be used over a wide pH range, as it can sorb both cationic and neutral

species. Future work will focus on the characterization of the fiber for other cationic compounds and expanding to biological fluids as a first step towards *in vivo* experiments. Prototype C18/SCX fibers were provided by Robert Shirey from Supelco, Sigma Aldrich (Bellefonte, PA, USA).

#### **TH067 Evaluation of the Pesticide Exposure in Surface Waters with Two Sampling Strategies: Active and Passive Sampling**

**C. Moschet**, Eawag / Uchem; H. Singer, Eawag; E. Vermeirssen, Oekotoxzentrum; J. Hollender, Eawag. Monitoring of polar organic pollutants in surface waters by passive sampling is considered more and more as an alternative to active water sampling.

Poly(styrenedivinylbenzene) copolymer (SDB) disks within Chemcatcher devices have been shown to be a promising receiving phase due to uptake of a broad spectrum of compounds with a suitable kinetic regime. However, using SDB-disks it is difficult to calculate time weighted average (TWA) concentrations. Although for some substances sampling rates have been determined in the laboratory, environmental factors during field exposures (e.g. flow velocity, biofouling) affect the uptake process. The aim of this study was to evaluate the performance of SDB-disks and active water sampling in a large field study for the screening of 50 organic pesticides. Five small agriculturally influenced streams were sampled from March to July 2012. Water samples were taken in two-week time-proportional composites (TPC). During the same time slots, SDB-disks covered with a polyethersulfone (PES) membrane were deployed at the same locations. Water samples were enriched with an offline solid phase extraction (SPE) using a mixed-bed multilayer cartridge. SDB-disks were extracted in acetone and methanol. Analytical measurement of both sample types was carried out using liquid chromatography with electrospray ionisation and high resolution mass spectrometry (LC-ESI-HRMS/MS). Extraction of SDB-disks required less effort (easier and faster) than water extraction using SPE. A qualitative assessment showed that, using SDB-disks, insecticide detections doubled and 50% more fungicide detections were achieved. This resulted from slightly lower detection limits with the SDB-disks due to fewer matrix effects. However, this is only relevant for substances that are frequently detected close to the detection limit (e.g., insecticides). A quantitative evaluation could be performed for half of the analysed substances. TWA concentrations were calculated using a generic sampling rate taken from literature data. Twenty out of 25 substances showed a good correlation between TPC and TWA concentration. However, the divergence of the concentrations were large. Only for 4 substances, the divergence between TPC and TWA concentration was less than a factor of 2 for less than 25% of all samples. To conclude, passive sampling is a very powerful tool for semi-quantitative screening. However, when exact quantification is needed, active sampling is essential.

#### **TH068 Different Approaches for the Monitoring of Pharmaceuticals and Personal Care Products in Waste Water**

**G. Fedorova**, University of South Bohemia in CB / Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses; O. Golovko, University of South Bohemia in CB / Lab of Environmental Chemistry Biochemistry; R. Grabic, University of South Bohemia in CB / Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses. Pharmaceuticals and personal care products (PPCPs) are introduced to the water environment by anthropogenic inputs, being only partially metabolized by human body. Such compounds are not effectively removed by waste water treatment plants (WWTP). Therefore, PPCPs are detected in WWTP effluent, consequently reaching surface waters. Among the sampling methods, spot sampling is the most frequently used one. The main disadvantage is that the information obtained from the sample is unique to the place and the time selected. To obtain more representative data automatic samplers can be used. Another option is passive sampling, which is less sensitive to accidental variations of the pollutant concentration and gives time-weighted average (TWA) concentrations. The application of two different approaches for the monitoring of waste water pollution was

evaluated. Content of 130 PPCPs was measured in both time proportional pooled water samples taken by automated sampler and extracts from 2 configurations of POCIS samplers. Passive sampling was advantageous regarding the limits of detection: more than 50 PPCPs were detected only in POCIS extracts but not in pooled water samples. One of the probable reasons for that could be loss of target analytes during the storage. In case of waste water, storage and preservation of the sample could be of great importance in order to get data that will reflect the real situation. Storage at higher temperatures can enhance bacterial growth in solution, resulting in losses of target analytes. Different regimes of storage were tested: fridge (+4

#### **TH069 A Passive Sampler for in situ Measurement of Pharmaceutical and Personal Care Ingredients in Water**

**W. Chen**, C. Chen, H. Zhang, K.C. Jones, Lancaster University / Lancaster Environment Centre; O.R. Price, Unilever / Safety and Environmental Assurance Centre; G. Ying, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences; N. Xu, Peking University Shenzhen Graduate School / School of Environment and Energy; H. Li, A.J. Sweetman, Lancaster University / Lancaster Environment Centre. Pharmaceutical and personal care products (PPCPs) contain a diverse group of emerging chemicals which have generated interest with both scientists and the public. As a result of their high consumption rates and continuous release into aquatic environments, they can achieve relatively steady state concentrations in the environment. However, the environmental fate and effects of these chemicals are poorly understood, in particular the bioavailable fraction and risks these chemicals may pose to aquatic organisms and humans via environmental exposure. A novel passive water sampler based on the theory of the diffusive gradients in thin films (DGT) has been developed for *in situ* sampling for a subset of chemicals, particularly, parabens, phenols and estrogens. The sampler provides a quantitative and time-integrated measurement of chemical concentration in aqueous systems without field calibration. Laboratory testing and performance characteristics of organic-DGT (o-DGT) have been carried out, with methylparaben (MeP), propylparaben (PrP) isopropylparaben (iPrP), ortho-phenylphenol (OPP), butylated hydroxyanisole (BHA), bisphenol-A (BPA), estrone (E1),  $\beta$ -estradiol (E2), estriol (E3), 17 $\beta$ -ethinylestradiol (EE2) and triclosan (TCS) as model compounds. The capacity of three types of binding resins (XAD18, HLB and SXA) have been tested and compared. Agarose gel (AG) was selected as the most suitable diffusive layer as it did not significantly adsorb the test substances. Uptake of chemicals by o-DGT increased with exposure time and with the inverse of diffusive layer thickness (0.25mm-2mm). o-DGT performance under different conditions, such as pH (4-9), ionic strength (0.001M-0.5M) and organic matter (0-8mg/L), has also been evaluated. *In situ* field measurements have been compared to grab samples collected in natural waters and wastewaters to determine the potential application of these novel passive samplers.

**TH070 Laboratory calibration of the POCIS and application to the passive sampling of 40 pesticides in rivers of an agricultural watershed in south of France** **g. poulier**, Irstea / Unité de recherche REBX; C. Adeline, S. Lissalde, R. Buzier, P. fondaneche, E. Renaudie, Université de Limoges / Groupement de recherche eau sol environnement; N. Mazzella, Irstea / Unité de recherche REBX; G. Guibaud, Université de Limoges / Groupement de recherche eau sol environnement; F. Delmas, F. Delmas, B. Delest, A. Moreira, G. Jan, S. Moreira, Irstea / Unité de recherche REBX. Pesticides have been widely used in agriculture since the 1950s to improve productivity. However, a part of these compounds is often driven to water bodies via hydrological processes such as runoff, leading to a large and diffuse contamination of aquatic environments, with possible toxic effects to biota. During the last decades there has been an increasing concern about the fate of pesticides in water bodies, as shown by the implementation of the European Water Framework directive (2000/60/CE). This legislation involves an efficient monitoring of water quality, what is not yet possible with conventional methods like analysis of grab samples, due to low sampling frequency and inadequate limits of

detection for some priority compounds. An answer could be the use of passive sampling devices like the polar organic chemical integrative sampler (POCIS). POCIS has been proven to be a very useful tool for screening, but a laboratory calibration step is necessary when quantitative data like time weighted average concentrations are needed. In our study we calibrated POCIS for 32 pesticides and 8 metabolites, commonly encountered in rivers. After this calibration step, several triplicates of POCIS have been successively exposed in three different rivers of an agricultural watershed in the south-west of France, over a period of 6 months (from March to September 2012). We observed high levels of metolachlor, an herbicide widely used for the treatment of corn and sunflower crops. Spring was identified as the most hazardous period for water quality, probably because of the succession of herbicides treatments and intense runoff after huge rain events. POCIS was able to integrate short variations of compounds concentrations, even for unexpected events like spates. In some cases we were also able to deduce the geographical origin of a contamination thanks to an adequate repartition of our POCIS on the watershed.

**TH071 POCIS Calibration for pesticide monitoring : from lab to in-situ experiments** a. togola, BRGM / Laboratory Division; I. Ibrahim, BRGM / Ecole des Mines d'Ales; C. Gonzalez, Ecole des Mines d'Alès. In order to estimate the water concentrations of pollutants from accumulated amounts in the sampler, laboratory or in situ calibration data are required in order to estimate the sampling rate (Rs) for each compound. The sampling rate of passive samplers depends on the physicochemical properties of the chemicals and the environmental conditions, such as temperature, water flow rate/turbulences and dissolved organic carbon. The challenge is to obtain TWA concentrations which are sufficiently representative of the real pollution levels in the aquatic medium. This goal is mainly dependent on the calibration of the passive sampler, generally conducted under controlled conditions at laboratory scale. However, as field environment is very different from laboratory conditions, use of inappropriate laboratory derived sampling rates for calculating TWA concentrations from passive samplers exposed in situ could lead to an inaccurate result of the real pollution levels. The aims of the present work were to study the uptake kinetics in surface water of a range of polar pesticides and metabolites by pharmaceutical POCIS samplers in order to determine sampling rates by in-situ calibration, to compare results with those obtained under laboratory conditions in order to assess the impact of environmental conditions on POCIS field performances. Finally, the objective is to evaluate the effectiveness of POCIS to determine TWA concentrations in the aquatic medium in comparison with the classical spot sampling methodology. The in situ experiment was conducted with samplers deployed in channel pilot system, an artificial irrigation canal bringing water from the Rhône River. Beside the numerous targeted pesticides, 13 compounds were detected in water samples including triazines, phenylureas, conazoles, chloroacetanilides, phenylamides and triazines metabolites, allowing the comparison between lab and *in situ* experiments. Accumulation during the 15 days exposure is linear for all compounds except DIA. For most of the compounds, the in-situ sampling rates were significantly lower by a factor of 3-5 than those from laboratory experiment, considering that field measured water velocity was 4 time lower than laboratory, the main effect of flow velocity on accumulation capability of POCIS is highlighted. By the measurements of spot sampling all along passive sampler exposure, integration of pollution event by POCIS has been demonstrated on metolachlor.

**TH072 Evaluating the use of performance reference compounds in polyoxymethylene passive samplers** B. Beckingham, University of Tuebingen / Civil & Environmental Engineering; J. Zhang, P. Grathwohl, University of Tuebingen / Center for Applied Geoscience. To determine environmental concentrations from passive samplers using simple partitioning coefficients, chemical sorption has to reach equilibrium. The time required to reach sorption equilibrium in the field can be a disadvantage, and furthermore the time to equilibrium is difficult to predict for compounds whose uptake is controlled by the

stagnant boundary layer because it can depend on site-specific conditions such as the degree of bio-fouling and wind or water flow. To overcome this, researchers may incorporate performance reference compounds (PRCs) into the sampler material and measure the loss of these compounds during exposure to track the extent to equilibrium, then applying models to develop a correction factor. This approach relies on the assumption that the PRCs are distributed evenly in the sampler and are lost from the sampler at the same rate as environmental chemicals are taken up, which has not been rigorously validated for many samplers currently in use. PRCs may appear to be released at a slower rate than other compounds from solution are being taken up, known as hysteresis, if there is a physical change or deformation to the polymer phase resulting from the loading process or if the PRCs are not initially homogeneously distributed within the sampler. In this study, validation of the PRC approach for polyoxymethylene (POM) film passive sampler material is evaluated in two ways. First, the uptake of target analytes and loss of PRC model compounds in POM is tracked simultaneously in batch tests using a passive dosing method to test the assumption that sorption and desorption is isotropic. Further, internal distribution of a fluorescent dye loaded in the POM sampler material such as done for PRCs is visualized over time during desorption using confocal laser scanning microscopy. Knowledge of the intricacies of the sorption and diffusion processes of organic chemicals to passive samplers and how methodologies may influence measurements will impart greater confidence in the proper use of these tools, which show exceptional promise for reliable assessment of the ecological and human health risks that chemicals may pose in the environment.

**TH073 Linking algal toxicity with freely dissolved concentrations of organic contaminants in the Firth of Forth Scotland** E.S. Emelogu, The Robert Gordon University Aberdeen / Engineering. The Firth of Forth in the east coast of Scotland receives inputs of a diverse range of trace organic contaminants from petrochemical, agro-chemical and pharmaceutical industries and discharges of urban waste water. Diffuse input through agricultural, urban and riverine run-off further adds to the contaminant pressure in the estuary. Due to recent legislation, direct discharges of organic materials into the Firth of Forth are in the decline. As a result, in addition to the low aqueous solubility of organic contaminants and dilution factors, the concentrations of most organic compounds monitored in the water phase using conventional sampling techniques are often very low. This, however, does not exclude their potential to cause toxicological effects on sensitive organisms, owing to their persistent, bioaccumulative and toxic potentials. Further, chemical analysis cannot assess the bioavailability or the combined toxicity of all the identified and unknown compounds to which organisms are exposed. In this study, silicone rubber passive sampling devices (SR-PSDs) were deployed in water at five sites along the estuary for ~2 months. Extracts from the deployed SR-PSDs were assessed for both algal growth inhibition and environmental exposure of wide range of organic contaminants including PAHs, PCBs and pesticides. Algal toxicity of the complex mixtures of freely dissolved organic contaminants were assessed on a native algal strain *Pavlova lutheri* in 24 well multititre plates for 72 hr. Time weighted average concentrations ( $C_{TWA}$ ) of freely dissolved (e.g. bioavailable) fractions of 40 PAHs and 32 PCBs were calculated using performance reference compounds (PRCs). The algal toxicity tests exhibited varied effects at the five sites suggesting the presence of algal growth inhibiting compounds in the estuary. Dissolved concentrations of the individual contaminants measured did not differ significantly at the five sites. However, slightly higher total dissolved concentrations were measured from the upper estuary to the inner Firth of Forth suggesting the influence of dilution. Further, pesticides of diverse polarities were identified in the extracts indicating sources from agricultural and riverine run-off or discharges from the agro-chemical industries. This study thus illustrates the value of combining bioassays and chemical analysis (with an effective sampling technique) for a realistic and rapid assessment of organic contaminants in the aquatic environment.

**TH074 Assessment of different passive samplers to predict**

**bioaccumulation of polycyclic aromatic hydrocarbons by carrot roots** J. Kokovic, University of Reading / Geography and Environmental Science. Polycyclic aromatic hydrocarbons (PAHs) belong to a class of hydrophobic organic pollutants, produced during the incomplete combustion or pyrolysis of organic materials from primarily anthropogenic sources such as fossil fuels and agricultural waste. The fate of PAHs in the nature is of great concern to human health as these contaminants are widely distributed in the environment and are known to be toxic, mutagenic and carcinogenic. In the past decades, various passive sampler devices (PSDs) have been developed to concentrate hydrophobic organic compounds (HOCs) from environmental matrixes and to mimic bioconcentration. Previous research has indicated that triolein embedded cellulose acetate membrane (TECAM) and petroselinic acid embedded cellulose acetate membrane (PECAM), types of lipid containing membranes have potential to accumulate certain polycyclic aromatic hydrocarbons (PAHs) from water and soil and to estimate the bioavailability of these pollutants to living organisms. The present study compares the ability of these passive samplers to mimic bioaccumulation of 15 PAH prioritised by the European Scientific Committee on Food (ESCF) in carrot roots grown in acid washed sand.

**TH075 Using performance reference compounds to evaluate freely dissolved concentrations of DDT and its degradates in contaminated sediments** A. Tcaciuc, Massachusetts Institute of Technology / Department of Civil and Environmental Engineering. Despite its ban in the 1970's, DDT and its degradates remain pollutants of concern at various sites in the world. Passive samplers have been used successfully for determining freely dissolved concentrations of various hydrophobic organic contaminants such as PCBs and PAHs, in pore waters of contaminated sediments. The losses of performance reference compounds (PRCs), which are preloaded into the sampler, are used to assess how close to equilibrium the sampler is after the deployment time. However, in our recent work involving *in situ* deployment of passive samplers in DDT-contaminated sediments, where the  $^{13}\text{C}$  labeled DDE, DDD and DDT were used as performance reference compounds, we observed a loss of the DDT PRC that was inconsistent with expectations based on its mass transfer properties and the behavior of the other PRCs. This was observed both in *in situ* and in lab exposures of PE to contaminated sediments. We believe that this loss is due to a reaction which is consuming the DDT PRC, and which has implications for the behavior of the DDE and DDD PRCs, the potential products of the reaction. Also this substantially complicates estimation of the correction factors needed to calculate the equilibrium concentration of the compounds of interest in the passive sampler. We have developed a reaction-diffusion model for understanding the behavior of the PRCs and for calculating the equilibrium concentrations in the sampler of the compounds of interest. To validate our model, we compare the results of this model against concentrations of these compounds from tumbling experiments done in the laboratory with the sediments collected from the deployment sites.

**TH076 Passive dosing in aquatic toxicity testing with the nematode *Caenorhabditis elegans*** S. Schaefer, Federal Institute of Hydrology / Biochemistry & Ecotoxicology. Nematodes are the most abundant multi-cellular organisms in sediment and soils. The bacterivorous nematode *Caenorhabditis elegans* - a well-studied model organism - is frequently used to assess the toxicity of sediments, soils and aqueous solutions (ISO 10872). As in many aquatic bioassays, toxicity testing of hydrophobic compounds is challenging due to their low aqueous solubility and the difficulties in maintaining constant freely dissolved concentrations. In order to control freely dissolved concentrations of analytes the standard nematode bioassay was modified by passive dosing of test compounds. Four chlorobenzenes (1,2,3-TCB, 1,2,3,4-TeCB, PeCB and HCB) as well as the disinfectant triclosan and its degradation product methyltriclosan were loaded on silicone O-rings as passive dosing reservoir. Since preliminary experiments with chlorobenzenes have shown that equilibration between medium and O-ring is not achieved in micro plates that are frequently used for this

bioassay, tests were run in 10 ml-headspace vials. The effect of the type of test vial – micro plate vs. headspace vials – on *C. elegans* was previously assessed. O-rings were loaded by partitioning from a methanolic solution containing the test compounds. The release of analytes from O-rings into nematode test medium was assessed for determining required equilibration times before start of nematode toxicity testing. Freely dissolved concentrations were measured with SPME fibres simultaneously exposed in the nematode medium inside the test vessels and compared to liquid-liquid extraction of aqueous samples. Analytes were quantified by GC with MS/MS detection. After determining the loading and release kinetics of the selected analytes, their toxicity on growth and reproduction of *C. elegans* was assessed after exposure through passive dosing. The data were compared to toxicity data obtained after active spiking of test compounds into the test using acetone as solvent.

**TH077 Availability of data on the persistence, bioaccumulation potential and toxicity for a selection of alternative brominated flame retardants** G. Stieger, ETH Zurich M. Scheringer, ETH Zuerich / Institute for Chemical and Bioengineering; C.A. Ng, K. Hungerbuehler, ETH Zurich. Brominated flame retardants (BFRs) comprise a wide range of brominated aromatic and aliphatic compounds. Two types of BFRs widely used in the last decades are polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD). Because of their concerning properties regarding persistence, bioaccumulation potential and toxicity (PBT properties), the use of these substances is now restricted or prohibited under various national and international legislations. However, PBDEs and HBCDD are often replaced by other BFRs such as decabromodiphenyl ethane, pentabromotoluene, pentabromoethylbenzene, and many more. Often, the chemical properties of these substitutes are only poorly known, thus concerns about their PBT characteristics remain. Here we investigate the availability, and furthermore the quantity and quality, of PBT property data for 36 alternative BFRs. In order to do so, we searched 25 publicly accessible databases for property data related to persistence (biodegradation half-life in soil,  $t_{1/2}(\text{soil})$ ), bioaccumulation potential (octanol-water partition coefficient, Kow, and bioaccumulation factor, BCF), and toxicity (chronic and acute aquatic toxicity, Tc and Ta). Overall, data were available for 19 of 36 BFRs. 46% of all collected database entries were for Kow whereas only 5% of the available values were persistence data. Values for Kow were found for 19 of the 36 BFRs; values for  $t_{1/2}(\text{soil})$  for 11 of the 36 BFRs, and values for BCF for 19 of the 36 BFRs. Values for Tc and Ta were found for 14 and 18 of the 36 BFRs, respectively. For all of these PBT properties, there are a few BFRs for which multiple values for the same property can be found, while for the other BFRs only one or no values are found for any of the properties. Among the few BFRs for which multiple entries were available for all PBT properties are tetrabromobisphenol A and 2,4,6-tribromophenol. Where multiple database entries were found for the same BFR and PBT property, the variability of the values was often substantial. As examples, the Kow values found for tetrabromobisphenol A range over five orders of magnitude and the Ta values found for 2,4,6-tribromophenol range over three orders of magnitude. Having investigated this set of 36 alternative BFRs, we conclude that PBT property data are scarce for persistence and toxicity. Also, data are unequally distributed: for some BFRs a lot of variable data is found whereas for many BFRs only very little information can be acquired.

**TH078 Bioaccumulation of different polybrominated diphenyl ethers by *Tubifex tubifex*** M. Kos Durjava, B. Kolar, L. Arnus, Public Health Institute Maribor; W.J. Peijnenburg, RIVM / Laboratory for Ecological Risk Assessment. Intelligent testing strategy approaches to make environmental risk assessments of large numbers of chemicals more efficient, require the development and application of in-silico techniques. Bioaccumulation tests of polybrominated diphenyl ethers (PBDE) on oligochaete species *Tubifex tubifex* were conducted to validate QSAR models on the toxic effect and the bioaccumulation potential of these lipophilic organic chemicals. Selective uptake of PBDEs by oligochaete allows assessing the bioaccumulation of

individual congeners in commercial mixtures. The bioaccumulation factor (BAF) at the steady state and the kinetic bioaccumulation factor ( $BAF_K$ ) determined in this study indicated a high bioaccumulation potential of the low PBDEs (tri, tetra, penta and hexa BDE). The BAF and  $BAF_K$  of highly brominated PBDEs (hepta, octa nona and deca BDE) were shown to be considerably lower. The bioconcentration factor (BCF) calculated from the ratio between the concentration of PBDEs in tubifex and surrounding water shows a similar pattern of bioavailability of these molecules. A comparison of these findings to bioaccumulation potentials in other species of annelids shows smaller differences between the bioaccumulation potential of low and high PBDEs. In our study the average values for BAF as well as for BCF for low and high PBDEs differed by less than an order of magnitude.

**TH079 Bioaccumulation of polybrominated diphenyl ethers in *Gammarus pulex*: importance of different exposure routes and modelling** J.D. Lebrun, Irstea; D. Leroy, A. Giusti, University of Liege; C. Gourlay-France, Irstea; J. Thome, University of Liege. Because of their intensive use in commercial and household products as flame retardants and their environmental persistence, polybrominated diphenyl ethers (PBDEs) become ubiquitous global contaminants, whose the final receptacle is water. Considering the potential toxic effects of PBDEs for aquatic organisms, the development of relevant tools for environmental risk assessment is of a great interest. In the environment, aquatic organisms exposed to chronic contaminations accumulate chemicals through water and diet. In the case of hydrophobic chemicals such as PBDEs, the uptake through diet may take a great importance due to their high adsorption on organic sediments and food. Since the process of bioaccumulation integrates the different exposure routes of organisms and chemical properties of contaminants, using biomonitors offers promising perspectives to quantify the fraction of PBDEs in water that is bioavailable and potentially toxic for biota. This study aims at investigating the potential use of an ecosystem engineer widely distributed in European freshwaters, *Gammarus pulex*, as exposure indicator of PBDEs. Characterizing its abilities to concentrate and regulate internalized PBDEs will allow the construction of a predictive model of bioaccumulation. In aquatic microcosms, the organism was exposed to a cocktail of main brominated congeners found in the environment (i.e., PBDE-28, 47, 66, 85, 99, 100, 153, 154 and 183) to assess its ability to bioconcentrate PBDEs. Results showed that all tested PBDEs were significantly internalized by *G. pulex*. Accumulated amounts were closely related to the partition coefficients of PBDEs. Then, internal levels of PBDE-47 were measured during or after initial exposure phase to establish the uptake and elimination rates from only aqueous exposure. We showed that elimination and metabolism efficiencies were negligible for PBDE-47 in this organism, which is in favour of its use as a quantitative biomonitor. Finally, bioaccumulation experiments were recorded with contaminated leaves, encaged or not, to determine the relative importance of aqueous and dietary routes in the contamination of the organism. Even if the water is the dominant exposure route, a significant uptake of PBDE-47 from food was established. We proposed a model integrating the two exposure routes to describe PBDE-47 bioaccumulation. To conclude, this ubiquitous amphipod could be used as early sentinel of the contamination of freshwaters by PBDEs.

**TH080 High levels of PBDD/Fs were detected in soot samples from a substation destroyed by spontaneous combustion – a negative consequence of using BFRs** J. Hagberg, MTM Research centre Örebro University / Dept. of Science and Technology; Y. Li, State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences. Incomplete combustion of materials containing brominated flame retardants, for example polybrominated diphenyl ethers, may result in substantial amounts of polybrominated dibenzo-*p* dioxins and furans, PBDD/Fs. Since the PBDD/Fs are pollutants with similar qualities as their chlorinated homologues, i.e. polychlorinated dibenzo-*p* dioxins and furans (PCDD/Fs), they are persistent, bio accumulative and toxic. In this

study, different soot samples were collected from a substation after a spontaneous combustion and were analysed for tetra- to octa substituted PBDD/Fs. By using a wipe testing procedure three soot samples were collected from inside surfaces and three samples were collected from the outside of the stainless steel cover. The substation was approximately 20 years old and all cables were isolated by plastics probably containing brominated flame retardants. The soot wipes were collected from surfaces ranging between 10-15 cm<sup>2</sup> in size and all samples had high to very high levels of PBDD/Fs, i.e. 200-4560 pg/wipe. The levels on the different wipes varied substantially possibly reflecting the occurrence of different temperature zones at which the soot particles and the flue gas had different possibilities to cool and adsorb. The contribution of low brominated furans were substantial with 2,3,7,8-TeBDF being the most dominating congener in most of the samples. Among the dioxin congeners the higher brominated compounds had the largest contribution. Two of the soot samples, one from a surface within the substation and one from an outside surface of the substation, had significantly higher levels than the other samples. Despite the elevated levels the congener pattern for these samples did not deviate from that of the lower level samples possibly indicating that these samples were taken from the same side of the substation where most of the flue gases and soot particles were more easily emitted to the surrounding atmosphere rather than indicating that the formation had been different at this side of the substation. TEQ based amounts (pg TEQ) established by using toxic equivalent factors for PCDD/Fs (WHO<sub>2005</sub>) ranged between 25-760 pg TEQ showing that the high PBDD/Fs levels in the soot may constitute a potential risk of occupational exposure to electricians working with restoring substations and other persons handling the waste material originating from substations destroyed by fire.

**TH081 Detection and Quantitation of Brominated and Chlorinated Hydrocarbons by DART with Linear Ion Trap and Triple Quadrupole Technology** C.P. Martins; c. yang; M. Blackburn, M. Prieto Conaway, Thermo Fisher Scientific; S. Harrison, AgResearch. Brominated hydrocarbons also known as brominated flame retardants (BFRs) have been used in various industries for decades. Recently several classes of BFRs have been detected in the biosphere. Chlorinated hydrocarbons have also been used for many years, primarily as pesticides (OCs). While most OCs have been banned in the United States, their use still occurs in developing countries. The continued use of BFRs and OCs as well as their persistence in the environment and potential deleterious activity therein makes the detection and monitoring of these compounds an important topic. Direct Analysis in Real Time (DART) as a simple, rapid, easy to use technique for detection and quantitation both BFRs and OCs. Standards for OCs and simple surface water sample extracts containing OCs and BFRs were dissolved in acetone at concentrations from 10ppb to 10ppm. Negative ion full scan mass spectral data was acquired on the LTQ linear ion trap with DART-SVP source. Initial studies were performed on the OCs Kepone and Mirex at 10ppm concentrations with negative ion full scan on the linear ion trap and a temperature of 100°C. Under these conditions two alternative modes of ionization were observed. Kepone (C<sub>12</sub>Cl<sub>10</sub>O) was ionized by the addition of a hydroxyl radical resulting in an <sup>10</sup>[M+OH]<sup>-</sup> species and Mirex (C<sub>12</sub>Cl<sub>10</sub>) ionized through the addition of a radical nitrogen anion and loss of a neutral chlorine radical resulting in a [C<sub>12</sub>Cl<sub>9</sub>N]<sup>-</sup> species. To further study the ionization modes a mixture of BFRs<sup>11</sup> and OCs was analyzed. DART grid voltage was maintained at 300V and several temperature gradients were run, optimum temperature to detect OCs and BFRs was determined to be 200°C. Under these conditions detection both the high molecular weight BFRs such as DBDPE (C<sub>18</sub>H<sub>11</sub>Br<sub>11</sub>) as well as the lower molecular weight OCs. The BFR DBDPE added the radical nitrogen anion and lost a neutral bromine radical to form a [C<sub>18</sub>H<sub>10</sub>Br<sub>10</sub>N]<sup>-</sup> radical anion. Sequential losses of halogen were observed when <sup>43</sup>Mn data was acquired for the BFRs and OCs. Using the preliminary data acquired on the linear ion trap precursors and transitions will be chosen for quantitation using the Quantum Access triplequad. Calibration curves will be created from stock solutions starting at 1ppm to 100ppt. LODs, LOQs, and linearity



will be determined based on 5 replicates at each level. In conclusion, a simple, fast, and easy to use method is described for screening and quantitation of OCs and BFRs from water samples.

#### **TH082 BROMINATED FLAME RETARDANTS AND DECHLORANES IN SEDIMENTS FROM THE GERMAN BIGHT**

**BIGHT** R. Sührling<sup>H.</sup> Wolschke, D. Milutinovic, Helmholtz-Zentrum Geesthacht / Coastal Research; R. Ebinghaus, Helmholtz-Zentrum Geesthacht. Sediments are habitats to a variety of species and as such of vital importance to the integrity of aquatic ecosystems. They can also function as sinks for numerous organic contaminants, that tend to adsorb to particles, due to their physicochemical properties, i.e. low volatility, low water solubility and high  $K_{ow}$ -values. Different sediment classes can be expected to have different binding capacities for organic contaminants thereby influencing the contaminant load, pattern and bioavailability of contaminants in one area. For this study halogenated flame retardants in sediments from the German Bight were analysed via liquid and gas chromatography coupled with mass spectrometry (LC-MS/MS and GC-MS/MS) in order to identify distribution and sediment class specific patterns of the contamination. The focus was on substitutes of the banned polybrominated diphenylethers (PBDEs), namely alternate BFRs and Dechloranes (Decs) as well as BFRs with very high production volumes such as hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA). The results show the lasting relevance of PBDEs as contaminants in coastal areas but also the growing relevance of emerging contaminants such as Decabromodiphenyl ethane (DBDPE) and Dechlorane Plus (DP). There are in many cases not enough data to evaluate the risk of the emerging contaminants yet many BFRs are expected to be bioaccumulative as well as toxic and might therefore pose a threat to the environment and especially aquatic organisms.

#### **TH083 Occurrence and behaviour of “classical” and alternative flame retardants in sediment, sludge and fish from Ebro and Llobregat river basins (Spain)**

**E. Barón**<sup>G.</sup> Santin, E. Eljarrat, D. Barcelo, Institute of Environmental Assessment and Water Research Studies (IDAEA). Classical flame retardants (FRs) such as PBDEs have been widely used for many years to increase the fire resistance of different materials. However, it has been demonstrated that they can act as endocrine disruptors and have a high bioaccumulation capacity. For that reason they have been banned and other FRs have been developed to meet the fire safety regulations. Some of these compounds are halogenated norbornenes (Dec 602, Dec 603, Dec 604 and DP) and other brominated FRs such as pentabromoethylbenzene (PBEB), hexabromobenzene (HBB) and decabromodiphenylethane (DBDPE). In this study, a total of 33 sediments and 7 sludge were analysed in Ebro and Llobregat river basins. Fish samples were collected in 10 different sites among the two river basins for a total of 50 samples from different species. This work was done in the frame of the SCARCE project. Several PBDEs and halogenated norbornenes were detected in sediments. Concentration levels of PBDEs in Llobregat river basin ranged from 1.5 to 44 ng/g dw, while in Ebro river basin ranged from nd to 38 ng/g dw. The most abundant congener was BDE-209, which is in agreement with other published studies. DBDPE was also detected, and its levels were similar or higher than BDE-209 levels, with concentrations between nd-26 ng/g dw and nd-32 ng/g dw in Llobregat and Ebro river basin, respectively. This may be due to the fact that DBDPE has been used as a substitute to BDE-209. Norbornene levels were lower than those of PBDEs, with levels up to 1.8 ng/g dw. These are the first reported levels of halogenated norbornenes in river sediments from Spain. As regards sludge, different PBDEs, halogenated norbornenes and DBDPE were also detected in samples from 7 wastewater treatment plants (WWTPs). PBDE levels (up to 342 ng/g dw) were considerably higher than halogenated norbornene levels (up to 19 ng/g dw). In fish samples, the most abundant compound of classical and emerging FRs was BDE-47 and Dec 602, respectively. Llobregat River (up to 520 ng/g lw) was more contaminated by PBDEs than Ebro River (up to 90.9 ng/g lw). Moreover, classical FR levels were higher than emerging FRs.  $F_{anti}$  (the ratio between the two DP isomers,

*syn* and *anti*) was lower than the value for commercial mixture, showing a different bioaccumulation capacity of two isomers. Biota to sediment accumulation factors (BSAF) were also evaluated in order to compare the risk of the emerging FRs. Emerging FRs seem to have lower BSAF than classical PBDEs.

#### **TH084 Transfer of emerging pollutants from amended agricultural soils: leachate and runoff**

A. de la Torre, I. Navarro, CIEMAT<sup>B.</sup> Eulalia M., INIA / Environmental; P. Sanz, CIEMAT; M. Porcel, G. Carbonell, INIA; M. Martinez, CIEMAT. Socio-economic changes of recent decades have resulted in a significant increase in organic waste production that could generate environmental problems. Although most pollutants are not necessarily a threat to the environment given their low concentration and/or availability to be metabolized by microorganisms, there are some organic compounds, including flame retardants such as polybrominated diphenyl ethers (PBDEs), decabromodiphenylethane (DBDPE), dechloranes (Dec 602, 603, 604 and 605), and some perfluorinated compounds (PFCs) among others, that do not break down easily during wastewater treatment and/or municipal solid waste (MSW) composting. Consequently, despite the use of MSW and sewage sludge composts, a common practice is to improve the chemical and biological characteristics of impoverished soils by supplying soil organic carbon content and nutrients. However, it is necessary to evaluate the transfer of these organic pollutants to different environmental compartments. This work aims to identify the presence of emerging organic pollutants in amended agricultural soils and their leaching and runoff after amendment with different biosolids. To fulfil this objective, a semi-field study was carried out with four biosolids (MSW composts, and sewage sludge: compost and thermal drying sludge) obtained from different Spanish urban waste treatment plants. Biosolid application rates were calculated by considering plant N requirements. The system consisted in 16 trays (2 x 2.5 m<sup>2</sup>), containing a 5-cm-thick soil layer, supported by a metal frame with a 10 % slope. Two individual systems (leachate and runoff) were connected to each trial for collecting rainwater after three rainfall events taking place from October 2011 to July 2012. Emerging pollutants content was analysed in all the samples and these results were used to evaluate the behaviour of pollutants in abiotic environmental compartments, their transfer capability and, subsequently, their fate. This information will be of much interest for environmental risk assessments. This study has been funded by Spanish projects CTM2010-19779-C02-01/02

#### **TH085 Occurrence of brominated flame retardants (PBDEs) in Antarctic soils, mosses and lichens at background areas of the Antarctica Peninsula**

A. Cabrerizo, IDAEA-CSIC / Department of Environmental Chemistry; J. Dachs, IDAEACSIC / Environmental Chemistry; D. Barcelo, IIQABCSIC; K.C. Jones, Lancaster University / Lancaster Environment Centre. The Antarctica is usually perceived as a symbol of the last great wilderness and remoteness. Although natural “barriers” such as oceanic and atmospheric circulation protect this region from lower latitude water and air masses, available data on concentrations of persistent organic pollutants (POPs) such as brominated flame retardants (PBDEs) evidence its ubiquitous presence in air, snow, water, vegetation or food webs organisms. The occurrence of these man-made synthetic chemicals in Polar Regions is just another manifestation of the multiple anthropogenic perturbations on the composition of the biosphere. Their long half-lives facilitate repeated cycles of volatilization and deposition resulting in progressive movement away from temperate and tropical source regions towards colder climate areas. Ultimately, these compounds may experience “cold-trapping” at the Polar Regions, where the colder temperatures further prolong persistence and enhance their accumulation in terrestrial compartments. Their extreme persistence, semi-volatility, bioaccumulation potential and adverse effects in wildlife and humans have led to develop international protocols regulating the use of some PBDEs, such as the Stockholm Convention and the UNECE LRTAP POPs protocol. Most of the literature available reporting PBDEs in Antarctic soils and vegetation are based on areas close to research facilities, which have additionally influence the levels of PBDEs in the

Antarctic environment, while there is no information available regarding the levels of PBDEs at background areas of Livingston and Deception Islands and Byers Peninsula (a special protected area). Therefore, the main objectives of this study were: i) to contribute with a large data set of PBDEs concentrations in soils, lichens, mosses and grass in Antarctica during austral 2008/09 summer; ii) to compare these levels of PBDEs with those found in soil samples collected in austral 2004/05 summer; iii) to study in detail potential local sources by assessing the PBDEs concentrations proximate to the currently used Spanish research station "Juan Carlos I", the Byers research camp in Livingstone Island and abandoned research stations in Deception Island.

#### **TH086 Halogenated Flame Retardants in human breast milk: an international study** C. Corcellas, IDAEA / Química Ambiental; J. Torres, O. Malm, Lab. of Radioisotopes, Biophysics Institute; W. Ocampo-Duque, Pontificia Universidad Javeriana; E. Eljarrat, D. Barcelo, IDAEA-CSIC.

Dechloranes are chlorinated flame retardants (FRs) which have been used as substitutes of Mirex. They include dechlorane 602 (Dec 602), dechlorane 603 (Dec 603), dechlorane 604 (Dec 604) and dechlorane plus (DP). In last years, some studies reported their presence in environmental and biological matrices. However, the studies related with the occurrence in human samples are very scarce. Xian *et al.* were the first authors reporting dechlorane levels in human blood samples, with concentration levels up to 43 ng/g lw. On the other hand, Siddique *et al.* analyzed for the first time these halogenated FRs in human milk samples from Canada, with levels up to 8 ng/g lw. Recently, Ben *et al.* correlated levels of DP in human serum and breast milk of Chinese women. The aim of our work was studied the levels of dechloranes in breast milk samples collected from mothers in different countries: Brazil, Colombia and Spain. At the same time, polybrominated diphenylethers (PBDEs), including the emerging ones such as pentabromoethylbenzene (PBEB), hexabromodiphenylether (HBB) and decabromodiphenylether (DBDPE), were determined in the same samples in order to compare the occurrence of "classical" and emerging FRs. Results showed the presence of Dec 602, Dec 603 and DP, being Dec 602 the most abundant. Dechlorane levels depended on the origin of the sample: in the rural zone of Brazil dechloranes were not found at quantifiable levels, while they have been detected at urban areas of Brazil (1,43 - 30,6 ng/g lw), Colombia (0,03 - 10,91 ng/g lw) and Spain (3,78 - 6,93 ng/g lw). These levels are in accordance with the previously found in Canada. The *fanti* <sup>the fraction of the</sup> *anti*-DP isomer to the total DP (*anti*- + *syn*-DP), was calculated. Results (from 0.50 to 0.86) indicated a similar value than those observed in the commercial mixtures (0.75). As regards PBDEs, 10 different congeners were detected, with BDE-209 being the most abundant. Contamination ranged from 13,1 to 261 ng/g lw. Emerging BFRs (PBEB, HBB and DBDPE) were not detected in any sample. Comparing PBDE levels with those obtained for dechloranes, and as expected, PBDE levels were up to 115 times higher than those of dechloranes. However, more studies focused on the impact of alternative flame retardants in human bodies are needed especially taking into account that contamination levels will increase over time, as DP is presented as an alternative to the banned deca-BDE.

#### **TH087 DETERMINATION OF HEXABROMOCYCLODODECANES (HBCDs) IN DIFFERENT POLYSTYRENE PRODUCTS** M. Rani, S. Hong, W. Shim, G. Han, M. Jang, Korea Institute of Ocean Science and Technology.

The widespread occurrence of 1,2,5,6,9,10-Hexabromocyclododecane (HBCD) in different environmental segments and food commodities has raised a concern among the environmentalists and food chemists. It is one of the most frequently used additive brominated flame retardants (BFRs) worldwide and mainly applied in expanded polystyrene (EPS) and extruded polystyrene (XPS) foams (0.7 to 2.5% HBCDs). EPS and XPS are used for making insulation board along with the packing and disposables. The industrial application of HBCD has increased during the last decade after restriction on PBDEs. To date, there is no restriction on the production or use of HBCD. Because of widespread use of HBCDs coupled with its persistence and toxicity, a database on

its level in polystyrene used for several applications is of paramount importance. Various products of EPS and XPS used in the area of food, construction, laboratory and packing of electrical and electronic appliance were collected. High-performance liquid chromatography coupled to atmospheric chemical ionization tandem quadrupole mass spectrometry was used for the analysis of selected emerging flame retardant. Results indicate that the level of HBCDs is found to be 4 times higher in EPS products than that of XPS. In both the cases, PS related to construction and laboratory has a significantly higher level of HBCDs which justify the use (12-2300 µg/g). Lower concentration were measured in most of samples belonging to the category of food (0.12-1.00 µg/g). The variation in level of HBCDs in each group shows the lack of proper protocol for the use of HBCDs in PS. This is supported by the higher concentration measured in shipping box (2400 µg/g), buoy used for oyster culture (140 µg/g) and tray used for packing of fish (42 µg/g) while lower concentrations in samples used for packing electrical appliance (1.63 µg/g). The sharp peaks for the TBBPA, BTBPE and DBDPE were also observed in most of XPS products although the concentration is lower than HBCDs (TBBPA=0.03-3.63 µg/g, BTBPE=0.01-1.02 µg/g and DBDPE= 1.1-21.0 µg/g). This reveals that HBCD is added to PS along with other BFRs that cannot be ignored. Therefore, a regulative and controlled method should be used for adding HBCDs. The database for HBCDs in PS products will help in refining a risk assessment for the exposure to HBCDs.

#### **TH088 Chronic and Acute Toxicity of Three Common Firefighting Foams on Dreissenid Mussels** C. Addis, Biological Sciences; D.R. Kashian, Wayne State University / Biology department.

Class A firefighting foams (CAFs) are widely used in both structural and forest fires, and can negatively impact the environments into which they are introduced. Recent studies have shown the surfactants in these formulas impose toxic effects on aquatic organisms by reducing oxygen availability. The few studies to date have focused on lethality, yet the sublethal impacts of these emerging contaminants are unknown. Therefore, the objective of this study was to investigate sublethal and lethal effects of acute and chronic exposure of three CAFs, Chemguard First Class (CGFC), Phoschek WD881 (PSWD881) and Phoschek First Response (PSFR) on dreissenid adults and veligers. Dreissenids are becoming increasingly ubiquitous, easy to collect, and exhibit planktonic and benthic life stages, making them an ideal bioassay organism. Veliger response was tested using a novel assay where individuals (n=12) were placed into 96-well microtiter plates and exposed to five concentrations (0, 10, 20, 30, 40, and 50 g/L) of each CAF in acute 4-hour assays, and eight concentrations (0, 0.001, 0.1, 0.25, 0.5, 0.75, 1, and 10 µg/L) in chronic 6-day assays. Mortality was determined by cessation of ciliary movement. All acute concentrations of CGFC resulted in 100% mortality after an average of 40 minutes of exposure, while the same level of mortality took approximately 145 minutes for both PSWD881 and PSFR. Probit analysis for the 6-day chronic veliger exposure estimated the LC<sub>50</sub>s for CGFC, PSWD881, and PSFR to be 0.3, 17, and 16 µg/L, respectively. Ten adult mussels (n=10) were exposed to the same concentrations to measure the effects on mortality and spawning intensity, induced with 10<sup>-3</sup> M serotonin. Spawning intensity was determined by the quantity of eggs produced or the optical densities of the sperm clouds. At these concentrations, there was no significant spawning effect (p<0.05), and no adult mortality occurred. This study is the first study of its kind to measure the effect of fire fighting foams on an early life stage of a freshwater mussel. Our findings indicate that veligers are substantially more sensitive than their adult counterparts to these firefighting foams, and that even at low concentrations these foams may have a significant impact on freshwater mussel populations.

#### **TH089 BIOACCESSIBILITY OF POLYBROMINATED DIPHENYL ETHERS IN INDOOR DUSTS** S. Garcia Alcega, University of Reading; A. Kucharska, S. Voorspoels, VITO.

The polybrominated diphenyl ethers (PBDEs) have toxic properties such as endocrine disruption, carcinogenicity and neurodevelopmental deficits (1, 2). Nowadays almost all the commercial mixtures of PBDEs have

been banned; this started with the restriction of penta and octa PBDEs in 2004 in Europe and in 2008 in the US, followed by the restriction of deca PBDEs in 2008 in Europe and in some states of America. By 2013 deca PBDE will be banned across the USA. However, products containing PBDEs are still in many homes, still marketed and disposed of in the environment. Moreover, Deca PBDEs which continue to be marketed can, through debromination, generate lower PBDE congeners which are more toxic. The intake of PBDEs mainly occurs via food, the ingestion of house dust and inhalation of indoor air. Household dust has been considered as a major source of PBDEs exposure in humans, particularly for young children, who tend to ingest more dust than adults and are more susceptible to some of the effects to these compounds (1). The objective of this research is to characterize the main components of different indoor dusts (lipids, carbon content and particle size) and to analyse the bioaccessibility of PBDEs from these dusts using a colon extended physiologically based extraction test (CE-PBET) (3) simulating fed and unfed conditions. Preliminary results indicate that for all the PBDEs the bioaccessibility increases in fed state, being more bioaccessible compounds close to a  $K_{ow}$  of 7, especially BDE-99 and BDE-153 ( $K_{ow}$  7 and 7.9) which are almost 30% bioaccessible in unfed conditions and 40% in fed.  $K_{ow}$  above 7.9 results in a decrease of bioaccessibility. In the case of BDE-209 ( $K_{ow}$  10) is only 10% bioaccessible in unfed state and 15% in fed. **Keywords:** bioaccessibility, BFRs, PBDEs, dusts **References:** Huwe J, Hakk H, Smith D, Diliberto J, Richardson V, Stapleton H, Birnbaum L (2008) *Environmental Science & Technology* 42: 2694-2700 Meng X, Zeng E, Yu L, Guo Y, Mai, B (2007) 41: 4882-4887 Tilston E, Gibson G, Collins C (2011) *Environmental Science & Technology* 45: 5301-5308

#### TH090 Linking BFRs identified in house dust to consumer goods

A. Golnouch, University of Toronto / Geography; E. Goosey, University of Toronto; A. Saini, University of Toronto / Department of Physical and Environmental Sciences; M.L. Diamond, University of Toronto / Department of Earth Sciences. Homes, offices and cars (n=35, 15, 10) were sampled in Toronto, Canada (2012) for the presence of old and new brominated flame retardants (BFRs). Dust samples were collected from living rooms and office floors, whilst a portable x-ray fluorescence (XRF) analyzer was used to detect the presence of bromine and chlorine (at the depth of 0.8 cm). Additionally, products within the rooms with a high bromine reading (> 10,000 ppm) were wiped with isopropanol impregnated glass fibre filters (n=50) to provide qualification (by GC-MS) for the type of BFR present. Results indicated the presence of 'novel' flame retardants (NFRs) in the dust at concentrations generally 1/10<sup>th</sup> that of polybrominated diphenyl ethers (PBDEs). However tris(1,3-dichloropropyl)phosphate (TDCIPP) was quantified at concentrations much higher than BDE-209 in some dust samples. In all samples the greatest PBDE contribution was from BDE-209. The XRF results indicated the presence of BFRs in numerous products throughout the home and office environments. Br content in the backing of televisions (both flat screen and cathode ray tube (CRT)) were consistently highest at > 100,000 ppm. Interestingly, these concentrations were higher than the Br content found in various occupational clothing articles from a fire station. Other products that had relatively high Br content were multiplugs, chargers (for electronic goods and batteries), internet routers (cable and wireless), DVD players, microwaves, and some foam chairs. Identification of BFR type using the product wipes was not always successful due to concentrations below the detection level. However in some cases where the product Br content was high, the wipe samples were able to provide a qualification of the BFR type, which correlated to the presence of BFRs in the dust.

#### TH091 Biodegradation of Organophosphorus Flame Retardants

S. Waaijers, R. Helmus, S. Jurgens, M. Kraak, University of Amsterdam / IBED; W. Admiraal, University of Amsterdam; P. de Voogt, J. Parsons, University of Amsterdam / IBED. Several brominated flame retardants (BFRs) have been banned due to their persistence, bioaccumulation potential, toxicity (PBT) and probable environmental risk. Hence, there is an urgent need for halogen-free flame retardants (HFFR). The European project ENFIRO has identified and evaluated a

number of halogen-free flame retardants that are potential replacements for brominated flame retardants. However, the PBT properties of most of these alternative flame retardants are poorly characterized. The aim of the present study was therefore to generate persistency data of selected organophosphorus flame retardants (OPFRs) by performing biodegradation studies with diluted waste water treatment sludge. To determine the aerobic biodegradability of the selected compounds, we performed a 28 days study based on OECD guideline 301 (1992). To monitor degradation each parent compound was analysed using HPLC-MS/MS. The OPFRs studied were triphenylphosphate (TPP), resorcinol bis(diphenylphosphate) (RDP), bisphenol A bis(diphenylphosphate) (BDP) and dihydrooxaphosphaphenanthrene (DOPO) with the easily degradable benzoic acid as positive control. In addition to primary biodegradation, expected breakdown products, such as the toxic compound bisphenol A, were also analysed. Our results will contribute to the evaluation of the environmental safety of these OPFRs and their potential as alternatives for the brominated flame retardants.

#### TH092 Toxicity and risk assessment of organophosphorus flame retardants in river water

J. Cristale, A.G. Vazquez, C. Barata, S. Lacorte, IDAEA-CSIC / Environmental Chemistry. Organophosphorus flame retardants (OPFRs) are worldwide used as flame retardants and plasticizers, and are ubiquitous contaminants in water environment. Their environmental levels in surface water and their toxicity were investigated in the present study. The OPFRs studied were tris(isobutyl)phosphate (TiBP), tris(n-butyl)phosphate (TBP), tris(2-chloroethyl)phosphate (TCEP), tris(2-chloro-1-methylethyl)phosphate (TCPP), tris(2-chloro-1-(chloromethyl)ethyl)phosphate (TDCEP), triphenylphosphate (TPhP), tris(2-butoxyethyl)phosphate (TBEP), 2-ethylhexyl diphenyl phosphate (EHDP), tris(2-ethylhexyl)phosphate (TEHP) and tricresylphosphate (TCP). A monitoring study was carried out in 3 rivers in Spain (Nalón (Asturias), Arga (Navarra) and Besòs (Barcelona)). OPFRs were detected in all the rivers, while TBEP, TCEP and TiBP were present at the highest concentrations, reaching the levels of 4.1  $\mu\text{g L}^{-1}$ , 1.9  $\mu\text{g L}^{-1}$  and 1.1  $\mu\text{g L}^{-1}$ , respectively. Since mixtures of OPFRs are often detected in water, toxicological effects of single and mixtures of these compounds were determined using *Daphnia magna* acute assays. Results evidenced that these compounds act by non polar narcosis, that means that their toxicity was proportional to their lipophilicity ( $K_{ow}$ ). Furthermore their joint toxicity was additive. These means that single and joint toxicity of these compounds can be predicted knowing their concentration levels in water using quantitative structure activity relationships (QSARs) and predictive mixture models. Based on these results, a risk assessment was performed calculating risk quotients (RQ) derived from the measured environmental concentration (MEC) and predicted no effect concentration (PNEC). The joint effect was estimated by the sum of RQs for each sampled site. No significant risk (RQs < 1) was observed for all the sampling sites monitored.

#### TH093 Fluctuating air pollution-associated asthma incidence in Taiwan

N. Hsieh, Department of Bioenvironmental Systems Engineering; C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering. Recent studies have implicated that air pollution has been associated with asthma exacerbations. The purpose of this study was to use the statistical indicators-based regression models to predict the time trend of asthma hospital admission in Taiwan appraised with time-varying air pollution data. Five major pollutants including particulate matters with diameter less than 10  $\mu\text{m}$  ( $\text{PM}_{10}$ ), ozone ( $\text{O}_3$ ), nitrogen dioxide ( $\text{NO}_2$ ), sulfur dioxide ( $\text{SO}_2$ ), and carbon monoxide ( $\text{CO}$ ) were selected. The asthma hospital admission was divided into 0-14, 15-64, and > 64 years to represent the subgroup of pediatrics, adult, and elder, respectively. We firstly used Spearman's rank correlation to test relationships between statistical indicators for air pollutants including coefficient of variation (CV), standard deviation (SD), skewness, and kurtosis with monthly asthma hospitalization. We further applied the indicators-guided Poisson regression models to evaluate and predict the impact of air pollution on asthma incidence. We found that SD of  $\text{PM}_{10}$  was the most correlated indicators for asthma hospitalization for all age group, in particular for > 64 years. The

skewness of  $O_2$  gives the highest correlation to adult asthma. The constructed regression model shows a better predictability in annual asthma hospitalization trends for 0–14 years group (mean absolute percentage error = 8.1 %) in Taiwan in the period 1998–2010. We conclude that the proposed study approach can predict asthma hospitalization effectively. Our analysis also identifies the particulate pollutant is likely to be the major risk factor that contributes to asthma hospital incidence.

**TH094 Risk assessment of airborne influenza virus particles and implications for control** C. Wang, Department of Bioenvironmental Systems Engineering; C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering. Influenza is a highly contagious indoor airborne infectious disease, leading to severe morbidity and mortality worldwide annually. The high infection risk may associate with the infection on human respiratory tract. Here we treated indoor environment as an ecosystem. The objectives of this study are: (i) to assess the indoor population transmission dynamics of influenza virus particles, (ii) to quantify the inhalation and deposition dynamics of virus particles in human respiratory tract, and (iii) to apply indoor environmental control and drug treatment for reducing influenza infection. This study used respiratory droplet experimental data to reconstruct the influenza virus particles size distribution. The compartmental susceptible-infected-recovery-environment (SIRE) model was used to simulate the dynamics of population transmission. We used the particle inhalation and human respiratory tract (HRT) models to assess inhalation and deposition of virus particles. A pharmacodynamic (PD) based dose-response model linked with a probabilistic risk assessment approach was used to assess influenza infection risk. We also examined the treatment efficacy after using antiviral drug on reducing influenza infection risk. Here we show that: (i) the mean diameter of particle distribution is  $7.68 \mu\text{m}$  for sneezing, (ii) the inhaled virus particle concentrations based on active physiological mechanism are  $1.75 \times 10^4$ – $4.09 \times 10^4$  and 450–954 virus particles  $\text{mL}^{-1}$  in respirable and inspirable transmission pathways, respectively, and (iii) indoor influenza virus particles are more likely to pose infection risk on upper respiratory tract. Our study also implicates that a proper ventilation system together with antiviral drugs could effectively control indoor airborne influenza virus particles. This study provides a novel risk assessment and control framework to better assess influenza infection risk in indoor ecosystem from a dynamic modeling point of view.

**TH095 OXIDATIVE STRESS STATUS IN NON-PETROL STATION DISPENSERS OF GASOLINE IN NIGERIA** O.M. Akinosun, University of Ibadan / Department of Chemical Pathology; S. Adamu, University College Hospital / Department of Chemical Pathology. Occupational exposure to toxic chemicals such as gasoline is a major public health concern worldwide. Gasoline is one of the most frequently utilized chemicals whose use is on the increase. When absorbed, the volatile constituents of gasoline generate free radicals leading to oxidative stress. Researchers have shown hematological alterations, increased risk of malignancies and other chronic diseases in humans and rats exposed to gasoline vapors. The risk is greatest in the developing countries, including Nigeria, where there are limited facilities to reduce over exposure and the toxic effects of the chemicals. Non gasoline station dispensers of gasoline, which are common sights in Nigeria especially in the Northern parts, often use mouth to create a vacuum pressure to dispense the product through pipes into the receivers and this may lead to high levels of exposure to gasoline vapors. Markers of oxidative stress including total plasma peroxide (TPP), total anti oxidant status (TAS), antioxidant vitamins (A, C and E), were compared between 90 road side dispensers of gasoline and 90 age and sex matched apparently healthy non gasoline dispensers serving as controls. Vitamins A, C and E assays were performed using an Ultra sensitive and specific HPLC. Total anti oxidant status, measured using the method by Koracevic et al., 2001, while Total Plasma Peroxide (TPP) levels were determined using the FOX2 method. The mean age of the exposed and control groups are  $29.03 \pm 3.7$  and  $29.24 \pm 3.5$  years respectively. The

mean plasma levels of TAS ( $0.60 \pm 0.33 \text{ mmol/L}$ ), vitamins A ( $54.45 \pm 6.4 \mu\text{g/dL}$ ), vitamin C ( $0.68 \pm 0.13 \mu\text{g/dL}$ ) and vitamin E ( $0.72 \pm 0.10 \mu\text{g/dL}$ ), of the exposed were significantly ( $p < 0.001$ ) lower than the controls ( $1.29 \pm 0.25 \text{ mmol/L}$ ,  $69.82 \pm 5.4 \mu\text{g/dL}$ ,  $1.06 \pm 0.13 \mu\text{g/dL}$ ,  $1.01 \pm 0.13 \mu\text{g/dL}$ , respectively). The mean plasma levels of TPP in the exposed ( $58.02 \pm 9.37$ ) is significantly higher ( $p < 0.05$ ) than the non exposed ( $47.46 \pm 10.28$ ). There is higher level of oxidative stress in road side dispensers of gasoline compared to the controls. This is an indication that road side gasoline dispensers are probably at greater risk of developing chronic diseases associated with increase oxidative stress and hence antioxidant supplementation may be of benefit. Key words: gasoline, total antioxidant status, total plasma peroxide

**TH096 Aggregate Risk Assessment on Xylene and Ethylbenzene** J. Seo, National Institute of Environmental Research; T. Kim, J. Kim, National Institute of Environmental Research / Risk Assessment Division; P. Kim, K. Choi, National Institute of Environmental Research. The aggregate risk assessment on xylene and ethylbenzene was carried out according to the guidance established newly in 2010 with the purpose of providing information for risk management. In human exposure assessment, the results indicated that lower ages were exposed more and that, in the interior space at home, the highest level of human exposure occurred via inhalation. At outdoor spaces, exposures via inhalation and drinking were less than 1%. In human health risk characterization, xylene showed HI (Hazard Index)  $< 1$  in all ages. When Reasonable Maximum Exposure was applied, HI for young children was 0.64. The HI of ethylbenzene was also below 1 (0.02–0.04) in all ages, indicating no potential risk. From this study, it is considered that xylene needs to be continuous monitoring with interest because this substance may be more sensitive on young age group. In addition, to reduce the uncertainty of the risk assessment, the Korean exposure factors on young age group such as infant, children had to be established as soon as possible.

**TH097 Organ-specific distribution of 7-chlorinated benz[a]anthracene and regulation of selected cytochrome P450 genes in rats** T. Ohura, Meijo University; H. Sakakibara, University of Miyazaki; T. Kido, University of Shizuoka; N. Yamanaka, N. Tanimura, National Institute of Animal Health; K. Shimoi, University of Shizuoka; K.S. Guruge, National Institute of Animal Health. Recently, chlorinated polycyclic aromatic hydrocarbons (CIPAHs) with larger 3-ring have been detected in the environment, which are possible to be concerned in hazardous pollutants. We previously reported that 14-day exposure to 7-chlorinated benz[a]anthracene (7-Cl-BaA), a new environmental pollutant, selectively induced hepatic cytochrome P450 (CYP)1A2 in rats, although treatment with its parent, benz[a]anthracene (BaA), induced CYP1A1, CYP1A2, and CYP1B1. In this study, to better understand the relative contribution of chlorination to the toxicity of polycyclic aromatic hydrocarbons (PAHs), we investigated the organ-specific distributions of 7-Cl-BaA and BaA in F334 rats. After 14 days of oral administration of 7-Cl-BaA or BaA at a concentration of 1 or 10 mg/kg body weight/day, both chemicals were detected in their plasma, which was collected 24 hours after the last administration, even at the lower dosage. Dose-dependent accumulation patterns were observed in the liver, muscle, kidney, spleen, heart, and lung. The 7-Cl-BaA concentrations in the organs were higher than those of the BaA. Furthermore, at the end of the exposure, 7-Cl-BaA specifically regulated several CYP genes in the heart more so than in other organs, although these inductions were not significant in the BaA treatment. 7-Cl-BaA might also stimulate the metabolic pathways of chemicals other than AhR-mediated metabolism, which is specific to normal PAHs, because of the alterations of CYP2J4, CYP4B1, and CYP17A1 expression in rats. In conclusion, our results imply that the chlorination of PAHs may change their organ-specific distribution and consequently alter their toxicological impacts compared to their parent PAHs.

**TH098 COMMON RADIOACTIVITY AND PESTICIDE HEALTH IMPACT IN CENTRAL ASIA** I. Hadjamberdiev, R. Tukhvatshin, Toxic Action Network Central Asia; I. Zhakipova, NGO

Ekoi. There are huge eco-health problems in Central Asia due dozens former USSR uranium tailings and abandoned obsolete pesticides warehouses. The 17% of TienShan-Pamir are worse polluted area, and the most in Ferghana valley (territory of three countries - Kyrgyzstan, Tadjikistan, Uzbekistan). Population (and part of physicians) don't clear its danger about and assessment of it. We studied radioactivity sources (gamma-wave level, uran-polluted food and water income, indoor radon) and counting body-income per years. Study points were 5 settlements of three countries. Additionally, we have determined content (in the same 3 settlements) – the other harmful toxicants - obsolete pesticides (still using illegally) - in vegetable and meat. It has been determine blood cells, proteins IgA/IgE, IgG, goiter hormones, liver ferments, urine tests. It has been concludes that immunity system and liver are very vulnerable in case of common two harmful impacts. Scientific results was implementing in the settlements (by school course, video and booklets in local languages).

**TH099 Polluting Ourselves: Methodological Considerations for the Curious Case of Risk Analysis for Endogenously Generated Compounds** B.P. DeMott, C.A. Williams, P.R. Gentry, S. Bullock, Environ International. Evaluations of risk assessment methodologies have repeatedly identified refined strategies for addressing cumulative exposures for particular compounds from multiple sources as a key element for next generation approaches. This topic has been discussed in both ecological and human health risk assessment contexts and by advisory bodies from the European Commission's Scientific Inter-Committee Coordination Group to the United States' National Academy of Sciences. One complexity recognized but not yet addressed via specialized alternative methods is the contribution of endogenous production to cumulative exposures. Beyond the initial problem of quantifying typical and episodic generation of metabolic byproducts and functional biochemicals (e.g., hormones), mode of action differences for internally generated versus externally applied doses and the regulation and capacity of detoxification pathways present complex challenges for risk assessment. We analyzed toxicological, toxicokinetic and risk characterization aspects of various endogenously generated compounds (EGCs) to develop a method for quantitatively distinguishing endogenous contributions from external sources. We identified the following key components for a useful method: 1) accounting for internal to applied dose conversion; 2) different approaches for EGCs present below effects thresholds versus endogenous exposures producing effects (e.g., hormones); 3) need to apply relevant mode of action characteristics for potential carcinogens. We present possible approaches for addressing each of these components illustrated by case studies with acetone, steroid hormones and formaldehyde, respectively. Using acetone, we demonstrate approaches for comparing normal circulating blood levels to equivalent inhalation exposure concentrations. Using estrogens, we demonstrate accounting for metabolic inactivation following ingestion exposure and relative potency considerations. Using formaldehyde, we demonstrate the lack of relevance of linear no-threshold extrapolation for EGCs. We conclude that it is reasonably achievable for new risk assessment methods to incorporate approaches distinguishing endogenous contributions from cumulative exposure and characterizing risks separately so that risk management of EGCs can be based on distinct and appropriate levels of concern compared to environmental exposures.

**TH100 ENZYME ACTIVITY AND OXIDATIVE STRESS IN AEDES (STEMOGYIA) AEGYPTI (LINNAEUS, 1762), EXPOSED TO ESSENTIAL OILS.** C.H. Soares, Universidade Federal de Santa Catarina / Bioquímica; Y. Simon, A. Becker, F. Cruz, A. Lindenberg, F. Casagrande, E. Silva, Universidade Federal de Santa Catarina. Dengue is a serious public health problem in many parts of Brazil. Increased outbreaks of the mosquito *Aedes (Stemogyia) aegypti* (Linnaeus, 1762) has been observed in recent times. So the search for the control strategies has become a priority for public health sectors in Brazil. The aim of this study was to evaluate the enzymatic activity related to oxidative stress in mosquito larvae exposed to essential oils. The oxidative stress response exhibited by larvae allows us to understand the

physiological mechanisms used to survive when they are exposed to natural larvicide. For this purpose, the activities of CAT, GSH and GPx of oxidative stress and enzyme such as cholinesterase, lactate dehydrogenase and alkaline phosphatase were evaluated. For the analyzes, the homogenate in water with Triton X100 (0.1%) and a pool of ten (10) mosquito larvae exposed to extract of essential oil for 24 to 48 hours at various concentrations (from 0.01 to 0,1 ppm) were used. In the same way, a control group no exposed was monitored. Regarding enzyme activities, significant differences were observed between the control group and the exposed larvae group, particularly the activity of lactate dehydrogenase which was higher. The activity of catalase and the concentration of reduced glutathione were higher for exposed larvae. The activity of glutathione peroxidase proved to be unviable biomarker for understanding the mechanisms of toxicological effects.

**TH101 Organotin contamination in market seafood and its implication for human health risk in Hong Kong.** K. Ho, Y. Mak, The University of Hong Kong / The Swire Institute of Marine Science and School of Biological Sciences; E. Mak, University of Ottawa / Department of Earth Sciences, Faculty of Science; K. Leung, The University of Hong Kong / The Swire Institute of Marine Science and School of Biological Sciences. Organotins (OTs), in particular tributyltin (TBT) and triphenyltin (TPT), have caused widespread adverse effects on marine organisms ever since their wide application as biocides in 1960s. For instance, TBT can induce the abnormal development of imposex in marine gastropods, and inhibit growth and development in oysters. A mandatory global ban on the use of OT-based antifouling systems has been enacted by International Maritime Organization to minimize their environmental impacts since September 2008. It is, therefore, anticipated that there will be a reduction of OT pollution in marine environments around the world. However, previously studies showed that OT are still heavily contaminating Hong Kong's waters. Humans can potentially uptake OTs via consumption of contaminated seafood, and high levels of these chemicals present in our body tissues may lead to health problems. In this study, we measured the tissue concentrations of OTs (i.e., mono-BT, di-BT and TBT, mono-PT, di-PT and TPT) in 12 commonly available seafood species in Hong Kong including three gastropod, three bivalve and six fish species. The highest total OTs concentration was 2325.8  $\mu\text{g kg}^{-1}$  dry weight in the tongue sole *Paraplagusia blochi*, while the Babylon shell *Babylonia areolata* also showed a considerably high amount of total OTs (1751.4  $\mu\text{g kg}^{-1}$  dw). TPT was the most abundant residue among the six OTs, accounting for 56-97% of total OTs. Following a standard risk assessment protocol, the non-cancer hazard quotients (HQs) and hazard indices (HI; i.e., summation of HQs) were determined. The highest HQ for TPT was 0.92 in *P. blochi* while the HQs for TBT and DBT were much less than 1. HI of *P. blochi*, however, is greater than 1 indicating that it is likely to have certain risks of consuming this species as seafood. Evidently, OTs are still persistent in Hong Kong's marine environments. TPT, in particular, should be the priority pollutant of concern. Appropriate management actions should be taken to control the use and release of OTs in the region in order to safeguard the marine ecosystem and human health.

**TH102 Differences Between U.S. EPA Reference Doses (RfDs) & European Chemicals Agency (ECHA) Long-term Derived No Effect Levels (DNELs) for Selected Metals** B.H. Magee, ARCADIS; K. Connor, ARCADIS / Risk Assessment and Environmental Science; B.J. Locey, ARCADIS / ESAP; R. Lemus-Olalde, Z. Yi, M. Jackson, ARCADIS. The European Chemicals Agency (ECHA) recently released manufacturer/importer REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) chemical registration files, making available the Derived No Effect Levels (DNELs) for many substances. The DNEL is defined as "the level of exposure above which humans should not be exposed" and needs to reflect the likely route(s), duration and frequency of exposure. Typically, DNEL values are developed by Industry following ECHA's guidance. The registrant has the final decision on the selection of the key studies, endpoints of concern, and the assessment factors to account for sources of

uncertainty. On the other hand, the U.S. Environmental Protection Agency (EPA) derives chronic reference doses (RfDs) and mandates that they be used by regulated parties. The RfD is defined as “an estimate (with uncertainty spanning perhaps an order of magnitude) of an exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.” For common metals that are present at waste sites, EPA’s RfDs have not been updated for many years. For instance, the RfD for copper is based on a 1965 study of acute copper sulfate poisoning in 48 people who attempted suicide by ingesting up to 4 ounces of copper sulfate crystals or powder. Chronic RfDs and long-term DNELs and their basis are compared for selected metals, such as copper, cobalt, iron, manganese, molybdenum, and selenium. In many cases, the DNELs are higher than the RfDs, such as the DNEL for copper of 0.16 mg/kg-day versus the RfD for copper of 0.04 mg/kg-day. Similarly, the DNEL for molybdenum is 4.85 mg/kg-day versus the RfD of 0.005 mg/kg-day. The differences between these toxicity values may be a result of differences in the processes by which they are derived, including differences in the availability of toxicity data, the entity (industry versus regulatory agency) responsible for generating the reference values, the methodologies and selection of default values used in the derivation, and the process by which the final values are accepted. These issues will be discussed, and recommendations will be made concerning which are the most scientifically defensible toxicological criteria for use in human health risk assessment.

**TH103 Particulates matter pollution in semi urban site** a.B. akachat, UMBB / Department of chemical engineering processes. The road traffic is the major source of air pollution in the agglomeration of Algiers. As part of the assessment and management of the ambient air quality, we have studied the impact of a petrol station located in a semi-urban site west of Algiers, a weighting of the frequency, the non-detection and the severity of the impact on air quality has been determined. The petrol stations were chosen for their wide setting up throughout the national territory, the proximity service they represent and the nature of services they provide (storage and distribution). The survey has enabled to identify six significant environmental aspects including a major emission of VOC known to be precursors of ozone in the presence of NO<sub>x</sub> we concluded that the petrol stations could be sites creating oxidant smog. A qualitative analysis by taking of dust by filter sampling system is in agreement with the obtained environmental weighting.

**TH104 Cadmium levels in house dust samples from Portugal** A. Sousa, University of Aveiro / Biology; M. Pastorinho, Universidade de Aveiro; T. Itai, Center for Marine Environmental Studies (CMES); S. Takahashi, Ehime University / Center for Marine Environmental Studies; S. Tanabe, Ehime University / Center for Marine Environmental Studies (CMES); L. Taborda-Barata, University of Beira Interior / CICS; A.J. Nogueira, University of Aveiro / Department of Biology & CESAM. Cadmium is a toxic metal classified as a human carcinogen. The general population is exposed to cadmium through consumption of food and drinking water, inhalation from ambient air or cigarette smoke, or ingestion of contaminated soil and dust. Generally, food is considered the major source of cadmium exposure for nonsmokers but inhalation and ingestion of contaminated house dust also plays an important role. Despite this, no reports on the levels of Cd in Portuguese house dust samples are available. In the present work, cadmium concentrations were quantified by ICP-MS (Inductively Coupled Plasma – Mass Spectrometry) on sieved dust samples collected from vacuum cleaner bags. A total of 28 homes from two Portuguese cities (Aveiro and Coimbra) were selected. Cadmium levels were highly variable, from 0.1 up to 8.3 µg.g<sup>-1</sup>, thus reflecting the diversity of sources in modern households. The calculated median value (1.1 µg.g<sup>-1</sup>) is similar to those published for European household dust and in the same order of magnitude for other locations worldwide. Despite the fact that for the studied households the average cadmium weekly intake is much lower than the Tolerable Weekly Intake (TWI) set by FAO/WHO for this metal, it is important to consider this exposure route while

characterizing cumulative exposures in potential health risk assessment. Considering the most contaminated house, for instance, 15% of the TWI is achieved only through dust ingestion.

**TH105 Polychlorinated biphenyl residues in human milk and umbilical cord blood in Vojvodina, Serbia** M. Turk Sekulic, Faculty of Technical Sciences, University of Novi Sad; J. Radonic, Faculty of Technical Sciences; M. Vojinovic Miloradov, Faculty of Technical Sciences, University of Novi Sad; Z. Grujic, Faculty of Medicine, University of Novi Sad; M. Prica, I. Mihajlovic, Faculty of Technical Sciences, University of Novi Sad. Polychlorinated biphenyls are ubiquitous toxic compounds in the environment with negative influences on the health status of human beings. Especially susceptible might be the fetus, which is exposed in utero, and newborn breast-fed infant, since both are exposed to relatively high levels of PCBs during a critical period of organ growth and development. A damaging of industrial and military targets in former Republic of Yugoslavia during the Balkan wars and the “Allied Force” operation resulted in the release of large amount of PCBs. No systematic and regular human biomonitoring of PCBs has been performed in the West Balkan Countries till now. In this study umbilical cord blood samples of healthy pregnant women aged between 20 and 39 years at the time they gave birth and human milk samples from the same women following delivery were collected between July and October 2012 from 20 participants living in an industrialized area of Vojvodina region. All samples were analyzed for their content of dioxin-like (PCB 118) and indicator (PCB 28, 52, 101, 138 153, and 180) congeners. Measurements were performed using ECD GC Varian 3400. Concentrations of selected PCBs were in the range of 4-70 ng/g <sup>lipid base</sup> for human milk. In all umbilical cord blood samples concentration levels of analyzed PCBs were under the LOD (< 0.5 ng/g, lipid adjusted). The four congeners 23’44’5-PCB (#118), 22’344’5-PCB (#138), 22’44’55’-PCB (#153), 22’344’55’-PCB (#180) contribute the main share to total body burden of PCBs. Higher chlorinated congeners were found in 50-fold higher concentrations in human milk in relation to the lower chlorinated congeners, while the levels of hexaCB were 2-4-fold higher than the levels of heptaCB. The body burden of PCBs increases with age and decreases over the total nursing period. Women who had lived outside highly industrialized areas showed lower concentrations of PCBs. Birth weights didn’t influence of PCBs concentrations on human milk burden. Unique and new information on bioaccumulation/biomagnifications in humans and also mother-child transfer by human milk and umbilical cord blood of PCBs is needed, since it controls incentive of breastfeeding, indispensable way for health infant’s development and growth. Acknowledgement: This study was funded by City Administration for Environmental Protection, Novi Sad (Project No. VI-501-2/2012-59)

**TH106 Uncertainty analysis of a Physiologically-based pharmacokinetic (PBPK) model parameters for PCDD** fabrega, Universitat Rovira i Virgili / Chemical engineering department; s. kumar; M. Nadal, University Rovira i Virgili; J. Domingo, Universitat Rovira i Virgili; M. Schuhmacher, Rovira i Virgili University. Polychlorinated dibenzodioxins (PCDD) are a family of compounds widely spread in the environment and biota around the world. Because the high human toxicity of some congeners a reliable risk assessment tools are necessary. In order to estimate the theoretical body burdens of chemical pollutants, physiologically-based pharmacokinetic (PBPK) models have been proved to be very useful. PBPK models are mathematical representations of the human body where organs are considered as compartments, where the distribution and accumulation of contaminants is predicted along time. In order to develop the PBPK models physiological and biochemical parameters are needed. Because the estimation of these parameters comes from experimental values a huge uncertainty has been reported in the PBPK models. The objective of the present work is to assess the uncertainty of the parameters associated to a PBPK model of PCDD. The uncertainty analysis has been done for a case study in Tarragona (Spain).

**TH107 Short- and long-term behavioral toxicity of an early**

**exposure to polycyclic aromatic hydrocarbons (PAHs)** G. Crepeaux, P. Bouillaud, J. Olry, URAFFA, INRA UC340, Université de Lorraine; J. Gaillard, UR AFFPA / URAFFA, INRA; C. Feidt, G. Rychen, R. Soulimani, H. Schroeder, URAFFA, INRA UC340, Université de Lorraine. Whereas PAHs have been recently established as potent neurotoxicants for both adult and developing brain, little is known about the neurotoxicity of an early exposure to such compounds through a contaminated food. In this study, we developed a model of exposure in pregnant and/or lactating female rats exposed to a daily ingestion of contaminated food pellets with a mixture of PAHs. This mixture included 16 PAHs referred as potentially toxic by the US-EPA. The proportion of each PAH in the mixture corresponded to the profile found in human contaminated food. Two levels of exposure were used: the lowest one (2 µg/kg b.w./day) was calculated to be representative of the mean daily human level of exposure whereas the second one was 100-fold higher. Two studies were performed: the first one occurred during both gestational and lactation whereas the second one was restrained to gestation only. Rat pups were tested for equilibration and motor coordination during the first 3 weeks of postnatal life. At the adult stage, the animals were evaluated for anxiety in the elevated plus-maze, for activity in the open field, and for spatial learning in the Y maze and the 8 arm maze. No short-term motor impairments were observed in pups whatever the period of PAH exposure. In adult rats, a gestational/lactational PAH exposure induced a locomotor hyperactivity and an increase in the anxiety level compared to controls. The same level of hyperactivity was observed in rats exposed during gestation but without any modification of anxiety. Finally, learning and memory performances remained the same in both rat groups of the two studies. The results of the present study show the ability an early PAH exposure occurring during critical periods of brain development to induce slight behavioral disruptions related to activity and anxiety and suggest the potent toxicity of the ingestion of PAH contaminated food during gestation and/or lactation. Discrepancies in the results obtained between the two studies suggest the role of the period of exposure in the modulation of the behavioral toxicity of PAHs.

**TH108 Kinetic study of polychlorinated biphenyl in lactating goats exposed to contaminated corn silage** A. Fournier, C. Feidt, Université de Lorraine / URAFFA, INRA; S. Jurjanz, Université de Lorraine / URAFFA; P. Marchand, ONIRIS / LABERCA; J. Pinte, Direction Générale de l'Alimentation; B. Le Bizec, ONIRIS / LABERCA; G. Rychen, Université de Lorraine / URAFFA, INRA. The persistent organic pollutants, like polychlorinated biphenyls (PCBs) or dioxins and furans (PCDD/Fs), may accumulate in tissues of organism. Ingestion of contaminated forage in ruminants results in a potential contamination of the animal products. Thus, there is real need to characterize the contamination and depuration process. This study was aimed to determine the kinetics of contamination and decontamination of PCBs in milk of lactating goats. Corn silage from the accidentally contaminated area of St Cyprien (France) with PCBs and PCDD / Fs (3.74 pg WHO<sup>-1</sup>TEQ DL-PCBs, 4.65 pg WHO<sup>-1</sup>TEQ PCDD / Fs + DL-PCBs and 9.57 ng ND<sup>1998</sup>L-PCBs per gram of silage recalculated at 12% moisture) was administered daily (0.6 ± 0.1 kg dry matter) to four lactating goats for a period of 39 days. The kinetics of PCB decontamination in milk was studied thereafter by replacing contaminated by uncontaminated corn silage during 20 days. DL-PCBs and ND<sup>1998</sup>L-PCBs have been analysed in milk, regularly sampled during the exposition and depuration period (ASE extraction and GC-HRMS equipped with a DB-5MS column using the SIM acquisition mode, limits of detection were 0.007 ng/g fat and 0.04 pg WHO<sup>-1</sup>TEQ/g fat for ND<sup>1998</sup>L-PCBs and DL-PCBs respectively). Concentrations of DL-PCBs + PCDD / Fs in milk exceeded rapidly (less than 15 days) the threshold limit of 6 pg WHO<sup>-1</sup>TEQ/g fat (Regulation 1881/2006/EU), and reached levels of 15-18 pg WHO<sup>-1</sup>TEQ/g fat after 5 weeks. Feeding uncontaminated corn silage resulted in a very fast decrease of the PCB levels in milk. The kinetics of decontamination was biphasic, including first an initial rapid elimination phase (less than 10 days) followed by a slower elimination phase, not exceeding 5 weeks. In these experimental conditions (physiological status: fatness, lactation),

20 days were sufficient to obtain a milk with a PCB level below the threshold concentration. Thus, the goats could be considered as decontaminated at the end of the experiment. This study reveals that in high milk producing ruminants, PCBs from environmental matrices may be considered as real concerns in terms of food safety. It also indicates that a decontamination process of these lactating animals remains feasible.

**TH109 Structural Alerts to Classify Inhalation Toxicity** R. Kühne R. Ebert, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry; I. Tluczkiewicz, S. Escher, Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM) / Chemical Risk Assessment; G. Schuurmann, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry. The concept of Thresholds of Toxicological Concern (TTC) defines generic human exposure threshold values for groups of compounds. In order to derive and to apply TTC values, a respective classification into particular groups is required. A classification scheme has been developed, which identifies structural features leading to low or high toxicity after inhalation exposure. The analysis is based on the TTC RepDose database, which contains 296 chemicals being tested in repeated dose toxicity studies. The classification is based on structural rules. An extended scheme additionally includes partition coefficients that can be estimated from chemical structure. The performance of the model is shown in terms of contingency statistics and toxicity ranges for the individual groups. Examples demonstrate the model use. The reliability and limitations of the approach are discussed. The scheme can be run fully automated within the OSIRIS edition of the software system ChemProp [1], that is publicly available free of charge due to a bilateral license agreement. In addition, the implementation yields the appropriate TTC value and provides information on the prediction uncertainty and applicability domain. The study has been financially supported by the CEFIC-LRI Project "Threshold of Toxicological Concern (TTC) for inhalation exposure: Improvement of the TTC concept for inhalation exposure and derivation of thresholds with the database RepDose". [1] Chemical Proper-ties Estimation Software System (ChemProp) 5.2.8, 2012. UFZ Department of Ecological Chemistry, <http://www.ufz.de/index.php?en=6738>.

**TH110 Risk Assessment of the dietary exposure to dioxins in Korea** J. SUH, Korea Food and Drug Administration / Department of Food Risk Assessment; Y. Kang, O. Paek, J. Yun, S. Park, S. Lee, National Institute of Food and Drug Safety Evaluation / Department of Food Risk Assessment. Dioxins are persistent organic pollutant derived from the environment. Many nations including our country devote to monitor of the level of them and to evaluate the dietary exposure of their population because of their endocrine disruptor action. Therefore, we investigated the risk of the dietary exposure to dioxins in Korea using data collected from national surveys conducted by the Korea Food and Drug Administration (KFDA) in 2000-2011. We included 1,362 foods (16 food groups of 66 food species) to evaluate risk assessment of dioxins. The mean level of dioxins (PCDD/Fs + DL-PCBs) was 0.275 pgTEQ/g wet weight (ww) and maximum level was 19.441 pgTEQ/g ww. The highest level of dioxins was found in the fish and shellfish groups. Dioxins exposure assessed by combining the dietary intake data of the forth Korea National Health and Nutrition Examination Survey (KNHANES IV-2) conducted by Korea Centers for Disease Control and Prevention in 2008. The mean estimated daily intake (EDI) of dioxins was 0.346 pgTEQ/kg body weight (b.w.)/day for the mean of the general populations and 1.270 pgTEQ/kg b.w./day for the 945 percentile (P95). EDI was lower than tolerable daily intake (TDI, 4 pgTEQ/kg b.w./day). The percentage of TDI for the mean and the P95 were 8.7% and 32.6%, respectively. The fish and shellfish group was the major contributor to dioxins for both the mean and the P95. The highest level of EDI was shown in 20-64 year old. The percentage of TDI was 10.4% and 36.5%, respectively for the mean and for the 95th in 20-64 years. The contaminations of dioxins by years were from 3.441 to 21.270 pgTEQ/g ww and EDI was between 0.135 and 0.609 pgTEQ/g ww. The percentage of TDI was shown from 3.4% to 15.2%. We



concluded no concern about the dioxins in Korea.

**TH111 Toxicity of amphotericin B conjugated with magnetic nanoparticles in *Paracoccidioides brasiliensis*.** D.C. Iocca, Universidade de Brasília; L.R. de Souza, University of Brasília; J. De Souza Filho, University of Brasília / Department of Genetics and Morphology; S.N. Bao, University of Brasília; C. Grisolia, University of Brasília / Genetics and Morphology; R.B. Azevedo, M.P. Garcia, University of Brasília. Nowadays technological development helps to reduce the size of the materials used in the field of biomedicine. Superparamagnetic iron oxide (SPIO) nanoparticles in drug delivery vehicles must address issues such as drug-loading capacity, aqueous dispersion stability, biocompatibility with cells and tissue, and its mechanism of toxic action. Magnetic fluid (MF) samples, mainly consisting of SPIO particles suspended in a carrier fluid, particularly maghemite, are the most used magnetic nanoparticles (MNPs) in biological and medical applications. Amphotericin B (AmB) is the most recommended treatment to routine use on severe *Paracoccidioidomycosis* (PCM), the most important systemic fungal infection found in Central and South America caused by *Paracoccidioides brasiliensis* (Pb). Unfortunately, AmB causes acute side effects following intravenous administration, which limits its clinical use. Thus, in this study we report *in vitro* tests of the MFLB-AmB complex, a new engineered nanosized magnetic drug delivery system comprising amphotericin B (AmB) adsorbed onto the outer surface of bilayer of lauric acid-coated nanosized maghemite (MFLB). Antifungal activity against *P. brasiliensis* strain 18 was determined by MIC (Minimum Inhibitory Concentration) assay. Cytotoxicity evaluated by MTT assay and genotoxicity was determined by DNA fragmentation evaluation in flow cytometer and comet assays, both against mice fibroblast cell line. The MFLB-AmB complex presented antifungal activity, with MIC of 1.25 µg/mL, whereas the free AmB showed MIC of 1.0 µg/mL. Cytotoxicity of MFLB-AmB was lower than AmB, with IC<sub>50</sub> of 19,8 e 6,1 µg/mL, respectively. IC<sub>50</sub> of MFLB-AmB induced less DNA fragmentation than IC<sub>50</sub> of AmB by comet assay. However, there is no difference between all groups on the flow cytometer assay. Hence, the results observed suggest that MFLB-AmB may be a new nanotool of treatment for PCM in humans, as the results indicate that its toxic action decreases when conjugated with MNPs. This model has presented a new possibility for more efficient handling of PCM in humans. There are promising results in this model (*in vitro*) but tests are still needed to verify their *in vivo* mechanisms of toxicity.

**TH112 Tools for modelling and assessment of exposure to dangerous substances in their whole life cycle** R. Prichystalova, Energy Research Centre; J. Dlabka, P. Danihelka, Technical University of Ostrava / Energy Research Centre. Chemical substances present a serious hazard for human health and environment during their whole life cycle, i.e. from the production, through the manipulation, transport and usage, to their disposal. To ensure the high standards of human health and environment protection, risk management of chemicals was introduced to optimize the risks. This review deals with the legislative instrument in the area of chemicals risk management, i.e. with Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals, abbreviated as REACH respectively. For risk assessment of substances during their whole life cycle, according to REACH, was created a lot of tools, which are able to simulated exposure scenario of substances, where data of exposure not exists. The aim of the review is to present selected tools with recommendation for which simulation of exposure scenario are useable or not (it means exposure scenario for workers, environment, consumers). This review was created with the help of the Ministry of Education Innovation for efficiency and the environment (CZ.1.05/2.1.00/01.0036).

**TH113 Hg environmental quality standards in different aquatic environmental matrices: Scientific underpinnings and regulatory implications** D.A. Vignati, CNRS / LIEC UMR; S. Polesello, CNR-

IRSA / UOS Brugherio; M. Carere, Istituto Superiore di Sanità; M.S. Bank, University of Massachusetts / Department of Environmental Conservation. Regulatory compliance for Hg pollution has long been assessed in terms of Hg concentrations in the water phase. In such frameworks, Environmental Quality Standards (EQS) are enforced as total Hg concentrations, although methylmercury (MeHg) is the form of highest concern. Water quality criteria based on total Hg levels in the water phase may therefore be underprotective against secondary poisoning, unless they are adjusted for MeHg levels; which is so far an uncommon practice. Furthermore, monitoring procedures are not necessarily harmonised and even simple filtration before analysis can result in marked differences in measured Hg levels. To correct for these potential biases, the European Union has introduced, in 2008, a major shift in Hg monitoring and management by adopting an EQS based on total Hg concentrations in biota as an alternative to the water EQS. The use of a Hg-EQS for biota is scientifically sound because it automatically takes into account the bioaccumulation and biomagnification processes that are the main concerns from Hg pollution. On the other hand, the proposed EQS numerical value (20 µg kg<sup>-1</sup> wet weight in fish) risks to create a situation of widespread non compliance in EU water bodies. The Proposal for a Directive of the European Parliament and of the Council as regards priority substances in the field of water policy (published in 2012) states that Member States may classify Hg as an Ubiquitous, Persistent, Bioaccumulative and Toxic (UPBT) substance. Provided that a robust monitoring baseline exists, Member States may present separate classification maps showing that the possible 'not good' status of a water body is caused by an UPBT. Hg classification as an UPBT substance must not relax the pressure on Member States to continue targeting important local pollution issues that can cause large differences in neighbouring water bodies. Rather, more state-of-the-art science must be brought in to refine the biota EQS. We have identified the following critical issues for consideration in further regulatory updates: dietary MeHg toxicity to the most representative/most sensitive fish species in EU waters, protective effects of Se against adverse Hg effects (i.e., simultaneous analyses of Hg and Se), updated data on risks for human health in relation to consumption of fishery products, and structure of the trophic chain must be elucidated to understand and estimate the actual risk to top predators.

**TH114 Bioaccumulation and biomagnification of mercury (Hg) to "at risk levels" in the fish community in the humic Lake Øvre Sandvann, SE Norway** L.S. Heier, UMB / Centre for Environmental Radioactivity (CERAD CoE); H. Myreng, Norwegian University of Life Sciences / Department of Plant and Environmental Sciences; T. Haugen, Norwegian University of Life Sciences / Department of Ecology and Natural Resource Management; O. Heier, The Norwegian Association of Hunters and Anglers (NJFF); B. Salbu, Norwegian University of Life Sciences / Department of Plant and Environmental Sciences; B.O. Rosseland, Norwegian University of Life Sciences / Department of Ecology and Natural Resource Management. There is a great international effort put into reducing the emissions of mercury (Hg) to the environment. Despite substantial national reduction of Hg emissions in Norway in last decades, the Hg concentration in freshwater fish in the south east of Norway is in general still increasing in lakes not affected by any point sources. This is hypothesised to be due to increased levels of organic carbon and/or reduction in "acid rain", which affects the speciation and methylation of Hg in the catchment and in the lakes. The fish Hg levels are high and for many lakes they reach levels that calls for restrictions related to consumption. The objective of the present work was to investigate Hg levels in fish in a pristine lake; Øvre Sandvann, in the SE highlands of Østfold, Norway. In addition, the concentration of selenium (Se) was determined to investigate the molar ratio between Hg and Se to assess the potential risk of toxicity of Hg to the fish. Collection of fish was done in the autumn of 2012 using pelagic- and benthic Nordic Gillnet series. Four fish species that inhabit the lake; brown trout (*Salmo trutta*), European perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), and European minnow (*Phoxinus phoxinus*) were caught. Muscle tissue were sampled for determination of stable nitrogen and carbon isotopes to investigate trophic position and feeding sources,

and for determination of total Hg and Se concentrations. Stable nitrogen and carbon isotopes were analysed using IR-MS, Hg was analysed using flow injection mercury system (FIMS) and Se was analysed using ICP-MS. The bioaccumulation of Hg was high and the highest levels were found in perch which reached a concentration of 2.5 ppm (ww) in the largest fish (1 kg). High levels were also detected in roach in which a fish in the weight range of 150 g reached levels as high as 0.7 ppm. The Hg levels in the fish were better correlated with fish age, compared to the size of the fish. The molar ratio of Hg:Se differed among the investigated species and is currently being analysed. The levels of Hg in fish inhabiting the lake is high which calls for consumption restrictions as well as a risk assessment of the toxicity of Hg towards the fish in the lake. This poses further questions such as: are the results transferable to all other lakes in same area, and further, do the differing hydrology in the lowlands (e.g. retention time, more complex ecosystems) reduce or increase the Hg levels in fish of the same species.

**TH115 Toxic effects of mercury on *Corbicula fluminea*: oxygen consumption, biomarkers, accumulation and recovery in organisms from two estuarine populations** P. Oliveira, Laboratory of Ecotoxicology and Ecology; A. Lirio, C. Canhoto, IMAR-CMA and Department of Life Sciences, University of Coimbra; L. Guilhermino, University of Porto, ICBA's & CIIMAR / Laboratory of Ecotoxicology and Ecology. Mercury is a most concerning environmental contaminant that may be accumulated by several species, thus increasing the risks of exposure and effects on their predators. The goal of this study was to compare the accumulation, recovery and effects of mercury in *Corbicula fluminea*, an exotic species in Europe and North America, from two populations from Minho and Lima Rivers (NW Iberian Peninsula) that have different levels of contaminants including mercury. Clams from both populations were individually exposed to inorganic mercury and fed with *Chlorella vulgaris* and *Chlamydomonas reinhardtii*. Treatments were: (1) control in dechlorinated tap water for human consumption (14 days) (2) control in dechlorinated tap water for human consumption (8 days) (3) continuous exposure (14-day) to 31.25 µg/L of mercury (4) continuous exposure (8-day) to 31.25 µg/L of mercury + 6 days in conditions similar to control. Effect criteria were: post-exposure oxygen consumption; and biomarkers (neurotoxicity, oxidative stress, energy production and biotransformation). Total body mercury concentrations were determined at 0, 8, and 14 days. Mercury induced toxic effects in organisms from both populations, including a reduction of oxygen consumption rate, and adverse effects on physiological functions assessed through biomarkers. After exposure recovery was observed. Significant differences between the two populations were found for some endpoints suggesting that pre-exposure to pollution may influence the toxic effects resulting from exposure to mercury in bivalves. Therefore these results indicate that mercury impairs several functions of *C. fluminea* with the outcome being influenced by historical exposure to pollution, that this species accumulates mercury even after a short-term exposure through water, and that some degree of recovery is expected if exposure is interrupted or ceased. This study was carried out in the scope of the project "NISTRACKS - Processes influencing the invasive behaviour of the non-indigenous species *Corbicula fluminea* (Mollusca: Bivalvia) in estuaries – identification of genetic and environmental key factors" funded by the Portuguese Foundation for the Science and the Technology (FCT) (PTDC/AAC-AMB/102121/2008) and FEDER COMPETE funds (FCOMP-01-0124-FEDER-008556). P. Oliveira had a PhD grant from FCT (SFRH/BD/82402/2011) funded by national funds of the MCTES and FEDER European funds (POPH-QREN-Tipology 4.2.)

**TH116 Life Cycle Thinking: not mainstream in management - A Systematic Literature Review** R. Rieckhof, TU Dresden. If not today, then in a close future, the challenges of climate change will increase incentives for organizations to integrate more sustainable sound choices in management over the whole decision process. For this reason, there is a need to conceptualize and operationalize "life cycle thinking" for managerial decision-making. It serves as a mindset to take accountability not solely for economic growth but also for impacts on

environment and society over the whole life cycle of a product or project (Heiskanen, 2002) and has evolved over time from growth orientation to impact orientation. The growth oriented concept explains market potentials, the impact oriented concept aims at developing solutions for the continuous improvement of the sustainable development of a product, process or service over the whole life cycle. In a systematic review, existing life cycle thinking methodologies, concepts or tools are identified and existing research streams, decision contexts, sustainability contexts as well as time dimensions covered are analyzed. For this purpose, strategic and operational decision process phases are combined, in order to identify the reach of the existing decision-supporting life cycle concepts. Moreover, the concept of sustainable development is applied which includes a double time perspective focusing on the whole product, process or service life cycle as well as the preservation of the economic, ecologic and social resources in present and in future. Results indicate that life cycle thinking still is no mainstream in managerial decision-making, so the integration of each decision process stage into adequate routines into the management control system is suggested. Judgments on intra-generational and inter-generational justice could be incorporated into decisions by taking into account industries and regions which contribute significantly to climate change as well as temporal scenarios. To summarize research on the inclusion of inter-generational justice, current applications of life year definitions, valuation and future studies have been presented. Typically, the life years are estimated on the basis of the useful life, economic life or technical life. For the temporal valuation approaches for temporal allocation (in particular discounting), dynamic modeling and time-related weighting are discussed. In order to model future studies, predictive, explorative and normative scenario models are suggested in literature.

**TH117 Bioenergy – A Review of Environmental Impacts** R. Rieckhof, TU Dresden. On a global scale, bioenergy plays a dominant role among renewable-energy options. Unlike fossil fuels, bioenergy can be carbon-neutral and will play an important role in the reduction of greenhouse gas emissions. While an assortment of reviews of biofuel Life Cycle Assessments (LCAs) is already available, only few of them are concerned with electricity and heat generation from biomass. Furthermore, the latter studies only include energy consumption and global warming potential as impact categories. We come to the conclusion that the insufficient consideration of impact categories in bioenergy LCA reviews constitutes a research gap. The goal of this study is to synthesise state of the art bioenergy LCAs and deduct further research needs. A systematic review is chosen as research method to gain a comprehensive and unbiased overview of state of the art bioenergy LCAs. We find that global warming potential, abiotic depletion potential, eutrophication potential and photo-oxidant creation potential are the impact categories most frequently addressed in bioenergy LCAs. The results of bioenergy LCAs show significant variations. Three main sources of variations are choices regarding allocation procedures, the system boundary and whether to include environmental impacts from capital equipment and transportation. There are still multifaceted discussions regarding methodological issues in bioenergy LCAs. No general consensus has been reached regarding the optimal functional unit for the environmental analysis of bioenergy, the ideal allocation of environmental impacts when more than one product is produced as well as how to treat alternative biomass fuel pathways such as decomposition if it is not used for energy purposes. The impact categories land use, biotic depletion, respiratory organics and respiratory inorganics are not often considered in bioenergy LCAs. A few LCAs already include an economic assessment whereas social factors have been widely ignored so far. This review is a first step in identifying sources of deviating results in bioenergy LCAs due to choices. We conclude that a stricter standardisation of environmental evaluations of bioenergy is recommended to improve their comparability. Furthermore, considering economic and social factors in LCAs will be an important step towards a holistic sustainability assessment of bioenergy pathways.

**TH118 Developments of social impact assessment method for water consumption considering educational opportunities and**

**employment** Y. Ono; M. MOTOSHITA, National Institute of Advanced Industrial Sci and; N. Itsubo, Tokyo City University. Water footprint is paid attention internationally because of the seriousness of water resource scarcity and increased population in developing country. International standard of the principle and procedure for water footprint is under development in ISO. In the process of water inventory, database considering the type of water and water form have been developing in recent years. About impact of water consumption in LCIA, several method focused on environmental aspects (ecosystem, resource and human health) have already been developed. However, there are still few methods considering social impacts caused by water consumption. Water scarcity can affect not only human health but also social aspect such as education and employment in some regions or countries, especially low income countries. Therefore, in this study, impact passways between water consumption and social impacts have been proposed. Social impact assessment method which considers water access and education was developed.

#### **TH119 ASSESSING ENVIRONMENTAL AND SOCIAL IMPACTS IN A WASTE MANAGEMENT SYSTEM: A CASE STUDY**

M.D. Bovea, Universitat Jaume I Department of Mechanical Engineering Construction; C. Coutinho-Nobrega, Universidade Federal da Paraíba; V. Ibáñez Forés, Universitat Jaume I; D. Bernad-Beltran, Universitat Jaume I. Dpt. Mechanical Engineering & Construction / Dpt. Mechanical Engineering & Construction; V. Perez-Belis, Universitat Jaume I. Dpt. Mechanical Engineering & Construction. This paper presents an environmental and social assessment of the evolution of the waste management system in the town of João Pessoa, Paraíba (Brazil). The aim of this work is to analyze different alternative scenarios for the management of municipal solid waste generated in the mentioned town, in order to obtain environmental and social indicators. From an environmental perspective, the methodological framework of LCA methodology is marked by the standard ISO 14040-44 (2006). Its application for the environmental assessment of waste management systems involves obtaining inventory data for each stage of their life cycle: pre-collection, collection and transport, treatment, recycling, recovery and/or landfilling. From them, environmental indicators are obtained for different impact categories (resource depletion, global warming, acidification, eutrophication, photochemical oxidation, ozone layer depletion, etc.), which made it possible to identify the key stages in the waste management system and the scenario that offers the best environmental behavior. From a social perspective, the methodological framework is not as defined as the environmental one is. Although several methodological guidelines have been defined (UNEP, etc.), so far no consensus has emerged on which are the social indicators or the way to calculate them. Therefore this paper presents the indicators that can be applied to the evaluation of a waste management system from a social point of view.

**TH120 Comparative life cycle assessment of certifiable and non-certifiable (coming from deforestation) wood** A. Deschryver, ETH; C. Guignard, Quantis; S. Humbert, Home. Current inventory databases, such as ecoinvent ([www.ecoinvent.org](http://www.ecoinvent.org)), do not allow to assess the difference in environmental impacts from using certified or non certified forest products within products or services. Therefore, the objective of this study was to assess the environmental benefits related to certified wood compared to non-certifiable wood. This study was done in collaboration with the Programme for the Endorsement of Forest Certification (PEFC, [www.pefc.org](http://www.pefc.org)). It was recognized there is no sufficient data to compare certified to non-certified forest across common environmental attributes. However, an important difference between certified and non-certifiable wood is the assurance that certified products are not derived from deforestation. As a first approximation, this sole characteristic is used to assess the environmental difference between certified and non-certifiable forest products. A global average approach is taken, meaning that when buying uncertified products it overall contributes to deforestation as there is no traceability to prove otherwise. First, the impacts of 1 m<sup>2</sup> of deforestation for wood extraction were modelled taking the ecoinvent process 'clear-cutting

primary forest to arable land' as a basis and adapting it to wood extraction. The impacts considered were e.g., machine use and CO<sub>2</sub> emissions from land use change. An effect on pluvial systems was identified but not enough data exists to assess this. Second, the amount of deforestation related to non-certified wood extraction was calculated from literature. For this, deforestation due to wood extraction at the global scale was allocated to the total non-certified wood production. Finally, the environmental impacts were calculated for certifiable and non-certifiable softwood, hardwood and paper using the impact assessment method IMPACT 2002+. Preliminary results show that on a global scale, assuming 10% of deforestation relates to wood extraction, 1 m<sup>3</sup> of non-certifiable wood accounts for 4.66 m<sup>2</sup> of deforestation. Due to the large impact of deforestation, the climate change effects from non-certifiable softwood are about 10x higher than from certified softwood, for ecosystem quality the impacts are 2.5x higher and for human health 10x higher. For paper the differences are smaller due to the contribution of other activities. Paper coming from non-certifiable wood is 5% to about 25% more impacting than full certified paper for climate change, ecosystem quality and human health.

#### **TH121 Spanish biofuels sustainability assessment, by means of life cycle analysis**

L. Herrera, CIEMAT / Energy Dpt. - Energy Systems Analysis Unit. Sustainability criteria have been set to biofuels to be consumed in Europe. Following these criteria, only biofuels that can prove greenhouse gas (GHG) emissions savings of at least 35% (and 50% from 2017 onwards) can be taken into account in order to (a) measure compliance with the requirements of Renewable Energy Directive (RED); (b) measure compliance with renewable energy obligations and (c) be eligible for financial support. In order to implement these sustainability criteria, the purpose of this study is to carry out updated and developed life cycle assessments of biofuels produced and used in Spain during 2010. This study included the current characteristics of the biofuels consumed in Spain. Furthermore, some default and typical GHG emission values for many biofuel pathways are provided disaggregated in the different stages of the cycle namely: raw materials cultivation, processing and transport & distribution. The study includes sensitivity analyses showing the impact on changed raw material and origin. Results show the best sustainable pathway for sugarcane in the case of imported ethanol and the barley for internal production. For biodiesel, soybean would be the best raw material for imported, and in the case of domestic production is the waste vegetable or animal oil the raw material with the best environmental behaviour.

#### **TH122 Sustainability assessment to guide the development of new biosolar technologies: defining the system first**

C. Van der Giesen, . The Dutch research program Toward Bio Solar Cells (TBSC) aims to develop technologies that make better use of solar energy by artificially improving the process of photosynthesis. To guide the development of these technologies, performance criteria for contributing to a sustainable energy future are identified and analyzed by performing a sustainability assessment. For this assessment the LCSA framework as presented in the CALCAS project ([www.calcas.net](http://www.calcas.net)) is used. This framework covers the assessing environmental, social and economic performance of technologies on micro, meso and macro scale levels and therefore a comparison of biosolar technologies with their competitors on the relevant sustainable performance criteria. Since little practical experience with LCSA exist, in this research a stepwise approach is applied. Here we focus on the first step: defining the broad system in which the technologies are expected to be implemented. This broad system description consists of a technical description of the specific technology under assessment, its intended use or function, alternatives or competing (existing) technologies and the societal context in which the technology will be implemented. Next steps will be (2) scenario building, (3) defining indicators and tools, (4) applying tools to calculate indicators and (5) interpreting results. TBSC technologies are still in their earliest stage of development which makes that the availability of data is limited. Therefore first the technical context and possible application of the technologies are defined. It is assumed that the theoretical concept of solar fuels provides an appropriate system for

analysis. Solar fuels can be described as liquid hydrocarbon fuels produced from solar energy, carbon dioxide and water. A first comparative LCA study focused on a comparison of different routes to produce solar fuels and existing (renewable) fuels. It provides a technical description and an assessment of the environmental performance of relevant alternatives to biosolar technologies on a micro scale. Additionally, stakeholder consultations are organized in which the LCA results are discussed and social-economic drivers for a sustainable energy future are identified through workshops. The outcomes are used to develop scenario's and to define environmental and socio-economic indicators that are relevant to assess the performance of biosolar technologies in the future and on meso/macro scales.

**TH123 Development of Social LCA database considering employment and labor accident** N. Matsunaga, . There are many study focus on evaluation of sustainability have been conduct in LCA. These sustainability assessment are based on Life Cycle Initiative proposed the method for sustainability assessment based on environmental aspects LCA, the economic aspects LCC, and the social aspects SLCA. International standards and guidelines for sustainability assessment have been created by ISO. On the other hand, ISO also mentioned that it is important to take into account the triple bottom line of environmental, economic and social aspects. However, there is no detail item or method was determined in the social aspects of sustainability assessment. Therefore, some method on LCA and LCC have been developed, but SLCA is still under development. In this study, a social LCA database considering employment and labor accident was developed. Finally, the database was verified by some case study.

**TH124 Development of reproductive risk assessment methods for birds potentially exposed to treated seed** A. Lawrence, Cambridge Environmental Assessments; J. Crocker, Independent Consultant. This presentation will provide an update on UK CRD funded project PS2373 "Development of reproductive risk assessment methods for birds potentially exposed to treated seed". The current avian long term risk assessment procedures are designed to ensure that, following a pesticide application, reproductive effects are unlikely and that there will be no long term repercussions for abundance and diversity (EFSA, 2009). The criteria for approval of plant protection products (Regulation EC 1107/2009) require that such substances shall have no unacceptable effects on the environment. The persistence of populations of species is implicitly assumed to be addressed by the requirement to protect the variability and interrelationships between species. Conventional avian reproductive risk assessments for treated seeds have a relatively high 'failure' rate (indication of potential risk), but the inherent conservatism would suggest a proportion of these failures are 'false positives'. The conservatism comes from an assumption of coincident breeding and drilling activity and a 'one field' approach to the risk assessment, in addition to the use of worst case toxicity endpoints, which may relate to a specific breeding phase which may not be exposed in reality. An improved approach would be to conduct such risk assessments at the population level, through inference from individual reproductive capacity and estimates of survival rates. This would address the protection goals of reproduction, abundance and biodiversity and would also add realism and ecological context to the assessment. This project seeks to provide a workable risk assessment tool which addresses this difficult scenario.

**TH125 Body burden modeling for bird and mammal risk assessment of pesticides: findings from the SETAC MODELINK workshop case study** A.J. Bednarska, Jagiellonian University / Institute of Environmental Sciences; V. Ducrot, INRA / Ecotoxicology and Quality of Aquatic Ecosystems; S. Hinarejos, Sumitomo Chemical Agro Europe SAS / Registration & Regulatory Affairs; G. Weyman, Makhteshim Agan (UK) Ltd; P. Thorbek, Syngenta / Environmental Safety. The current risk assessment for birds and mammals is based on measurements of external exposure which is used to determine whether an organism will be affected by a toxicant in the environment. However,

it is normally the internal concentration which drives the toxicological effect. Internal concentration is the net result of absorption, distribution, metabolism and excretion (ADME), and toxicokinetic (TK) models are mathematical descriptions of these processes. To improve our understanding of the relationship between the external and internal concentrations of pesticides, we need TK models which translate an external concentration of toxicant, which may change over time, to an internal concentration at target site. Within the registration process of plant protection products often ADME data within rat, live-stock or hen are available. Based on the ADME data, a simple body burden model was developed and then applied to a variety of feeding scenarios, including one tested in a standard laboratory test on rats. The model considers the absorption of a pesticide across intestinal wall after oral uptake into systemic circulation and its elimination from the body. Body burden models are considered as a potential tool for higher-tier risk assessment for birds and mammals (EFSA, 2009). However, currently body burden-type models are a research area rather than an established methodology in environmental risk assessment. The applicability of the current body burden approach as a refinement option in bird and mammal risk assessment was discussed during the SETAC MODELINK workshop. This presentation will outline the main data and findings of TK/TD vertebrate workgroup working on a case study for a hypothetical pesticide applied as seed treatment. This case study served as an example to explain the main assumptions behind the body burden model, its advantages and limitations, and to indicate where and how it should be extended or adapted. This case study was also used to illustrate the consequences of different feeding scenarios on internal concentration of pesticide. Participants from academia, industry and regulators have worked together to consider the critical properties needed during the development of a body burden model, such as species (focal, surrogate or indicator species) and their feeding behavior. We will highlight the conclusions and recommendations of the workgroup and discuss the developed guidance document for when and how to apply a body burden model to regulatory risk assessment.

**TH126 Using population models in ecological risk assessment for the common vole (*Microtus arvalis*): A case study in context of the "MODELINK" workshop** F. Bastiansen; W. Schmitt, Bayer CropScience AG / Environmental Modelling; M. Ebeling, Environmental Safety; R. Luttik, . The use of population models in risk assessment has increased in recent years and has already been subject of discussion in 2007 at the SETAC "LEMTOX" workshop. At the "MODELINK" workshop in October 2012 efforts were made towards a definition and harmonization of population modelling approaches for risk assessment use in ecotoxicology. In the small mammals case study group possibilities of using population models were discussed on the basis of models on wood mouse, field and common vole. For the common vole analyses have been conducted using the RIFCON "eVole" model, which is an individual-based, spatially explicit model. It implements the ecological processes relevant for the population dynamics of the common vole: Survival, reproduction, home range behaviour and dispersal. The processes that are influenced by potential effects of plant protection products are represented mechanistically; thus the potential exposure and the resulting effects can be estimated with lower uncertainty. Since the population dynamics emerges from the fate of and the interaction between individuals, the impact of the voles' individual fate on the dynamics on population scale can be assessed. The model works on a landscape scale and runs on custom landscapes that are composed of different habitats. Habitats are defined by the seasonal dynamics of the vegetation cover and vole relevant food resources they provide. For the workshop a case study was defined that comprises one spray application of a hypothetical substance in winter cereals at a rate of 750 g/ha at BBCH 40-45. This substance was defined to show three sub-lethal effects: Delayed sexual maturity in the F1 generation, F1 number at birth and the number of F1 individuals at day 28 after birth. For each of these effects dose response functions were derived. A probabilistic exposure calculation was conducted, resulting in a probabilistic distribution of daily exposure values (DDD). Using this distribution, for each sub-lethal effect a distribution of effect values was

determined using the respective dose-response function, which were then applied in the population model on a generic worst-case landscape comprising treated fields and surrounding off-crop areas. Treatment (annual 'application of the effects') and control (no 'application of the effects') simulations were conducted and the results were compared with regard to the viability of the populations in development over time. The detailed results are shown and discussed.

**TH127 Population modeling of small mammals as a higher tier approach in ecological risk assessment: a case study** F. Bastiansen;

J. Ludwigs, Rifcon GmbH; I. Barber, Dow Agrosciences. Interest in the use of small mammal population models for ecological risk assessment has increased in recent times. In particular, individual-based population models offer an opportunity to understand the potential impact of effects on individual animals at the population level before deciding on the need for field evaluation. In the case study at hand the potential impact of an insecticide on small herbivorous mammal populations was evaluated. Based on toxicological studies, it was shown that the effect of this insecticide on pup survival is restricted to a small time window during gestation. In our modeling approach, first, the exposure of individual females was simulated using a probabilistic approach taking into account the individually varying time until the critical time window is reached per female. This time period, among other factors, determined the exposure to the insecticide during the critical time window. Second, using data from two-generation toxicological laboratory studies, a dose-response curve was generated. Third, from the exposure distribution that was calculated in the probabilistic approach the effect on pup survival was calculated via the dose-response function. The resulting probabilistic effect distribution was applied in the eVole Model, a common vole (*Microtus arvalis*) population model, which is an individual-based, spatially explicit model. The model mechanistically implements the ecological processes relevant for the population dynamics of the common vole: survival, reproduction, home range behaviour and dispersal. It works on a landscape scale and is able to run on custom landscapes that are composed of different habitats, which are defined by the seasonal dynamics of the vegetation cover and food resources they provide. The effect values determined in the probabilistic calculation were applied in the population model, i.e., the respective vole individuals in the model were subjected to the effect. The effect was applied at the beginning or the end of the application period of the insecticide, respectively. The probabilistic calculation of the effects resulted in an effect distribution showing a mean effect on pup survival of 23%. The distribution was used in the population model in which simulation runs were conducted with and without the effects. The resulting population densities of treated and control vole populations are shown and discussed.

**TH128 Demographic risk estimation of endocrine disrupting chemicals based on effects to reproduction and sex ratio of *Daphnia* - Population simulation** Y. Tanaka, National Institute for Environmental Studies / Research Center of Environmental Risk; S.

Oda, H. Watanabe, N. Tatarazako, National Institute for Environmental Studies. One of the most important merits of the ecological risk assessment at the population level is to quantify ecological risks which are measured by different endpoints in a unified ecologically-relevant criterion, e.g. intrinsic rate of natural increase. We attempted to estimate the population-level effect of an endocrine disrupting chemical (pyriproxyfen as a model substance) by an extended *Daphnia* reproduction test which incorporated distortion of offspring sex ratio as an alternative endpoint. For predictive purposes, a time-dependent toxico-dynamics model was made based on the receptor occupancy model that simulated sex change due to toxicant exposure at a sensitive life stage. A DEBtox model was also used to jointly predict toxicants' effects on reproduction (the number of offspring). All model parameters which related exposure concentrations to probability of sex changes and reduction in reproduction were estimated by Markov chain Monte Carlo simulations using reproduction test data with *D. magna*. These two dose-response models were implemented to an age-structured population model which simulated population growth of a *Daphnia* population in

the asexual phase. The effect of sex ratio distortion on the intrinsic rate of natural increase was assumed to be mediated by reduction of asexually reproducing females. Simulations of population growth indicated that 250 ppb stationary exposure of pyriproxyfen reduced the intrinsic rate of natural increase by 75 % due to the inhibition of reproduction and by 21 % due to the sex ratio distortion, and approximately one fourth of the total reduction of the intrinsic rate of natural increase was explained by the sex ratio distortion under various exposure schemes.

**TH129 Incorporating ecological scenarios into population modelling for use in aquatic risk assessment - A simulation study for the emerging insect *Chaoborus*** T. Strauss, RWTH Aachen University / Research Institute Gaiac. The aim of MODELINK, a SETAC Europe

Technical Workshop on the potential use of ecological models to link ecotoxicological tests to protection goals in chemical risk assessment, is to give recommendations how to apply ecological models to regulatory risk assessments. In the case study for aquatic invertebrates, the focus was on potential effects of variable exposure on aquatic invertebrate populations. Adverse effects on population density as well as on time to recovery are considered as important endpoints. As a model substance, a pyrethroid (MODELMethrin) with fast dissipation in the water column but with multiple applications per year was used in this case study. The multivoltine aquatic phantom midge *Chaoborus crystallinus*, which is a common pelagic invertebrate predator in fishless ponds and ditches, is the most sensitive organism in this case study. Emerging insects such as *C. crystallinus* can recover from inside the polluted water bodies (autochthonous recovery), and by the immigration of adult insects emerged from other water bodies (allochthonous recovery). In principle, isolated populations (e.g. in individual ponds) are more vulnerable to pesticide exposure due to the lack of immigration from outside than spatially closely connected populations (e.g. in large ditch systems). A mechanistic individual-based population model for *Chaoborus crystallinus* was used which had been validated before with experimental data from aquatic outdoor mesocosm studies. In order to predict ecotoxicological effects on the population dynamics of *Chaoborus*, this model was coupled with a toxicokinetic-toxicodynamic (TK/TD) model following the General Unified Theory for Survival (GUTS). FOCUS exposure profiles for MODELMethrin were used as inputs to the model. The simulated ecological scenarios should take into account the spatial proportion of affected and unaffected populations in the field depending on the migrating radius of *Chaoborus*, as well as the amount of food resources. Simulation results for FOCUS scenarios combined with ecological scenarios will be analysed and discussed in relation to the general need of defining scenarios for ecological models used in pesticide risk assessment.

**TH130 Combined application of As and Cd to *Cynara cardunculus* L.: phytotoxic effects** B. Sánchez-Pardo, Univesidad Autonoma de Madrid; D. Carracedo, P. Zornoza, Universidad Autónoma de Madrid.

Phytoremediation technologies can be used to treat soils contaminated by heavy metals. However, most contaminated soils contain high concentrations of several contaminants, and the negative environmental effects of their combination are different to those caused by each contaminant individually. In this regard, experiments to assess the phytotoxic effects of heavy metals are more realistic if they are undertaken with such mixtures. The aim of the present work was to study the response of *Cynara cardunculus* L. to a combined supply of As and Cd. Plants were subjected to five treatments - 0 mM As + 0 mM Cd (control), 1 mM As + 1 mM Cd, 1 mM As + 5 mM Cd, 5 mM As + 1 mM Cd, or 5 mM As + 5 mM Cd - in a randomised block design trial with three replicates. Forty nine days after beginning treatment, the plants were divided into their shoots and roots, weighed, and their As and Cd concentrations determined by ICP-MS. The plants of all As-Cd treatments showed smaller canopy development, smaller leaves and chlorosis, although these symptoms were strongest in plants treated with combinations containing 5 mM Cd. A thickening and darkening of the roots was also seen in plants grown with the 1 or 5 mM As + 5 mM Cd combinations. In both organs, toxicity symptoms were most pronounced

in plants treated with 5 mM As + 5 mM Cd. Shoot fresh weight was reduced mainly by treatments containing 5 mM Cd. Root fresh weight was increased in treatments involving 5 mM As. Root and shoot As and Cd concentrations increased with the concentration of these elements in the nutrient solutions. However, the 5 mM Cd dose slightly reduced the final accumulation of As in root tissues, while the 5 mM As dose reduced the shoot Cd concentration. Finally, the shoot appeared to be the organ most affected by the combined application of As and Cd, especially in plants receiving treatment involving 5 mM Cd; As accumulated mainly in the root, but 50% of the Cd accumulated in the shoot. The application of 5 mM Cd with either 1 or 5 mM As has phytotoxic effects in *Cynara cardunculus*; these become especially noticeable when both heavy metals are applied at 5 mM.

**Acknowledgement** - This work was funded by the Spanish MCyT (project CTM2010-21922-C02-02/TECNO) and the Madrid Regional Government (project S2009/AMB-1478).

**TH131 Modeling of competitive adsorption of copper, calcium and hydrogen ions to soil and plants** H. Kurasawa; R. Shoji, Tokyo National Col. of Technology. Soil pollution by heavy metals is a serious problem in these days yet. Heavy metals released to the terrestrial environment are competitively adsorbed by soil ligands and plant ligands. When plants accumulate heavy metals, the toxicity was occurred against organisms in soil ecosystem, such as growth inhibition of terrestrial plants. The bioavailability of heavy metals and the amount of adsorption to soil particles should be changed with the soil solution pH and the concentration on the activity of the existing ions. In this study, the influence of pH and competing ions on copper adsorption to soil are analyzed by SLM (Soil Ligand Model) developed on the basis of the concept of the BLM (Biotic Ligand Model). The objective of this study are to examine the influence of pH and competing ion on the copper adsorption to soil, and to examine the relation between copper ion activity in the soil solution and amount of accumulation of copper to in a winter barley *Hordeum vulgare*. The amount of copper adsorption to soil becomes smaller by the addition of calcium in the tested soil amended with a certain amount of copper. The amount of copper adsorption to soil is decreased by decreasing the soil solution pH. Binding constants calculated by the SLM are derived as follows:  $\log K_{Cu} = 0.425$ ,  $\log K_{Ca} = 0.247$ ,  $\log K_{H} = 1.28$ . Those binding constants indicate that hydrogen ion is more binding easily to soil particle than copper ion. The amount of copper accumulation in *Hordeum vulgare* increased with the copper ion activity in the soil solution. The heat of adsorption of heavy metals was calculated by the van't Hoff equation derived by the derived adsorption isotherms at three different temperatures (293K, 303K, 313K). The heat of adsorption on the pH4 is the largest among three different pHs (pH4, 5, 6). The copper ion which shows weaker bonding to the soil ligand is displaced by hydrogen ion which shows stronger bonding to the soil ligand. The heat of adsorption at pH=4 is the largest among three different pHs. Copper adsorption to the soil particles is decreased by decreasing the soil solution pH, though the copper ion activity in the soil solution is increased by decreasing the soil solution pH. Controlling soil solution pH, it is necessary to consider the accumulation and toxicity of heavy metals against terrestrial organisms.

**TH132 Uptake, depuration and bioconcentration of arsenic, zinc and copper mixtures in juvenile milkfish (*Chanos chanos*)** M. Lin, Department of Natural Biotechnology; Y. Yeh, Nanhua University / Department of Tourism Management. This study is aimed to investigate the bioconcentration of individual trace elements by juvenile milkfish, *Chanos chanos*, following exposure to a mixture containing arsenic (As), zinc (Zn) and copper (Cu). The time-integrated uptake and depuration of As, Zn and Cu by the juvenile milkfish during exposure to each of these three elements and in various combinations were examined. These three elements used during the experiments were chosen to represent the pollutants found in the culture ponds of juvenile milkfish in the blackfoot disease (BFD) area, southwest Taiwan. A 14-day exposure experiment under controlled laboratory conditions was conducted to assess the uptake rate constants ( $k_1$ ) and depuration rate constants ( $k_2$ )

as well as the bioconcentration factor (BCF) of juvenile milkfish based on a simple 1<sup>st</sup>-order one-compartmental model. The interactions among the elements and the subsequent uptake and depuration rates associated with the individual elements were analyzed. The effects of the individual elements in the mixture also were analyzed. The resulting data demonstrated that the levels of As, Zn, and Cu in juvenile milkfish increased with increasing exposure time before reaching an equilibrium status. The depuration rates for As and Zn was higher than for Cu, which means it takes longer time to depurate Cu from the fish than As and Zn. Tests for the significance of differences of the model parameters ( $k_1$  and  $k_2$ ) among the individual pollutants and the binary mixtures were performed based on the sum of squares reduction test (SSRT). In spite of the decreasing effects of the Zn additive on As accumulation and the Zn additive on Cu accumulation, the interactive effects among the As, Zn and Cu on each other were all not statistically significant. It shows that Zn can reduce the accumulation of As and Cu because of the high Zn accumulation ability of milkfish. The competition of Zn with As or with Cu may cause the reduction of accumulation in juvenile milkfish of the latter two pollutants.

**TH133 Kinetics of metal uptake in the zebrafish (*Danio rerio*) in single and multi-metal exposure scenarios using stable isotopes** P. Van Den Plas, Universiteit Antwerpen / Systemic Physiological and Ecotoxicological Research, Department of Biology; V. Mubiana, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology; E. Smolders, K.U. Leuven / Division Soil and Water Management; K. De Schampelaere, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology. The uptake and toxicity of metals in aquatic systems strongly depends on the environmental conditions and exposure scenario. To account for these effects in the environmental risk assessment of metals different models have been developed which take into account chemical speciation, exposure route and species sensitivity to set site specific quality criteria. Most of the efforts made so far focus on single metals with little attention for multi-metal exposure scenarios which are in fact the most realistic and frequently occurring situations. In the current work we characterize the kinetics of metal uptake in waterborne exposure scenarios in the zebrafish (*Danio rerio*) over a broad range of metal concentrations and combinations of metals (representative for natural and toxicological scenarios). Metals included are copper, zinc, nickel and cadmium and uptake rates are determined using the stable isotopes <sup>65</sup>Cu, <sup>67</sup>Zn, <sup>115</sup>Cd and <sup>62</sup>Ni. The use of the stable isotopes allows to determine the unidirectional flux of the metal and also to determine uptake rates of metals which are under homeostatic control (e.g. in case of the essential elements copper and zinc). Metal analysis was performed using high resolution ICP-MS which combines high sensitivity and resolution. Metal uptake rates obtained under the different exposure scenarios were analyzed using a membrane transport model that also incorporates different types of interactions such as competitive and non-competitive inhibition. In quantitative terms results are expressed in terms of binding constants and maximum transport rates. Results obtained so far allowed to identify the metals showing clear interactions in combined exposure scenarios and the concentrations ranges within which these interactions occurred. Results show that significant interactions may already occur at relative low exposure concentrations. Both antagonistic and synergistic effects were observed on metal uptake in mixture scenarios. The results were used to develop a metal uptake model that predicts metal uptake rates under multi-metal exposure scenarios in zebrafish.

**TH134 Effect of cadmium on copper uptake by gills in zebrafish *Danio rerio*** I. Komjarova; N. Bury, Kings College London. Understanding of environmental toxicology of metals is essential for setting adequate site specific water quality criteria. In natural environments aquatic organisms are constantly exposed to a variety of metals via water and solid phases. In living organisms metal ions regulate an array of physiological mechanisms. The excessive amounts

of essential and non-essential metals entering the cell disrupt tightly regulated metabolic processes and cause toxic effect. Present as a mixture in surface waters, essential macroelements and trace metals enter a cell via common or metal specific uptake routes and may share or compete for the uptake site. These interactions on the cell surface can significantly affect metal intracellular accumulation and ultimately lead to disruption of essential metabolic processes. Previous work has shown that such interactions between essential and nonessential metals exist even at low environmentally realistic metal concentrations in water. Alterations of metal uptake/accumulation rates in aquatic organisms exposed to waters containing different metal mixtures compared to single metal exposure were demonstrated. While some of these interactions, i.e. Cd/Ca or Ag/Cu could be explained by ion sharing/competing for an ion channel or a metal transporter, other effects such as changes in Cd/Cu uptake rates in mixed metal exposures, were more puzzling as no common uptake routes are currently known for these two metals. In the current work, the effects of combined Cu/Cd exposure on the metal accumulations in zebrafish *Danio rerio* were investigated using molecular and stable isotope approaches with the aim to elucidate the mechanisms behind Cu and Cd transport into the cell during multimetal exposure and identify possible changes in the expression of the candidate metal transporter genes in the gills of zebrafish. The organisms were exposed to single metals (0.005-0.025  $\mu\text{M}$  Cd<sup>106</sup> and 0.05-0.45  $\mu\text{M}$  Cu<sup>65</sup>) and their mixtures. The expressions of the candidate metal transporter genes (DMT1, ECaC, CTR1, ATP7A, NaHE-2, Zip3 and Zip10) in the gills of zebrafish were evaluated by conducting qPCR on target genes, while metal uptake rates were determined based on metal content of the gill tissue determined by ICP-MS analysis. The results indicated presence of metal interactions affecting Cu uptake and elimination processes, although particular mechanism of the interaction has to be further investigated.

**TH135 Concentration level-dependent interactions in Ni and Zn mixtures: development of a chronic binary mixture Biotic Ligand Model for *Daphnia magna*** C. Nys, University of Ghent / Environmental Toxicology and Aquatic Ecology; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; E.E. Smolders, Katholieke Universiteit Leuven; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology; K. De Schamphelaere, Ghent University UGent / Environmental Toxicology and Aquatic Ecology. Risk assessment of metals is generally based on ecotoxicity test results with individual metals. Although, organisms in the environment are mostly exposed to mixtures of metals. Biotic Ligand Models can be used to predict toxicity of metal mixtures. However, as we still lack knowledge about the interactions between metals in mixtures, we are not able to incorporate metal mixture models in environmental risk assessment procedures. Here, we tested the combined effects of Zn and Ni on chronic (21 day) toxicity to *Daphnia magna* using a full-factorial design. The results were analyzed with the independent action model by evaluating if the concentration response curve of one metal changes in the presence of the other metal. The results suggested concentration level-dependent interactions between Zn and Ni. Zn and Ni did not interact with each other at low exposure concentrations, while at concentration above a certain threshold of either individual metal (i.e. EC20) a synergistic interaction was observed. The data were used to develop a Biotic Ligand Model, equipped with an additional parameter accounting for this synergism. This model is able to predict chronic toxicity of binary Zn and Ni mixtures based on the existing chronic *D.magna* Biotic Ligand Models for individual Zn and Ni toxicity.

**TH136 The implications of using concentration addition for the risk assessment of metal mixtures.** T. Van Regenmortel, Lab of Env. Tox & Appl. Ecol.; F. De Laender, Université de Namur ASBL / Lab of Env.Tox&Appl.Ecol.; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; K. De Schamphelaere, Ghent University UGent / Environmental Toxicology and Aquatic Ecology. Ecological effects of chemical mixtures can be assessed using various model formulations of which the concentration addition model

(CA) is most frequently applied. This study evaluates the effects CA predicts when freshwater communities are exposed to a mixture of Ni, Zn and Cu, each present at their HC<sub>5</sub>, i.e. the concentration that is hazardous for 5% of the species in a community. The CA model was applied for a wide range of bioavailability-determining water characteristics such as pH, Ca and DOC. For all three metals, high-quality NOECs (published in the peer-reviewed literature) and the corresponding water characteristics, were compiled in a database. Using biotic ligand modeling, all NOECs were normalized to 125 realistic and physico-chemically distinct EU water bodies, yielding 125 site-specific HC<sub>5</sub>s per metal. Using CA, the identity and fraction of potentially affected species per water body was evaluated. Preliminary results indicate that CA predicts risk of metal mixtures in all of the 125 water bodies tested, with at least one species affected per water body. The fraction of potentially affected species exposed to a mixture of Ni, Zn and Cu, each present at their HC<sub>5</sub>, showed an increasing trend with increasing pH. A slightly declining trend in the fraction of potentially affected species was observed with increasing calcium concentrations. The amount of DOC did not seem to influence the fraction of potentially affected species. This study is the first to demonstrate that using CA for the risk assessment of metals implies that environmental risk will be predicted for a substantial amount of water bodies and species. However, the validity of these predictions needs to be further evaluated using a combination of modeling studies and dedicated laboratory and field studies.

**TH137 Prediction of mixture toxicity for metals in soil: assessment of low effect levels** K. Oorts, F. Verdonck, ARCHE; V. Verougstraete, Eurometaux. Only a few models are available so far to predict toxicity of mixtures (dose/concentration addition and independent action), and the concentration addition approach seems to be commonly selected as a default for regulatory purposes because of its higher protection level and the limited information about the mode of action of most substances. The applicability of these models in a regulatory framework for assessing the ecological effects of naturally occurring elements, such as metals, in soil may not be straightforward, however. While research on potential interactions among metals is often focused at relatively high (e.g.50%) effect levels, the correct prediction of effects or risks of metals at low or background concentrations also is a crucial aspect for assessing potential toxicity of metal mixtures. For several metals, the predicted no-effect concentrations are indeed close to their natural background concentration in soil. Overestimation of risks of individual metals at background concentrations will lead to over-protective results and e.g. identification of risks in uncontaminated natural soils when predicted risks of individual metals are added in order to assess potential mixture effects. The concentration addition approach will therefore be evaluated for its potential to correctly predict effects at low doses based on the species sensitivity distributions for several metals and data for total metal concentrations in a wide range of arable land soils across Europe. Summation of predicted risk ratios (ratio of exposure over predicted no-effect concentrations) is compared with summation of predicted potentially affected fractions as estimated by the species sensitivity distributions after correction for differences in metal bioavailability of metals among soils. Since the application of the concentration addition approach on PNEC level is not straightforward, the appropriateness of the concentration addition concept on species sensitivity distributions and PNEC level will also be evaluated based on the comparison of the results with concentration addition of effects on species level.

**TH138 Classifying watersheds for assessing bioavailability and ecological risk of metals using DGT** W. Naito, AIST Tsukuba West / RISS; M. Kamo, AIST / RISS. It is generally accepted that the bioavailability and toxicity of metals in surface water to aquatic organisms depend on the speciation of metal, not on total or dissolved metal concentration. Metal speciation is a function of water chemistry including temperature, pH, organic content, and the compositions and concentrations of ions. To develop efficient and effective methods of assessing and managing the risk posed by metals to aquatic life, it is



important to determine the effects of water chemistry on the bioavailability of metals in surface water. We have been collecting information regarding bioavailable fractions of metals in several water systems by employing the technique called diffusive gradients in thin-films (DGT). Chemical equilibrium model (e.g., WHAM) is also used to determine bioavailable fraction of metals in the rivers. Target water systems include metal-contaminated rivers adjacent to abandoned mines, industrial areas and MWWTP area. In this presentation, we will present what types of watersheds and metals required to consider bioavailability when assessing risks on aquatic life. In addition, we will examine the impact of incorporation of bioavailability on risk assessment of metals (or metal mixture) using the obtained data.

**TH139 Investigating Toxicity Using Singular and Metal Mixture BLM Models: A Case Study at Ross Lake** S. Yacoub, University of Toronto; M.L. Diamond, University of Toronto / Department of Earth Sciences; R.C. Santore, Hydroqual Inc; C. Gueguen, Trent University / Department of Chemistry; H. Sonnenberg, Stantec Consulting. Virtually all metal releases from mining operations occur as mixtures, but knowing which metal could exert the greatest toxicity is important when developing pollution prevention plans. We report on a case study in which we used field measurements, equilibrium speciation modelling of single metals and metal mixtures, and toxicity testing to try to determine the source of toxicity in a mine-impacted lake. Ross Lake has received Zn and Cu enriched mine tailings effluents for over 80 years and is now a net source of Zn to downstream water bodies. Only sparse benthos live in the lake. Laboratory toxicity testing on *Hylella azteca* found Ross Lake sediment to cause 100% mortality, and exposure to Ross Lake water to cause 30% mortality. WHAM estimated that 70-75% of Zn in the water column was in its truly dissolved form and hence, bioavailable form. DGT (diffusive gradient thin film) measurements were within an order of magnitude. WHAM modelling estimated that 5-50% of total Cu in the water column was in the truly dissolved phase. DGT measured concentrations were more than one order of magnitude higher than the corresponding WHAM-estimated concentrations. DGT-measured concentrations of Zn were generally below BLM-calculated LC50 for Zn<sup>2+</sup> whereas DGT-measured concentrations of Cu<sup>2+</sup> were above the LC50. This led to the conclusion that high measured calcium values of 150 – 400 mg/L ameliorated Zn toxicity, whereas low pH caused Cu toxicity. A different story emerged when metals were modelled using the BLM for metal mixtures. When the mixture was considered, Cu and Zn in the water column were not considered toxic, likely the result of competition between metals. The metal mixture BLM did estimate Cu, Zn and Pb toxicity in sediment porewater. Although none of the analytical methods can unambiguously determine the cause of toxicity in Ross Lake, it is likely that the metals mixture modelling provides a more realistic assessment of toxicity than either the single-metal modelling or DGT measurements.

**TH140 Amendments application to reduce arsenic and metals mobility in a multi-contaminated mine soil.** R. Manzano, Department of Agricultural Chemistry; J. Penalosa, Universidad Autónoma de Madrid / Agricultural Chemistry; E. Esteban, Universidad Autónoma de Madrid. It is well known total concentration of trace elements are poor indicator of metal(loid) toxicity. Recently, a field based technique with rhizon-samplers has been suggested as an efficient mechanism to monitor available fractions of trace elements in soils. Amendment incorporation in soils may alter trace elements mobility by adsorbing, complexing or co(precipitating) them. The aim of this work was to assess the labile fraction of arsenic and metals using rhizon samplers in a multi-element contaminated soil amended with several materials. 2 L pots were filled with control soil plus dumping material from an ancient arsenopyrite mine (Bustarviejo, NW Madrid, Spain) in a 60:40 proportion (w/w). Several amendments were mixed with the soil materials: FeSO<sub>4</sub> 1% w/w ('Fe'); CaCO<sub>3</sub> 1% w/w ('Ca'); FeSO<sub>4</sub> 1% w/w + CaCO<sub>3</sub> 1% w/w ('Fe+Ca'); paper mill 1% w/w ('PM'); FeSO<sub>4</sub> 1% w/w + paper mill 1% w/w ('Fe+PM'). Not amended treatment was also included in the design ('NA'). *Festuca curvifolia* and *Arrhenaterum elatius* subsp. *Bulbosum* were sown. Pore water was sampled during five weeks using

a rhizon soil sampler (Eijkelkamp, 10 cm long, diameter 2.5 mm). Arsenic and metals in pore water were analysed by ICP-MS. The lowest pH values in pore water corresponded to the 'Fe' treatment, while 'Ca', 'Fe+Ca' and 'PM' showed the highest values. Pore water collections from 'Fe+PM' presented similar pH values to those obtained in 'NA'. Paper mill and CaCO<sub>3</sub> incorporation increased As concentration in pore water significantly (290 and 400 % increase respectively compared to 'NA'), as well as reduced Zn, Mn and Cd concentrations, according to the increase of the pH values. 'Fe+PM' and 'Fe+Ca' reduced effectively As soluble concentrations (88 and 70% reduction respectively compared to 'NA'), probably because of the formation of iron oxyhydroxides at pH values higher than those obtained when only iron was applied. An extreme increase of metals concentration (Cu, Zn, Mn and Cd) in pore water in 'Fe' and 'Fe+PM' was observed. Rhizon samplers were valid devices to assess the potential mobility of trace elements in soils. The effect on metal(loid)s lability was appropriately reflected in the soil solution extractions.

**TH141 Effect of iron plaque on arsenic uptake and distribution in *Lupinus albus*: its possible role in arsenic phytostabilization in aerated soils.** T. Fresno, Universidad Autónoma de Madrid / Department of Agricultural Chemistry; J. Santner, BOKU - University of Natural Resources and Life Sciences / Department of Forest and Soil Sciences; T. Prohaska, BOKU - University of Natural Resources and Life Sciences / Department of Chemistry; J. Penalosa, Universidad Autónoma de Madrid / Department of Agricultural Chemistry; E. Moreno-Jimenez, Universidad Autónoma de Madrid. Addition of iron amendments to As-contaminated soils has shown to immobilize this metalloid. Nonetheless, the management of contaminated soils still requires further knowledge of the interaction of these two elements in the rhizosphere. In wetlands, iron plaque is commonly formed, and has been reported to have a strong affinity for As and to block As accumulation in plants. Although there are examples of the effect of iron plaque on As distribution and speciation in wetland conditions, little is known about the effect of Fe nutrition on aerated-rhizosphere plants. The hypothesis is that As uptake and distribution is linked to Fe nutrition in aerated soils, and managing Fe will impact As biogeochemistry in terrestrial eco-/agro-systems. The aim of this work is to investigate the formation of an iron plaque on the root surface of *Lupinus albus* and its effect on As uptake and distribution in the plant. *Lupinus albus* plants were grown under controlled conditions in a complete nutrient solution with 3 mg L<sup>-1</sup> of iron as a Fe-chelate. After 4 weeks, iron plaque was induced in a half of the plants adding iron as 30 mg L<sup>-1</sup> of ferrous ion (as FeSO<sub>4</sub> · 7H<sub>2</sub>O). The other half of the plants was supplied with the same iron source, the Fe-chelate, to avoid iron plaque formation. After inducing iron plaque for 3 days, having plants from both treatments were exposed to two doses of arsenate: 5 µM (Fe<sup>plaque</sup> As5 / Fe<sup>chelate</sup> As5 treatments) or 20 µM (Fe<sup>plaque</sup> As20 / Fe<sup>chelate</sup> As20 treatments) for 10 days. A control without As was also established for each Fe treatment. At the harvest, a brownish coating was observed on the roots of Fe(II)-treated plants, suggesting precipitation of Fe oxides on the surface. This coating was not observed in Fe-chelate plants. Iron plaque was extracted using the dithionite-citrate-bicarbonate (DCB) technique and Fe and As were analysed in the extracts and in the whole roots. Analysis of the extracts showed that above 5% of total As in roots was sorbed on the plaque of Fe(II)-treated roots. Analysis of the root cross sections using laser ablation coupled to ICP-MS (LA-ICP-MS) showed as first results a greater co-accumulation of Fe and As on the surface of the roots treated with Fe(II) compared to roots treated with the Fe-chelate. Further analysis will be carried out to study the effect of this plaque on As distribution in *Lupinus albus* plants.

**TH142 Heavy metal hyper accumulation by some plant in some mining sites in Abakaliki, Nigeria** I.N. Onwurah, University of Nigeria / Biochemistry; O.E. Ogbu, UNIVERSITY OF NIGERIA, NSUKKA / Biochemistry. Exploring plants with an exceptional metal tolerant property and the potentials to accumulate heavy metals, on the basis of which heavy metal tolerant species are classified as hyper accumulators, indicators or excluders, is an ongoing current research

interest. This work examined some plants found in the vicinity of mining, abandoned mining and sites not having any history of mining in Abakaliki, Enugu State of Nigeria. Analysis of bioaccumulation profile in the plants growing in these sites revealed hyper accumulation of Arsenic by *D. oliveri*, *A. Tectorum* and *A. indica*. Cadmium was observed to be hyper accumulated by *D. oliveri* and *A. indica*. All these plants belong to hyper accumulator families except *A. indica*. Spectral properties of the chlorophyll showed slight deviation in the chlorophyll spectrograph especially at 533 nm, which remained constant after chlorophyll destruction. Bioaccumulating plants growing at both the mining and abandoned mining sites showed non-significant difference in chlorophyll content, when compared with plants in non polluted control sites. The abilities of these native plants to hyper accumulate metals should be explored in the process of phytoremediation. *D. oliveri*, *A. Tectorum* and *A. indica* which have a wide ecological distribution in the tropics and displayed ability to survive in multiple metals polluted sites should be useful for bioremediation (phytoremediation) of heavy metals' polluted environment, particularly agro soil in the tropics.

**TH143 Assessment of the colloidal stability of C60 and a fullerene derivative in the presence of dissolved organic matter and electrolytes** J. Haftka, University of Utrecht / Institute for Risk Assessment Sciences; E. Emke, KWR Watercycle Research Institute; P. de Voogt, University of Amsterdam / IBED; P. Bauerlein, KWR. Because of their chemical and electrical properties, carbon-based nanoparticles such as fullerenes have been widely applied in personal care products, drug delivery systems and solar cells. The properties of manufactured nanoparticles have been increasingly studied because of their potential risks to the environment and human health. In commercial applications such as organic photovoltaic cells, derivatised fullerenes are used to modify their solubility and electronic properties. Most studies have focused on the fate and properties of C<sub>60</sub> fullerenes and there is currently limited information available on the environmental properties of derivatised fullerenes. The colloidal stability of two fullerenes (C<sub>60</sub> and [6.6] diphenyl C<sub>60</sub> bis-butyric acid methyl ester) was therefore studied in the presence of organic matter and different electrolytes (NaCl and CaCl<sub>2</sub>). Aqueous suspensions of fullerenes were made by solvent exchange using toluene. Static experiments were performed in time and fullerenes were extracted from the water phase with toluene. The samples were subsequently analysed with liquid chromatography coupled to high resolution Orbitrap mass spectrometry. Aggregate size of the fullerenes was measured with dynamic light scattering and flow-field-flow fractionation coupled to a multi angle light scattering detector. The results show that the concentrations of the particles are stabilised by relatively low concentrations of dissolved organic matter (2 mg C/L) if NaCl is present as electrolyte. Precipitation of organic matter however occurs when CaCl<sub>2</sub> is present in solution and sorption of the fullerenes to organic matter causes co-precipitation of fullerenes. Size measurements show increasing particle sizes of both nanoparticles in the presence of electrolytes with a larger influence of divalent compared to monovalent cations. The results imply that the colloidal stability of (derivatised) fullerenes is influenced by already low concentrations of organic matter.

**TH144 Capabilities of aF4-MALS to differentiate Carbon Nanotubes and Soot in natural samples** A. Gogos, Agroscope / Analytical Chemistry; T. Bucheli, Agroscope ART / Analytical Chemistry; R. Zenobi, ETH Zurich / Department of Chemistry and Applied Biosciences. Multiwalled Carbon Nanotubes (MWCNTs) are increasingly used in various sectors of modern technology and have a high likelihood of entering environmental compartments, such as soils. Methods to characterize and quantify MWCNTs from soil samples are still scarcely available to date. In addition, quantification –depending on the used method – may be biased by soot ubiquitously present in soils and sediments at the milligram per gram concentration range. Differentiating between MWCNTs and soot is a big challenge, as they are chemically very similar, e.g. in terms of thermal stability or density. However they do differ in shape; MWCNTs come in needle-like to curled forms, whereas soot particles tend to be spherical to semi-

spherical. Detection of scattered light at multiple angles (Multi-angle light scattering, MALS) allows absolute determination of both molar mass and the root mean square radius of a particle based on light scattering theory. Additionally, from these two parameters, the shape of particles (e.g. random coils, spheres or rods) can be extrapolated. Here we test the potential of MALS coupled to asymmetric Flow Field-Flow Fractionation (aF4) to size-separate dispersions of MWCNTs and soot as well as differentiating between their different shapes. We analyzed dispersions of different MWCNTs, as well as soot and mixtures in various ratios of both. In addition, we spiked a standard reference soil (LUFA 2.2) with the respective particles and extracted them using ultrasonication and different surfactants. The resulting soil extracts were then analyzed by aF4-MALS and offline Electron Microscopy. First results indicate that shape differentiation of CNT and soot extracted from spiked soils is possible.

**TH145 Are current fish toxicity tests fit for purpose for nanoparticles? Preliminary results from the MARINA project.** B. Shaw, School of Biomedical and Biological Sciences; C. Liddle, K. Windeatt, Plymouth University; T. Henry, R. Handy, University of Plymouth / School of Biomedical and Biological Sciences. Whilst there are several well established toxicity tests used to routinely assess the hazard and risk of traditional chemicals in the environment, it is currently unclear whether these are fit for purpose for nanomaterials (NM). This work, as part of the European Commission's MARINA project, aimed to evaluate the OECD 210 Fish, Early-life Stage Toxicity Test for selected nanoparticles (NP) and improve them where necessary. Experiments were carried following the standard OECD protocol using zebrafish (*Danio rerio*). Briefly, viable embryos were exposed in a semi-static regime from 2-4 hours post-fertilisation to either TiO<sub>2</sub> NP (anatase structure, 8 nm mean particle size (MPS)) or Ag NP (15 nm MPS with capping agent) along with appropriate bulk or metal salt controls for 6 days (until first feeding). Water changes (100 %) were carried out every 24 h with subsequent redosing. Survival, exposure concentrations, hatching rate and success, abnormal appearance and behaviour, and morphometrics were assessed along with routine water quality. Exposure to 0-160 mg l<sup>-1</sup> TiO<sub>2</sub> NP or bulk did not result in high levels of acute toxicity (highest mortality was seen in animals exposed to 80 mg l<sup>-1</sup> TiO<sub>2</sub> NP where 28.33 % died by day 6), with no significant differences between the acute toxicity of the two materials (GLM, *P* > 0.05). Both Ag NP and AgNO<sub>3</sub> were acutely toxic to zebrafish, though the latter was more toxic (6-day LC<sub>50</sub> values of 58.6 and 5.0 µg l<sup>-1</sup> respectively). Evidence of delayed hatching was seen in fish exposed to Ag NP along with changes to total length in hatched larvae within this treatment (e.g., length significantly decreased by 13.3 % in fish exposed to 100 µg l<sup>-1</sup> Ag NP compared to controls, ANOVA, *P* < 0.05). Oedema (swollen yolk sacs) was also seen in fish from both treatments with, for example, mean yolk sac volumes of 17.7, 35.53, and 39.82 µm<sup>3</sup> for the control, 100 µg l<sup>-1</sup> Ag NP and 5 µg l<sup>-1</sup> AgNO<sub>3</sub> treatments respectively. Problems with the standard OECD 210 test protocol included the inability to maintain the test solutions within 20 % of nominal concentrations (e.g., < 7 % for TiO<sub>2</sub> NP after 24 h), with pronounced settling of the NP at the bottom of the beakers. On-going work is focused on the development of exposure chambers designed to maintain concentrations without impeding the life cycle or health of the animals, along with an assessment of the suitability of a combined embryo to larval stage test; and the sensitivity of sub-lethal end points.

**TH146 Advanced understanding of nanoparticle shape applying multi-detector approaches** S. Wagner, University of Vienna / Department of Environmental Geoscience; A. Dudkiewicz, Food and Environment Research Agency; F. von der Kammer, T. Hofmann, University of Vienna. Properties of nanomaterials are designed according to the application requirements. The variation of the particles shape e.g., can influence their reactivity and accordingly also their toxicity. With the increasing application the need of methodologies to detect, characterize and quantify these nanomaterials rises. Several techniques that are able to provide information on size and particle mass concentration are currently available. One widely applied technique is

Field Flow Fractionation (FFF) coupled to multiple detectors. During FFF separation particles are (ideally) separated according to diffusion coefficient (FlowFFF) or buoyant mass (CentrifugalFFF) and subsequently characterized and quantified by a combination of e.g. UV-absorption, fluorescence, multi angle light scattering (MALS), and ICP-MS. Furthermore, transmission electron microscopy (TEM) as an imaging technique provides information on particle size and possibly on their elemental composition. For non-spherical or heterogeneous particles the equivalent spherical diameters (ESD) determined with different techniques cannot be compared in terms of determined size because the measurements are attributed to different physical principles. However, the differences among the resulting ESD-values are able to deliver information on the degree of non-sphericity and particle shape. In this study we deduced valuable information on the dependency of the particle shape on determined particle size comparing size data from different analytical techniques. In turn, shape information was derived from the variation of the different ESDs. Particle size separation in a suspension of poly-disperse fumed SiO<sub>2</sub>-nanomaterial was examined by means of FlowFFF, CentrifugalFFF and centrifugal liquid sedimentation (CLS). Subsequently, online detection and quantification was performed by MALS and ICP-MS. The hydrodynamic radius was determined based on the FlowFFF data. Volumetric size information resulted from the CentrifugalFFF and CLS data. The radius of gyration and the hydrodynamic radius were calculated from the MALS/DLS signal. The resulting hydrodynamic radius ranged between 10 and 130 nm with an average value of  $62 \pm 2$  nm. Preliminary experiments employing FFF-MALS-ICP-MS analysis suggest that particles are non-spherical. Supporting information from TEM analysis reveals that size of primary particles ranges from 5 to 20 nm and remains constant over the entire aggregate size range.

**TH147 Stability of pristine and functionalized titanium dioxide nanoparticles in the environment – Investigation using DLS and zeta potential measurements** M. Bjergström Environment and Toxicology; C. Wei Na Aw, M. Winther-Nielsen, DHI / Environment and Toxicology. The continuous release of nanoparticles (NP) into the environment causes concerns about their distribution and the adverse effects they may cause on organisms or entire ecosystems. The knowledge of the behaviour of NP in the environment is still insufficient. The environmental behaviour of NP is affected by the surface chemistry of the NP itself and the condition of the environment such as pH, ionic strength and interactions with natural organic matter. In this study we investigated how different combinations of salinity, pH, concentration of dissolved organic matter and concentration of NP affected the surface charge (zeta potential) and agglomeration (DLS) of engineered NP with different functionalization (TiO<sub>2</sub>, TiO<sub>2</sub>-OH and TiO<sub>2</sub>-octyl) but with the same primary size. The raw NP were characterized by several physical-chemical methods including XRD, DLS, zeta potential and BET. A range of concentrations of NP were dispersed in solutions with different salinities (0, 20 and 30 PSU) and different contents of dissolved organic carbon (0 and 10 mg/L) mimicking natural conditions. The pH of the solutions was adjusted to 4, 6, 8 and 10, respectively and DLS and zeta potential of the solutions were measured shortly after pH adjustment and after 24 hours. The results show that the dispersions were unstable with zeta potentials in the range of -30 - +30 mV in general. A content of dissolved organic carbon had a stabilizing effect on all three types of the NP with zeta potentials shifting closer towards the stable region. Increasing the concentration of NP only has minor influence on the stability of pristine TiO<sub>2</sub> and TiO<sub>2</sub>-OH while a decrease of the zeta potential for TiO<sub>2</sub>-octyl show a tendency toward less stability. The pH affected the NP differently showing a decrease in zeta-potential for all three NP between pH 4 and pH 8 but with an increase at higher pH for the pristine NP, only. The effect of salinity follows the same trends for all NP showing an increasing zeta-potential from 0 to 20 PSU followed by a stabilization or a slightly decrease from 20 to 30 PSU. The time dependence of the zeta potential was of minor importance in our experiments. However, the results of the DLS measurements showed a trend towards slightly larger particle sizes from the start of the experiment until the termination

after 24 hours. The tendency to agglomeration was in general higher for pristine TiO<sub>2</sub> than for the functionalized TiO<sub>2</sub>.

**TH148 TRACKING Ag NP TRANSFORMATIONS AT ENVIRONMENTALLY-RELEVANT CONCENTRATIONS IN SYNTHETIC, NATURAL, AND PROCESSED WATERS USING SINGLE PARTICLE (sp)-ICP-MS** D.M. Mitrano, EMPA Technology Society Lab / Chemistry and Geochemistry; C.P. Higgins, Colorado School of Mines / Civil & Environmental Engineering; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry. Accurate data on engineered nanoparticle (ENP) environmental behavior and the interplay between ENP size, surface area, and dissolution rate is critical if one is to appropriately characterize the risks these novel materials may pose to environmental health. The advancement of the single particle ICPMS (sp-ICP-MS) technique is a great benefit for the study of ENPs in natural systems at environmentally relevant (ng/L) concentrations. Previous studies may have obscured environmentally-relevant dissolution rates because of the artificially high ENP concentrations used in the experiments, an insufficient variety of particle types, or incomplete assessment of water chemistry factors. Here, we analyzed 60 nm and 100 nm Ag ENPs (citrate, tannic acid, and polyvinylpyrrolidone (PVP) surface coatings) for changes in size (diameter) as a function of time. Both short term (< 24 hour) and intermediate term (1 week) dissolution was examined. Dissolution was measured directly as dissolved Ag and by computation from the reduction in measured particle diameter as a function of time. We systematically controlled environmentally relevant water chemistry components including chloride, sulfide, and dissolved organic carbon. Additionally, we investigated NP behavior in both natural (moderately hard reconstituted laboratory water, Clear Creek water) and processed (tap, waste water treatment plant) waters. We found water chemistry to significantly affect the stability of the particles over time, ranging from near complete dissolution in tap water within several hours to significant particle persistence in waters containing chloride, sulfide, or dissolved organic matter. Particle capping agents were variably effective in decreasing the dissolution rate, with tannic acid-coated particles dissolving the fastest. These findings can be compared to previous studies at higher particle number concentrations, to elucidate the importance of working at far-from-equilibrium conditions to isolate kinetic effects.

**TH149 Pepper Nigrum as a fixator of Curcumin, administrated against aluminium neurotoxicity and Alzheimer's disease** K. Zerrouki, Univ Motaganem / Biology; N. DJEBLI, Department of biology, health & sciences faculty- Univ of MOSTAGANEM. The objective of this study is to clarify the role of Pepper Nigrum with Curcumin as a protective and therapeutic agent against neurodegenerative diseases including Alzheimer's disease caused by aluminum chloride AlCl<sub>3</sub>. The mice were randomly divided into four groups; each group containing seven mice (for each experience: neurotoxicity, Alzheimer's model): control, neurotoxicity and Alzheimer model, intoxicated/Alzheimer treated groups and the control treated groups. AlCl<sub>3</sub> was dissolved in distilled water administrated orally (100 mg/kg) for the intoxicated/Alzheimer's model groups, and intoxicated/Alzheimer's treated groups, with a D-Galactose (200mg/kg) for the Alzheimer's model given for 11 weeks; in parallel of Curcumin/Pepper administration (45mg/15 orally-200/66mg/kg IP respectively for the intoxicated treated group and Alzheimer disease model. The control treated groups received the same doses of curcumin/Pepper. Functional behavioral assessment is required as part of tested the nervous statue (manifest determinations). These guidelines apply to animals in special tests. The assessment of animal memory using different types of mazes has been Longley used in neurosciences. Several models have been proposed recently, mainly trying to evaluate accuracy of choice between the alternatives presented in the same day of the session, instead of looking for the accumulated learning through successive days of training. Mice from both studies were sacrificed with an overdose of Chloral in order to realize histological study. Behavioral

and memory tests show a remarkable difference between the intoxicated treated/Alzheimer treated and the intoxicated/Alzheimer groups estimated from the significant results. The results of the histological study show that there are typical neuropathological changes in almost of treated intoxicated mice's brains. In this investigation the effect of curcumin with over load of aluminum chloride to mice lead to reduction of neurotoxicity and Alzheimer's disease appeared as shrunken decreased of pyramidal cells, reduced effect of decreasing number of the pyramidal cells. In this research preventive effect of curcumin with a fixator of absorption (Pepper N) was evaluated on chronic neurotoxicity of aluminum, as well as Alzheimer's disease induced (subacute / subchronic), and study through the use biological models of the behavioral, memory, biological analysis and histological status of nerves.

**TH150 Loss of Life Expectancy related to temporal evolution of PM<sub>2.5</sub> considered within energy scenarios in Europe** I. Blanc, Mines ParisTech / Centre for Energy and Processes; M. Lefevre, B. Gschwind, T. Ranchin, Mines ParisTech; K. Drebszok, A. Wyrwa, AGH University of Science and Technology. People exposure to particulate matter can have various health effects as described in scientific publications in the area of observational epidemiology. This study estimates the Loss of Life Expectancy (LLE) related to PM<sub>2.5</sub> concentrations corresponding to a selected baseline energy scenario derived from the GAINS model. Most often energy scenarios are defined as consistent pathways towards a long-term target defined by a set of criteria that describe a sustainable energy supply. This study accounts for the temporal evolution of PM<sub>2.5</sub> concentrations along the time frame from 2005 till 2050. The analysis was carried out for 43 European countries with a spatial resolution of 50 x 50 km. LLE was considered over the whole life time of the population older than 30 years in year 2005. We propose an algorithm for the computation of LLE for population exposed to PM<sub>2.5</sub> based on the approach recommended by the Task Force on Health described in IIASA's Report and accounting for the Pope exposure-risk parameter. The LLE computation is based on the difference between the life expectancy with no exposure to particulates and life expectancy with exposure to observed particulates. We considered in our algorithm the temporal evolutions of PM<sub>2.5</sub> concentrations along the scenario, as well as population densities. LLE results were derived using different PM<sub>2.5</sub> concentration profiles from 2005 to 2050. The first PM<sub>2.5</sub> concentration profile corresponds to a constant PM<sub>2.5</sub> concentration with values fixed to the 2005 situation. The second PM<sub>2.5</sub> concentration profile corresponds to the temporal evolution of the selected energy scenario. LLE is then derived for both situations: the fixed PM<sub>2.5</sub> concentrations case and the variable PM<sub>2.5</sub> case as defined for the baseline scenario. LLE results are significantly different between the two cases with a decrease by half for most European countries. The Netherlands and Eastern Europe are the only regions where LLE exceed 300 days/person of life lost. Applying this new feature of temporal evolution of PM<sub>2.5</sub> is of interest for assessing the potential impacts of scenarios accounting for the possible technical evolution of energy pathways. Results are provided on line in numerical form as well as in form of LLE maps.

**TH152 PBDES AND THEIR STRUCTURAL ANALOGUES IN TERRESTRIAL ENVIRONMENT** U. Kim; N.T. Yen, Pusan National University; H. Moon, Hanyang University; S. Choi, UNIST; J. Oh, Pusan National University. Thanks to Stockholm convention, penta- and octa- PBDE based flame retardants already phased out from the market in the globe and a lot of leading researches about the physicochemical characteristics, impact, toxicity and fate of PBDEs in the environment were performed. However, the whole mechanisms or chemical fates of PBDEs in environment are still unknown due to difficulties in interpretation of their complex transformation pathways. As known for many researches results, the originally released PBDEs are transformed to structural analogues like hydroxylated or methoxylated brominated diphenyl ethers or debrominated diphenyl ethers and etc. Therefore, in this study, we investigated the parent mono- to deca- PBDEs and their methoxylated and hydroxylated structural analogues in the terrestrial environment to understand the fate of PBDEs

by comparing distribution and transformation ratio between parent PBDEs and their transformed compounds in each environmental matrix. To understand and interpret the occurrence and distribution of structural analogues of PBDEs in terrestrial environment, typical four compartments in in-land as air, water, soil and vegetation were selected in this study.

**TH154 Mechanism-specific effects of selected heterocyclic PAHs typically found at taroil-contaminated sites** M. Brinkmann, RWTH Aachen University Institute for Environmental / Department of Ecosystem Analysis; S. Schiwy, K. Bluhm, RWTH Aachen University, Institute for Environmental Research; G. Hinger, Aquatic Toxicology and Ecology Section / Department of Zoology; A. Sagner, A. Tiehm, Water Technology Center / Department of Environmental Biotechnology; A. Eisentrager, Federal Environment Agency / Department IV 2: Pharmaceuticals, Chemicals, Experimental Studies; H. Hollert, RWTH Aachen University, Institute for Environmental Research. Heterocyclic aromatic hydrocarbons containing nitrogen, sulphur or oxygen heteroatoms (NSO)

**MOPC01 Data quality and uncertainty in LCA: Case study of biotechnological and petrochemical production of lactic acid** Q. Mrani, TECHNISCHE UNIVERSITÄT DARMSTADT / BauIng; L. Schebek, Technische Universität Darmstadt / Industrial Material Cycles. Biotechnology is a promising way for a future "greening" of the chemical industry. Based on biological feedstock, bulk chemicals as well as specialty chemicals may be produced by microbiological processes. A change from today's chemical production to biotechnological processes – often termed as "Biorefinery" - will be a far-reaching change. However, at different stages within single processes as well as in full production chains advantages just like disadvantages to environmental impacts may be encountered. We use LCA in order to assess process-chains of future Biorefinery concepts. As one example, the production of lactic acid is investigated. Since a long time uncertainties have been a very important issue in LCA. In scientific research, methods are usually optimized and adapted to reduce uncertainties. In LCA you can observe a movement in the opposite direction. In relation to time, the scientific methods have to deal with increasing uncertainties on data, analysis methods and variables. The reason is the flood of inconsistent data and methods that have rapidly been developed. They make the LCA-practitioner uncertain and the results inconsistent. Assessment of uncertainty is so an important, but also challenging issue. Comparing biotechnological versus petrochemical production of lactic acid, very diverse reasons for uncertainty of data will be encountered. Specifically, biotechnological production needs a large effort in pre-processing as well as cleaning and post-processing. Taking into account this different outline of processes, their effects in the model is analyzed as to consequences for uncertainty. The parameters with major contributions are determined through sensitivity analysis that uses variable distributions instead of point values (Monte Carlo simulation). Based on findings of the relevant processes, a generic description of sources of uncertainty for the investigated type of biotechnological processes is derived. The LCA of lactic acid is carried out mainly using the Ecoinvent database. Uncertainty due to the missing data is investigated. In principle, the missing data is calculated as a value of zero, instead of a different value. A list of the missing data will be generated. The study will be concluded with critical discussion, review and improvement of the relevance rankings for uncertainty in LCI datasets.

**MOPC02 Statistical Models of Energy Consumption in Chemical Batch Plants** C. Pereira, Departement of Biology; S. Papadokonstantakis, K. Hungerbuehler, ETH Zurich. The relevance of energy consumption as an indicator of sustainability in the chemical sector, has lead to the development of evaluation tools - which include energy use as a metric - , not only in academia but also in the industry. The use of these evaluation tools requires process specific data of energy utilities consumption. However, data of energy use at the process level is usually scarce, particularly in multiproduct and multipurpose batch

plants, even when the production is already implemented. This is due to the fact that measurements are costly and time-consuming to perform. In this work shortcut models of process steam consumption in multipurpose batch plants, were developed by means of statistical analysis of production data. These models take the form of generic data ranges with a median, and an interquartile interval derived from the model parameterization. The use of these models requires only knowledge of the synthesis reaction type, for example alkylation, reduction, etc. If additional input data regarding operational parameters are considered, like the maximum temperature achieved during the reaction and work-up processes, the model predictions can be refined, meaning that the generic intervals become narrower. Thus, the models proposed in this work can be used according to different levels of data availability. The performance of the models was tested by means of cross-validation and further assessed in a case study, showing that good estimations of process steam consumption in multipurpose batch plants are provided. In this study we focused on steam consumption, due to the fact that this is the energy utility with highest saving potentials, however an extension of this methodology to other energy utilities like cooling water and brine should be straightforward. The models presented in this work allow the use of environmental evaluation methods, which require steam consumption as an energy metric. This is the case when Life Cycle Assessment (LCA) of chemical production processes are performed.

**MOPC03 Selective introduction of temporal dynamic based on the sensitivity analysis of economic and environmental flows** P. Collet, IFPEN; L. Lardon, INRA; J. Steyer, INRA / LBE; A. Helias, INRA. Life Cycle Assessment (LCA) is an assessment tool usually based on steady state processes: the temporal and spatial variations of extractions, usage and emissions are aggregated during the Life Cycle Inventory (LCI) step. This approach significantly reduces the environmental relevance of some results, as it has been underlined for the case of climate change. As the recent development of dynamic impact methods is based on dynamic inventory data, it seems essential to develop a general methodology to achieve a temporal LCI. However, the systematic introduction of the dynamic in the inventory faces two major drawbacks: (i) the definition of at least one dynamic model for each process which can be very time consuming and the source of many approximations, (ii) the capacity to manipulate an important set of dynamic systems which can generate computational problems. This study presents a method to select sub-systems, in the whole process network, for which dynamics have to be considered while the others are approximated by steady state representation. The selection procedure is based on the sensitivity of the impacts to variation of environmental and economic flows. Once these flows have been identified, their own timescales are compared to the inherent timescales of the impact categories affected by the flows. The timescales of the impacts are divided into three categories (day, month and year) based on the time step on which the emissions and removals are aggregated in the ReCiPe method. If the timescales of the environmental and economic flows are shorter than the one of the involved impact, the flow is not selected. In the other cases, the flow is selected and a temporal dynamic could be introduced. This approach is illustrated by the LCA of ethanol from sugarcane from the Ecoinvent database. It leads to the selection of a limited number of environmental and economic flows where the introduction of a temporal dynamic is relevant. The need of future developments of time integration in the LCI is pointed out in order to deal with the recurrent problem of waiting times.

**MOPC04 What is the most important process in ecoinvent 2.2?** C. Mutel, ETH Zurich / Institute of Environmental Engineering. Life cycle assessment inventory databases attempt to model the entire industrial economy, and each database iteration adds new processes. However, the number of updated processes is usually relatively small, and is not driven by a focused effort to reduce overall uncertainty in database LCA results. We develop and implement two approaches to identify priority datasets. In both cases, we use ecoinvent version 2.2 as the case study database. First, we apply the PageRank algorithm to the technosphere

matrix. This algorithm uses an iterative approach to weight processes, giving importance to a process by the number and importance of processes that utilize this process as an input. Preliminary results show the importance of rail and lorry transport, and medium voltage UCTE electricity. The second approach is a systematic analysis of the contribution of each technosphere process to result uncertainty for all processes in the case study database and a large number of selected impact assessment methods. Because of the large and asymmetric uncertainty, primarily lognormal, distributions used in ecoinvent, we do not use an analytical approach, but instead perform a large Monte Carlo analysis using the Method of Elementary Effects and Contribution to Variance. We identify the processes that contribute the most to overall database and database category LCA result uncertainty. Both approaches are implemented in the open source Brightway2 life cycle assessment software. Identifying the processes that contribute, on average, the most to overall database uncertainty can provide database developers with a list of priority datasets whose data quality can be improved in future releases. In cases where these datasets have high inherent variability, the importance of these datasets can be communicated to database end-users, and the datasets could be split if such splitting would reduce variability or uncertainty.

**MOPC05 Reducing the uncertainties in adapting prior LCI and flow data set databases to the ILCD-recommended characterisation methods** E. Lees-perasso, CODDE; A. Quesne; M. Jacquot, Bureau Veritas CODDE. In the past years, the Joint Research Centre (JRC) conducted a state of the art of the existing environmental characterisation methods in order to recommend the most relevant ones. This study led to the selection of 17 ILCD-recommended mid-point and end-point impact indicators. They are and will be widely used as compulsory characterisation methods in national and European directives and standards. Therefore, Life Cycle Assessment (LCA) practitioners and tools have strong incentives to perform LCAs based on these methods. The selected characterisation methods have been adapted to comply with the ILCD recommendations concerning the dataset documentation and the list of ILCD elementary flows. This framework reduces the uncertainties inherent to possible incompleteness and inaccuracies in ILCD-compliant databases and limits the risks of misinterpretation. Yet, most of the existing LCA tools and LCI databases were developed in the past decades and were based on different methodologies. They are not always compliant with ILCD recommendations. Therefore, there is a gap between the existing databases and the recommendations on different levels: available elementary flows, nomenclature, classification, localisation, differentiation of generalized flows (e.g. Hydrocarbons (unspecified)), database completeness, etc. This gap leads to possible uncertainties in LCIA results. The goal of this presentation is to show how to adapt an existing database to ILCD recommendations in order to reduce this gap. First it will show how to identify the sources of the uncertainties in LCIA results, then how to reduce them, and finally how to establish a methodology to base the future data development on the ILCD framework.

**MOPC06 Environmental Input-Output Analysis: Hybrid Monetary-Physical vs. Monetary models. Differences highlighted considering French household consumption** A. Beylot; A. Gautier, S. Vaxelaire, J. Villeneuve, BRGM. Hybrid Input-Output Life Cycle Assessment (Hybrid IO-LCA), here referring to the assessment based on the combination of process and environmentally-extended economy-wide Input-Output inventory data, has encountered an increasing interest in the LCA community in the last decade. Hybrid IO-LCA is indeed claimed to provide better system completeness than process-LCA by avoiding arbitrary cut-offs. On the one hand, Monetary Input-Output Tables (MIOT) are regularly provided by national statistical services. On the other hand, a considerable amount of process Life Cycle Inventories has been compiled in physical units such as mass and further gathered in standard databases. Hybridizing MIOT with statistical data in physical units to substitute for monetary data, and accordingly deriving Hybrid Monetary-Physical Input-Output Tables (Hybrid M-

PIOT), therefore appears as one of the ways to ease and develop Hybrid IO-LCA (which is in that case “twice hybrid”: mixing process/Input-Output data and monetary/physical data). In this context, this work aims at 1) applying Hybrid Monetary-Physical Input-Output Analysis (Hybrid M-PIOA) to specifically evaluate the environmental impacts caused by products consumed by French households, 2) putting the results obtained from Hybrid M-PIOA in perspective with the results obtained from purely Monetary Input-Output Analysis (MIOA) and 3) qualitatively discussing the pros and cons for the use of Hybrid M-PIOT as opposed to MIOT with respect to the uncertainty entailed in the final results. First, Hybrid M-PIOT are constructed for France for the year 2006, in a framework distinguishing 59 activities per 59 products. The construction of tables in mass units requires the compilation of data of multiple origins and distinct precisions. The initial tables do not balance and are accordingly reconciled using constrained weighted least-squares optimization, which accounts for the input data uncertainties estimated by expert judgment. Then, fossil CO<sub>2</sub> and resource consumption caused by French household consumption are estimated using Hybrid M-PIOT on the one hand and MIOT (directly extracted from environmentally-extended Eurostat statistics) on the other hand. Finally, the differences in results are compared and discussed with a specific focus on the product groups mainly contributing to the impacts. The main sources of uncertainties are qualitatively assessed and compared from one method (Hybrid M-PIOA) to another (MIOA).

**MOPC09 The Impacts of REACH on the Critical Data Scarcity Problem in Chemicals Assessment** G. Stieger, ETH Zurich / Institute for Chemical and Bioengineering; M. Scheringer, ETH Zurich / Institute for Chemical and Bioengineering; S. Stempel, ETH Zurich; C.A. Ng, ETH Zurich / Institute for Chemical and Bioengineering; K. Hungerbuehler, ETH Zurich / Institute for Chemical and Bioengineering. As early as the establishment of the classification and labeling Directive (Directive 79/831/EEC) in 1979 there has been an expectation that chemicals regulation within Europe would enable the identification of potentially hazardous chemicals and prevent ‘mis-investments’ in substances that could delay or otherwise hamper effective environmental protection. With the entry into force of REACH in 2007, current expectations are even higher. Of key importance are the reporting requirements for the registration dossiers, which promise to help with the critical data scarcity situation regulators now face in chemicals assessment. What evidence exists that chemicals legislation has or will be able to fulfill these expectations? What are the lessons learned from the implementation of Directive 79/831/EEC? Regarding REACH, we are now five years beyond its entry into force. The period for pre-registration has passed, as has the deadline for registration dossiers on chemicals produced or imported at ≥1000 tonnes, CMR substances at ≥100 tonnes, and chemicals classified as very toxic to aquatic organisms. Presumably, data have now been collected for a large number of chemicals. How available are these data? Where can they be found and what do they tell us about the impact of REACH so far on the data scarcity situation and, more broadly, on our ability to prevent ‘mis-investments’ in potentially hazardous chemicals? In this work, we investigate the location, availability, and ease of extraction of chemical property data resulting from the implementation of REACH over the last five years. In addition, we make an initial survey of the perceived quality of these data in order to assess whether a relationship exists between the increasing data *quantity* under REACH and the availability of *quality* data. Higher quality data could be used both directly by regulators in chemicals assessment as well as by the scientific community to develop better structure-activity models, thus further aiding the goal of REACH to reduce the need for animal testing.

**MOPC10 Bioaccumulation assessment: Use of data from fish dietary studies for regulatory purposes** L. Wollenberger, C. Prevedouros, J. Caley, S. Barmaz, J. Hokanen, J. Peltola-Thies, J. Tarazona, B. Versonnen, European Chemicals Agency. Information on bioaccumulation is a standard data requirement under the REACH Regulation (EC 1907/2006) for substances produced or imported above 100 tonnes per year. The information is used for secondary poisoning

and persistence, bioaccumulation & toxicity (PBT) assessment. Bioaccumulation potential is also reported in the safety data sheet. Under the Regulation EC 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP) bioaccumulation potential is considered for environmental classification and labelling using either Log K<sub>ow</sub> or the bioconcentration factor (BCF). Fish are generally used as the key indicator for bioaccumulation in laboratory studies. In those studies fish are exposed to the chemicals dissolved in water in flow-through systems and the BCF is calculated as the ratio of the concentration in the fish to the dissolved concentration in water at steady state or by the ratio of the uptake and depuration rate constants. However, aqueous exposure studies may be difficult to conduct with poorly soluble substances. In October 2012, the OECD adopted a revised bioaccumulation test guideline 305, “Bioaccumulation in Fish: Aqueous and Dietary Exposure”. The revised guideline includes the additional possibility of dietary studies for substances with properties that create difficulties for aquatic exposure testing. In the dietary study fish are exposed via food spiked with the test substance. This route of exposure allows the derivation of a biomagnification factor (BMF). The BMF is calculated as the ratio of the concentration in the fish to the concentration in the food. However, REACH and CLP use the BCF as a threshold to indicate a high bioaccumulation potential for PBT assessment and classification and labelling. This makes test results from the dietary exposure study difficult to use directly since the dietary study generates a BMF, as the only route of exposure is via the diet. Data from the REACH registration database, previous regulatory frameworks and from the literature were analysed in order to discuss the selection of dietary versus flow-through study for fish bioaccumulation testing and the interpretation of results from the dietary test and their use for regulatory purposes.

**MOPC11 The application of Ecotoxicological Thresholds to a typical chemical inventory of a Fast Moving Consumer Goods (FMCG) company** S. Gutsell, Unilever; G. Hodges, Unilever Research; S. Marshall, Unilever; J. Roberts, Unilever Research. In human health risk assessment the Threshold of Toxicological Concern (TTC) describes a level of exposure to an untested or unidentified chemical that is likely to be without harm, such that chronic exposures below the threshold can be assumed to be without appreciable risk over a lifetime. This concept is equally relevant for chemicals with limited or no (eco)toxicological data, potentially allowing a no-effect concentration to be established for groups/classes of chemicals. No significant toxicity is expected to occur at exposures below this value. Threshold approaches offer a key advantage over other prediction methods such as QSAR in cases where the chemical structure or Mode of Action (MoA) is unable to be defined. In such cases a threshold-based approach allows an early tier assessment to be completed indicating whether environmental exposure to a chemical may cause concern and whether further testing is required. However, existing published thresholds all require that the chemical be classified according to either a chemical class or MoA. The most thorough investigation to date of such a threshold approach for use in environmental risk assessment was conducted by De Wolf *et al* who derived Environmental Thresholds of No Concern (ETNC) for the aquatic compartment and classified chemicals according to the Verhaar categorisation scheme. However, in cases where such classification is not possible, a generally applicable threshold value could be useful to allow an assessment to be made. Here we address the question of whether previous threshold values are applicable to a typical chemical inventory of a Fast Moving Consumer Goods (FMCG) company. Aquatic toxicity studies were reviewed from an internal dataset covering over 500 chemicals and ETNC values generated for chemical functional classes. Broadly these ETNC values were comparable with those determined by De Wolf *et al.*. An exception to this were the cationically charged chemicals which gave rise to a lower ETNC value.

**MOPC12 The Potential for Reducing the Uncertainty of Deterministic Environmental Quality Standards (EQS) by Increasing the Minimum Ecotoxicity Dataset** G. Merrington,

Environment Agency; D. Leverett, A. Peters, P. Simpson, WCA Environment. A review of guidance documents from Australia, Canada, the US and Europe on setting long-term aquatic Environmental Quality Standards (EQS) has shown that the minimum dataset requirement (i.e. the base set of data) is smallest for Europe, where an annual average EQS may be derived using a deterministic approach from just one chronic data point (so long as acute data are available). The European data requirements for the derivation of an EQS using a probabilistic approach are also the largest of all the jurisdictions reviewed (10-15 chronic values from 8 taxonomic groups). A review of the EQS for the current 33 priority substances under the Water Framework Directive (WFD) shows that those derived using deterministic approaches (i.e. where data for too few species are available to allow the use of a Species Sensitivity Distribution) have assessment factors ranging from 2 to 500 and that 5 of the 33 substances have EQS derived from datasets which include five or fewer chronic data points. Four example substances with relatively large chronic datasets were used to assess the effect of dataset size on the EQS derived according to the current Technical Guidance Document for deriving EQS under the WFD (EC 2011). The variability of the EQS calculated from randomly selected data points from each dataset was shown to sharply decrease when using seven or more chronic data points from three trophic levels, even when the same assessment factor (10) was used. Given the implications of introducing an EQS, we suggest that it would be appropriate to require more data for the derivation of an EQS. From the data reviewed here, it appears that the use of more chronic values from more trophic levels in deterministic calculations will reduce uncertainty in the derived EQS (even though this would not be reflected in the size of the assessment factor which would remain at 10). A significant reduction would be achieved with perhaps seven chronic values from three trophic levels.

**MOPC13 Using alternative methods and non testing strategies for environmental risk assessment in REACH 2013 dossiers: how far can we go?** G. Deviller, Rhodia, Member of Solvay / Direction Responsible Care; E. Cohet, A. Mandrillon, C. Mathon, F. Palais, R. Patoux, M. Enrici, Rhodia, Member of Solvay. All phase-in substances manufactured or imported in the EU at or above 100 tonnes/year have to be registered by the 31 st May 2013 under REACH regulation. The lack of data on the hazardous properties of chemicals was the driving force behind the development of a new chemicals policy in the EU nevertheless one of the objectives of REACH is to promote alternative methods for the assessment of hazards of substances both to reduce animal testing and to reduce the costs. Rhodia, Member of Solvay, as a chemical company applied for registration of more than one hundred REACH substance dossiers including several chemical classes such as organic, inorganic substances, mono/ multi constituents and UVCB. The aim of this work is to illustrate how far non testing techniques could be used for filling data gaps in order to perform the environmental hazard assessment for the second tonnage band registration. Existing data coverage, reasoning for waiving tests, adequacy of possible alternative methods (QSAR modeling, *in vitro* and read-across), evaluation of existing data as part of a weight-of-evidence approach and experimental testing strategies are addressed. A similar exercise performed on first tonnage band in 2010 (Deville & al, 2010) revealed that almost half of the endpoints were fulfilled by experimental data's and the other half by waiving strategy. It was expected that lower data availability and higher tier tests requirement for the next registration deadline and further development of tools and guidance would increase the use of these techniques. Since then, the OECD Toolbox had been improved to facilitate its use under REACH (e.g. QPRF downloadable into IUCLID...) and several lines of guidance have been launched by ECHA. Although there is a clear objective of facilitating the use of alternative methods, those guidelines are sometimes inconsistent with actual possible approaches. The final objective is to identify the most reliable approaches which have gained regulatory credibility and their impact on the reduction of animal testing and costs, based on our experience, in the context of the environmental risk assessment for REACH registration dossier.

**MOPC14 Comparison of acute aquatic ecotoxicity data generated on cosmetic ingredients by the OECD QSAR Toolbox v3.0 and laboratory testing** L. Colombe, L'OREAL / Environmental Research; J. CHENEUBLE, L'OREAL / Environmental Research; D. GEORGIEVA, Laboratory of Mathematical Chemistry / Laboratory of Mathematical Chemistry, University Prof. As. Zlatarov; O. MEKENYAN, Laboratory of Mathematical Chemistry; J. L'HARIDON, L'OREAL. Substances used as cosmetic ingredients show a large chemical diversity. European Union (EU) regulations (REACH, Cosmetic Directive ...) support more and more determination of intrinsic properties of substances by *in silico* models (QSAR, read across ...) to avoid unnecessary studies, reduce animal testing and lower the cost of regulatory dossiers. This trend is also supported by the increasing involvement of companies in sustainable development. Some intrinsic properties of substances address aquatic ecotoxicity and are taken into account in regulatory hazard classification and assessment of potential risks to the environment at different stages of their life cycle. Since long, L'Oréal has developed and uses *in silico* methods for screening cosmetic ingredients towards both human safety and potential environmental impacts. However, the prediction of aquatic ecotoxicity from *in silico* models does not appear sufficiently reliable for many chemicals used in cosmetics today. One of the most promising tools for assessing *in silico* aquatic ecotoxicity of substances is the OECD QSAR Toolbox. In this study, hair dyes, UV filters and surfactants were selected from the EU CosIng list. Their acute toxicity on aquatic species, assessed in standard laboratory tests, has been compared to data generated with version 3.0 of the OECD QSAR Toolbox. Results and conclusions of this study are presented.

**MOPC15 Variability in Environmental Quality Standards: what are the causes?** L. Ceriani, B. Marks, WCA Environment; M. Junghans, S. von Arb, Swiss Centre for Applied Ecotoxicology Eawag-EPFL; P. Whitehouse, Environment Agency of England and Wales; I. Johnson, WRc. The Water Framework Directive (WFD) aims to achieve good surface and groundwater status by 2015. One of the tools used to deliver this aim are Environmental Quality Standards (EQS). Furthermore, the WFD requires that Member States (MS) identify Specific Pollutants and establish national EQS according to Annex V of the Directive. Since MS have access to similar sources of data and approaches to EQS setting are set out in the recent EU Technical Guidance, most people would expect assessors to arrive at the same EQS when faced with the same data and methodology. This research examines whether this is the case. Ten substances have been identified as Specific Pollutants common to at least six MS. EQS dossiers for the ten Specific Pollutants were obtained, where possible, from MS. For each chemical the number of ecotoxicity data points collected and the number remaining after reliability and relevance evaluation was determined along with information on the choice of critical datum, the extrapolation method adopted, and the choice of assessment factor. These have been compared in order to understand their contributions to any differences in the final long-term EQS values for freshwater, expressed as an annual average. This study highlights that EQSs developed under previous regimes (e.g. Dangerous Substances Directive) and retained vary from those developed following the WFD Annex V principles. The quantity of ecotoxicity data retained after quality assessment for a substance can differ greatly between MS. It is not clear if this reflects differences in investment in data searching or differences in the rigour with which reliability assessment of the data is undertaken. Where a common data source has been used, e.g. European Risk Assessment Report, variability is typically reduced. The identification of the critical datum also differs between MS. This can be due to differences in quality assessment of data, especially for endpoints with a lack of standard test guidelines and a reliance on expert judgement. In order to reduce EQS variability between MS, it is recommended to improve consistency through training based both on the 2011 Technical Guidance and on the quality assessment of data, and to incentivise informal exchange and discussion of data interpretation, and use of expert judgement.



**MOPC17 Toxicokinetics of pharmaceuticals and biocides in freshwater crustaceans** J. Jeon, Eawag Aquatic Research / Environmental Chemistry; D. Kurth, Eawag (Swiss Federal Institute of Aquatic Science and Technology); R. Ashauer, University of York / Environment; J. Hollender, Eawag / Dept Environmental Chemistry. Biotransformation (BT) is a biological process modifying organic molecules to derivatives with generally increased hydrophilicity, thus promoting the chemical excretion from the organisms, resulting in less bioaccumulation and potential for toxicity. The processes of uptake, internal distribution, biotransformation and elimination of xenobiotic substances in organisms are termed toxicokinetics (TK) and each process can be quantified by rate constants. In the present study, TK parameters (e.g. uptake, elimination, and transformation rate) in *Gammarus pulex* and *Daphnia magna* were estimated for the pharmaceutical tramadol and the biocides irgarol, and terbutryn. Bioaccumulation factor (BAF) for parent compounds and retention potential factor (RPF) for biotransformation products (BTPs) were also calculated. For toxicokinetics of irgarol, *G. pulex* showed greater uptake/excretion rates and BAF/RPF values than *D. magna*. The total sum of RPFs representing the ratio of the total amount of BTPs to the parent at equilibrium was 0.56 and 0.17 for *G. pulex* and *D. magna*, respectively. In comparison between compounds, tramadol was transformed more and faster than irgarol, resulting in the greater sum of RPFs for tramadol. O-demethylation and N-oxidation were major BT mechanisms for tramadol while hydroxylation was the most rapid reaction for irgarol. The slight difference in the chemical structure between irgarol and terbutryn led to the relatively large discrepancy in the BT rate and RPF of relevant BTP.

**MOPC18 Implementing Ecopharmacovigilance Approaches to Support Post-Launch Product Stewardship** P. Robinson, AstraZeneca / Brixham Environmental Laboratory; N. Budgen, AstraZeneca / Essential Safety Health and Environment; C. Coleman, AstraZeneca / Brixham Environmental Laboratory; G. Holm, AstraZeneca / Essential Safety Health and Environment; R. Murray-Smith, J. Snape, AstraZeneca / Brixham Environmental Laboratory. Ecopharmacovigilance is a developing science associated with the detection, evaluation, understanding and prevention of adverse effects of pharmaceuticals in the environment. In Europe and North America, a new regulatory submission or a line extension is normally accompanied by an Environmental Risk Assessment (ERA), but there is no regulatory requirement to monitor the environmental risks of medicines post-launch. Nevertheless, researchers continue to report instances of pharmaceutical residues in the environment from a range of sources, particularly after patient use. Whilst there are major challenges for signal detection and relating cause and effect, there are some practical steps that can be taken to review the significance of these emerging data. This poster describes processes that have been developed within AstraZeneca (AZ) for managing the environmental risk of products throughout their life. Environmental Risk Management Plans have been implemented; these 'live' documents take account of all relevant information that may impact the environmental risk profile. Active literature monitoring is undertaken to search for new data relating to AZ products: ecotoxicological data (including non-standard biomarkers) that could impact the Predicted No Effect Concentration (PNEC) and information on fate and exposure that might impact the Predicted Environmental Concentration (PEC). A database is maintained of all reported occurrences of AZ's Active Pharmaceutical Ingredients (APIs) in the environment, along with relevant metadata (e.g. location/source, duration of sampling, robustness of analytical method), which help to make decisions on how these findings should be interpreted. The database has been linked to a Geographical Information System so that the data can be interrogated, prioritised and visualised on global maps. These tools are used to help understand the nature of the potential risks identified and decide what follow-up measures should be adopted (e.g. updates to the ERA assumptions, further research). Reported measurements of pharmaceuticals in the environment are prevalent in the literature; approximately 5000 instances of AZ APIs have been recorded to date. However, presence does not equate to risk. After

further scientific scrutiny of the data, less than 2% required detailed investigation to better understand any potential risks. Furthermore, these cases are predominately related to only 2-3 active ingredients.

**MOPC19 Monitoring synthetic surfactants and pharmaceutically active compounds in the unsaturated zone from aquifers systems (SW, Spain).** C. Corada-Fernández; P. Lara-Martin, University of Cadiz; J. Jimenez-Martinez, University of Rennes I; L. Candela, Polytechnic University of Catalonia; E. Gonzalez-Mazo, University of Cadiz. Soil and associated groundwater contamination is a worldwide environmental problem with disastrous consequences. Although regulated compounds such as pesticides, polychlorinated biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs) are frequently monitored, there are many non-regulated pollutants that could also access to groundwater without our knowledge. In this study, we have determined the presence of emerging contaminants such as synthetic surfactants and pharmaceutically active compounds (PhACs) in the unsaturated zone from aquifers systems located at Jerez de la Frontera (SW, Spain). These chemicals may be affecting the porous media as a consequence of the reuse of wastewater and sludge from the sewage treatment plant (STP) at Jerez de la Frontera (200 000 inhabitants) in the surrounding crops. Soil samples were extracted from several stations up to a depth of 2 m. Disturbed samples were obtained using hand drilling, and undisturbed samples by means of soil sample rings inserted by percussion. Physical, chemical, mineralogical and hydraulic properties were determined. Emerging contaminants were determined by two different analytical procedures based on the use of pressurized liquid extraction (PLE) and solid phase extraction (SPE), followed by later quantification by liquid chromatography - tandem mass spectrometry (LC-MS/MS). Surfactants were found in all samples, showing a wide range of concentrations: between 73 and 1300 µg/kg for linear alkylbenzene sulfonates (LAS), from 329 to 1090 µg/kg for alcohol polyethoxylates and between 155 and 279 µg/kg for nonylphenol polyethoxylates (NPEOs). The presence of LAS and AEOs homologues with longer alkyl chain was predominant due to their greater hydrophobicity. Over 60 pharmaceutical compounds belonging to various therapeutic groups (analgesics, antihypertensives, lipid regulators, psychiatric drugs, antibiotics...) were analyzed too. Only 7 of these chemicals were detected occasionally (diclofenac, metoprolol, fenofibrate, carbamacepine, clarithromycin, famotidine and hydrochlorothiazide) at really low concentrations (from 0.1 to 1.3 µg/kg).

**MOPC20 Binding affinity of antidepressants to the serotonin reuptake transporter as a predictor of adverse behavioral effects observed in fish** L.E. Sweet, Institute of Environmental Toxicology; J.H. Bisesi, University of Florida / Environmental and Global Health; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX). Widespread use of antidepressants and their incomplete removal during wastewater treatment has resulted in increased detection of antidepressants in many surface waters. Research in our lab has shown that exposure to the antidepressant fluoxetine, a selective serotonin reuptake inhibitor (SSRI), decreases brain serotonin levels, which correlates with an increase in time for a predator (*Morone saxatilis* x *M. chrysops*, hybrid striped bass) to capture its prey (*Pimephales promelas*, fathead minnow). Similarly, exposure to the antidepressant venlafaxine, a serotonin and norepinephrine reuptake inhibitor, causes brain serotonin levels to decrease, resulting in a significant increase in time to capture prey. However, exposure to the non-serotonin targeting drug bupropion, a norepinephrine and dopamine reuptake inhibitor, did not affect predation behavior. These results indicate that there is a mechanistic link between SSRI antidepressant exposure, brain serotonin levels, and alterations in predation behavior. The objective of the current study was to determine the relationship between the binding affinity of SSRI antidepressants to their target receptor, the serotonin reuptake transporter, and adverse behavioral responses in fish. A zebrafish serotonin reuptake transporter plasmid construct was overexpressed in mammalian cell lines to produce sufficient protein for receptor binding affinity assays. Radioligand binding assays were performed with the transfected cell line and hybrid striped bass brain homogenates. The

antidepressants fluoxetine, venlafaxine, and bupropion were used as competing antidepressants in the presence of [<sup>3</sup>H] Citalopram for 1 hour at 25°C. The binding affinity (K<sub>d</sub>) of each antidepressant was quantified using radioligand binding assays<sup>1</sup>. These binding affinities were correlated with brain serotonin concentrations and observed behavioral effects from hybrid striped bass exposed to the respective antidepressants. Furthermore, the binding affinity of sertraline and citalopram were investigated to provide *in vitro* predictions of brain chemistry and behavioral alterations observed *in vivo*. Results from this study will provide a valuable tool for predicting the effects of antidepressants on subtle behaviors that directly influence ecological fitness and population survival; but are not captured using traditional toxicity testing endpoints.

**MOPC21 Evaluating the potential cytotoxic and genotoxic effects of pharmaceutical effluents from KP Pharmaceutical industry in Anambra State, Nigeria** A.C. Udebuani, Federal University of Technology / Department of Biotechnology; O.A. Offoma, Federal University of Technology, Owerri / Biotechnology; I.N. Onwurah, University of Nigeria / Biochemistry. The potential genotoxicity and cytotoxicity of water effluent from a pharmaceutical industry in Ogidi, Anambra State, Nigeria was evaluated using *Allium cepa* Linn assay, an alternate first-tier assay to experiments on animals for preliminary toxicity screening in accordance with the council directive 86/609/EEC art-23 that encourages research on alternative techniques to animals' use. The cytotoxic effects was evaluated on the basis of strong growth retardation in high concentrations of the effluent which resulted in root growth inhibition (5.33 to 1.33) and decrease in mitotic index from 68.4 to 52.8 and these results were statistically significant (p< 0.05). Genotoxicity based on chromosomal aberrations induced in the onion root tips include sticky chromosomes, bridges, laggard, vagrant, polar deviation and polyploidy. Result also showed the concentrations of heavy metals Zn, Cd, Pb, Cr, Ni and Cu, in the polluted sample to be 0.010, 0.006, 0.003, 0.300, 0.018 and 0.020 g/kg respectively. Chromium in the effluent was found to be the highest and may be responsible for the observed genotoxic effects on the onion root tip cells. The study established the potential cytotoxic and genotoxic effects of this pharmaceutical waste in the environment.

**MOPC22 Environmental risk assessment of veterinary medicinal products – a new concept for a plant test with more realistic exposure scenario** U. Kuehn, Federal Environment Agency; I. Ebert, Umweltbundesamt / Pharmaceuticals Department; M. Simon, Fraunhofer IME; M. Herrchen, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME); B. Foerster, ECT Oekotoxikologie GmbH (ECT); J. Roembke, N. Graf, ECT Oekotoxikologie GmbH. The poster presents the regulatory need and the background of a special more realistic terrestrial plant testing approach for veterinary pharmaceuticals. Environmental effects of veterinary medicines are assessed according to the guidelines of the European Medicines Agency (EMA) and the VICH GL 38 (ECOTOXICITY PHASE II). According to the guideline a terrestrial plant test in phase II is required because residues of pharmaceuticals release with dung and manure from treated animals on agriculture land. The terrestrial plant test is conducted using the standardised test protocol OECD 208 (Seedling Emergence and Seedling Growth Test). The current regulations take into account only the parent compound but do not consider transformation products and NER (Non Extractable Residues). This might result in incorrect estimation of risk in case of substances applied on agricultural soils with manure. Therefore, the German Federal Environmental Agency (UBA) has initiated a research project to develop a special terrestrial plant test with a more realistic exposure scenario. The test substance is applied in manure and stored over a defined period prior testing. Then this mixture is tested in a standard terrestrial plant test according to OECD 208 in order to evaluate the potential phytotoxicity of transformation products and NER. This new approach is currently developed with different plant species, different manures and storing periods by two research partners: Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) Schmallenberg and ECT Oekotoxikologie GmbH Flörsheim.

## **MOPC23 What happens after fish stop taking the birth control pill?**

**The recovery phase of a whole lake experiment** K.A. Kidd, University of New Brunswick; P. Blanchfield, B. Park, Fisheries and Oceans Canada; V.P. Palace, Stantec Consultants Ltd.; K. Wautier, Fisheries and Oceans Canada. Sewage treatment works effluents contain hormones and mimics that interfere with sexual development and reproduction in individual fish. To assess the effects of these endocrine disruptors on fish population sustainability, whole lake additions of the synthetic estrogen ethynylestradiol (EE2) used in birth control pills were done from 2001-2003 at the Experimental Lakes Area, northwestern Ontario, Canada. In the first year of the additions, male fathead minnow (*Pimephales promelas*) and pearl dace (*Margariscus margarita*) became feminized, with delayed spermatocyte development and high concentrations of vitellogenin, an egg yolk protein precursor. In the second and third summers of EE2 additions, reproductive failures occurred for the shortest-lived fish species, the fathead minnow, with a subsequent collapse in the population. Modest reductions in catch-per-unit-effort (CPUE) and loss of small size classes were observed for pearl dace. To assess potential recovery, from 2004-2010 we continued to monitor these populations in the spring, and opportunistically collected individuals for vitellogenin and gonad histology. In 2005 and 2006, vitellogenin concentrations were back at baseline levels in male pearl dace and fathead minnow, respectively. The incidence and severity of gonadal abnormalities decreased with time post-exposure, and were absent in fathead minnows and pearl dace three and six years post-exposure, respectively. Size-frequency distributions and CPUE of fathead minnow returned to pre-experiment levels by the spring of 2007. These results indicate that, given time, fathead minnow and pearl dace can recover from EE2 exposure at the biochemical through population levels.

## **MOPC24 Early Life Stage (ELS) Tests with Diclofenac in Rainbow Trout and Zebrafish**

J.C. Wolf, Experimental Pathology Labs Inc; J. Hardisty, EPL, Inc.; A. Hartmann, Novartis Pharma Stein; U. Memmert, Eurofins Regulatory AG; K. Weber, AnaPath GmbH. 1. Introduction Diclofenac (DCF) is a widely used non-steroidal anti-inflammatory drug (NSAID) which is regularly detected in surface waters. Current aquatic risk assessments for DCF are based on several fish studies that use as yet unvalidated endpoints such as histopathology or biomarker, although histopathological evaluations do not typically provide population-relevant data for environmental risk assessment. 2. Materials and methods ELS tests were conducted in both rainbow trout and zebrafish. Population relevant endpoints were evaluated, such as hatching, growth and survival, plus histopathological effects in potential target organs in the trout study 3. Results and discussion 3.1. ELS in rainbow trout In the rainbow trout ELS test, the no observed effect concentration (NOEC) including histopathology was 320 µg/L. 3.2. ELS in zebrafish The effect of DCF on zebrafish growth was less clear, and the NOEC can be interpreted as 10 µg/L. A moderately reduced growth of zebrafish exposed to concentrations of up to 320 µg/L was not repeatable. 4. Conclusions It was concluded that DCF has, with high probability, no adverse effect on both fish species up to 320 µg/L. That is a sufficient safety margin for fish populations, since concentrations of DCF in European rivers are in the ng/L to low µg/L range. Published studies with DCF in salmonids identified lesions in liver, kidney and gills at concentrations of 5 µg/L based on histopathological investigations (1, 2). The recent ELS test with DCF in rainbow trout did not yield lesions up to 1000 µg/L. To better understand the differences between the recent ELS study and the previously reported study results, a pathology working group (PWG) will be convened to review those studies. The results of the PWG are presented. 5. References [1] Schwaiger J, Ferling H, Mallow U, Wintermayr H, Negele DR. 2004. Toxic effects of the non-steroidal anti-inflammatory drug diclofenac Part I: histopathological alterations and bioaccumulation in rainbow trout. *Aquatic Toxicology* 68:141-150. [2] Triebkorn R, Casper H, Heyd A, Eikemper R, Köhler H-R, Schwaiger J. 2004. Toxic effects of the non-steroidal anti-inflammatory drug diclofenac Part II. Cytological effects in liver, kidney, gills and intestine of rainbow trout (*Oncorhynchus mykiss*).

**TUPC01 Chemical footprint analyses provides clear views for managing chemicals at various scales** L. Posthuma, RIVM / Lab. for Ecological Risk Assessment; M. Zijp, RIVM / DMG; D. van de Meent, RIVM / Institute of Wetland and Water Research; A. Hollander, Radboud University. The footprint concept has been coined to express whether gross chemical emissions (separate compounds or mixtures) are larger than the capacity of the environment to accommodate emissions, with too big a footprint implying undesired impacts. Aspects of a generally accepted method for deriving a chemical footprint have been discussed in a Special Session at SETAC-Berlin in 2011, without showing results of 'the footprint of all chemicals for global diversity' nor for chemical footprints of regions, industries of certain kinds, or products. In adopting the footprint concept as final idea for presenting cumulative chemical impact of large groups of compounds, we developed a methodology for chemical footprint quantification, tested for two situations: organic chemicals in the EU, and plant protection products in the catchments of western-European rivers. The footprint derivation asks for data and approaches to handle emissions, fate, sensitivity and mixtures, as well as spatial data and, finally, boundary principles. The latter is key: a footprint either or not surpasses a boundary, and the definition of a boundary thus requires specific attention. This presentation will show the approaches followed for two case studies, shows how the methods depends on spatial aspects, and especially highlights the key role of boundary definitions. We show how a regulatory boundary can be derived, given choices made in the existing chemicals' policies, as well as from boundaries originating from biodiversity considerations. In doing this, relative and absolute interpretations of the results are discussed, showing that the steps provide robust results for chemical management priorities, despite the possibility that absolute prediction of global biodiversity impacts of all chemicals are beyond reach. The footprint method is shown to be informative for management with two example cases. Applications at the product-, organization- and regional levels can be foreseen.

**TUPC02 The role of plastic additives in the economy wide Chemical footprint – the example of Sweden** T.V. Rydberg, IVL Swedish Environmental Research Institute; H. Andersson, IVL Swedish Environmental Research Institute Ltd; M. Rahmberg; J. Wester Dahl, IVL Swedish Environmental Research Institute. The economy-wide annual emissions of over 250 specified additives in plastic and rubber articles have been previously calculated. The current study reports on the further assessment of the contribution of these emissions to the total chemical footprint of the Swedish economy, using established LCIA methods such as USEtox and USES-LCA. As expected, the actual contribution of individual substances and substance groups to the total impact score changes when comparing with their contribution on a mass basis. Thus, for example the plasticisers constitute around 50 % of the total emissions of additives from plastics, while the impact score for the plasticizers after applying characterisation factors makes up significantly lower share of the total, about 10 %, for USEtox aquatic ecotoxicity. Further validated results will be presented and discussed. In order to achieve these results, several new LCIA characterisation factors had to be developed. This was done with the use of substance data obtained with quantitative structure-activity relationship (QSAR) models. Although it can be concluded that this is a feasible way to gather substance data for large datasets, further improvements of the methods used are however needed to achieve sufficiently accurate results. For example, several potentially important substances could not be calculated as they were outside the applicability domain. The data needs and data availability will be further described and discussed. Various approaches towards the Chemical footprint concept is also discussed in the content of the study.

**TUPC03 Approaching the planetary boundary for chemical pollution through a chemical footprint indicator – exploring feasibility via two case studies** A. Bjorn, Technical University of Denmark / Department of Management Engineering; M.Z. Hauschild,

Technical University of Denmark (DTU) / Dept. of Management Engineering; M. Birkved, Technical University of Denmark; M.L. Diamond, University of Toronto / Department of Earth Sciences. As the global pressures on the environment are generally increasing it becomes important to quantify environmental limits. These limits can be understood as physical thresholds that, if exceeded, lead to serious changes in the states of ecosystems. The Planetary Boundaries concept is the most recent attempt to quantify environmental limits. Nine planetary boundaries define the playing field that humanity should stay within in order not to cause any unacceptable global environmental changes. Of these nine boundaries chemical pollution has not yet been quantified. This study investigates how the impacts from chemical emissions may be implemented in the ecological footprint framework, in which consumptions of renewable resources and emissions of waste are expressed in land occupation (ha\*year). A tentative methodology is tested on two cases: 1) The annual emissions of chemicals in Europe and 2) The annual application of pesticides by farmers in Denmark. USEtox is chosen as characterization model of the emission inventories. When converting the unit of the USEtox damage indicator,  $\text{PAF} \cdot \text{m}^3 \cdot \text{day}$ , into a footprint, the physical interpretation of an emission based footprint is necessary. The footprint of most emissions can be interpreted as the physical area needed to absorb the emissions in the environment. However for non-degradable emissions (such as persistent organic pollutants and heavy metals) the footprint should be interpreted as the physical area needed to make up for the loss of biocapacity resulting from the chemical emission. The possibilities and final choice of operationalizing these interpretations in a chemical footprint indicator will be presented. The results of the two case studies will be presented and interpreted with respect to: 1) Identifying the chemicals contributing the most for each case 2) Comparing the obtained chemical footprints with their corresponding (planetary) boundaries 3) Comparing and validating the results to empirical measurements to the extent possible 4) Comparing the obtained chemical footprints with other types of footprints (built-up land, cropland, fishing, etc.). Based on the cases studies the chemical footprint indicator will be evaluated in terms of robustness, data demands and decision context in which it may be applied.

**TUPC04 About the interest of a distinction between surface and ground water in water availability footprint – The case of thermal electricity production** A. Prieur Vernat, GDF SUEZ / CRIGEN; A. Boulay, CIRAIG / Department of Chemical Engineering; J. Mertens, GDF SUEZ / Laborelec. Accounting for impacts on water resources due to electricity production is needed to have a complete view of its environmental performance. The modelling of impacts related to water is still not consensual and many methods differ in specific modeling choices. One of them is the distinction between surface and ground water when assessing changes in water availability. While most methods do not assess them separately, it is also argued that they should be kept separate as they are not available in the same proportions, do not serve the same users and require different technologies to be used. Boulay et al. (2011) developed a method that assesses the stress caused on the resource based on its source of abstraction, distinguishing surface, ground and rain water, but also provides values independently of the source when it is not known. This was used on a case study on the production of electricity as part of the periodically updated Life Cycle Assessment of GDF SUEZ. The water availability footprint for two types of power plants from its 2011 electricity production is presented, including both water used at the power plant and water used for the fuel supply chain and infrastructures. Those results show the impact of a distinction between surface and ground water at midpoint: while in most regions the difference is not high, it can be significant in other regions, where the gap between indexes for the various types of water is high. More conceptually, distinguishing surface and ground in a water stress index is important if it serves as factor in the impact pathways which will differ as the users deprived will differ: ecosystems and human users (transport, fisheries, hydropower) are deprived when using surface water. However, endpoint results may be inverted if groundwater is scarcer but surface water deprives more users, which may question the

relevance of using scarcity as a midpoint indicator.

**TUPC05 Assessing water deprivation at the sub-watershed scale in LCA including downstream cascade effects** P. Loubet, Veolia Eau d'ÎledeFrance; P. Roux, Irstea / Research Unit: Information and Technologies for Agro-processes; M. Núñez, UR050, Laboratoire de Biotechnologie de l'Environnement; V. Bellon-Maurel, Irstea / Research Unit: Information and Technologies for Agro-processes. Human freshwater uses are in competition with water availability for ecosystems and can lead to impacts evaluated by LCA. Physical water deprivation at the midpoint level is currently assessed in water-related LCIA methods using indicators (withdrawal-to-availability and consumption-to-availability) that represent water scarcity at the watershed scale. Although these indicators have brought a great improvement to assess water impacts in LCA, significant challenges still remain to improve their accuracy and relevance. Issues such as the spatial and temporal resolution as well as the sensitivity to additional significant water consumption should be addressed. This is especially true for large countries or watersheds with very heterogeneous internal water availability and for studied systems where water is expected to become the key environmental issue. The proposed framework focuses on the impacts caused by surface water (from river and renewable groundwater) consumption, commonly named blue water consumption. The framework is constituted by two different steps that define, at the sub-watershed scale, (1) the consumption-to-availability ratio (CTA) and (2) the water deprivation characterization factor (WDCF). While CTA shows current water scarcity of a sub-watershed, WDCF assesses the effect of water deprivation in a sub-watershed on the impacted downstream sub-watersheds. CTA is based on a water balance conducted at the sub-watershed scale and on the ratio between water consumption and water availability. Then WDCF of a specific sub-watershed is the weighted sum of each downstream sub-watershed effects, using CTA. It represents the cascade effects (i.e. water deprivation) on downstream potential usages and environmental requirements. Resulting WDCF are midpoint indicators that can be used to assess water deprivation of a studied system water consumption in LCA. Results are presented including WDCF computed for several watersheds and their application to the LCA of different water withdrawals and releases scenarios for a megacity.

**TUPC06 Spatialized Life Cycle Assessment of US Dairy production: from regional to national scale** A. Asselin, University of Michigan, School of Public Health, Environmental Health sciences; O. Jolliet, University of Michigan / School of Public Health; A.D. Henderson, University of Texas School of Public Health / Environmental Science; M. Heller, University of Michigan, School of Public Health, Environmental Health sciences; G. Thoma, University of Arkansas / Department of Chemical Engineering; M.D. Matlock, University of Arkansas / Centre for Agricultural and Rural Sustainability; M. Margni, Ecole Polytechnique de Montreal / Department of Mathematical and Industrial Engineering; R. Saad, CIRAI, Ecole Polytechnique de Montreal; L. Lessard, S. Vionnet, Quantis; J. Dettling, Quantis International; S. Humbert, Home. Milk and dairy product production, at the national scale, is a distributed production system that entails great geographic diversity with respect to inputs and outputs. Milk therefore represent a very interesting case study to develop and test multi-scale spatialized life cycle approaches for both inventory and impact assessment. This presentation illustrates such a spatialized method, presenting the results of a large milk LCA project carried out for the national US dairy industry, focusing on water footprint (water use and water quality). This paper first develops an original matrix approach that enables the consistent combination of the impacts from an inducer perspective (impact per kg milk produced in 18 US watershed), from an emission perspective (impact per kg feed produced in each watershed) and from a receiver perspective. Water use impacts show very high spatial variations. Because a large fraction of milk production takes place in western watersheds, where many crops also are irrigated, production of milk in those states will induce water consumption for crops produced locally or regionally, leading to a national average of

120 L competition per kg milk produced at farm gate. For water quality, a detailed spatialized analysis for a 50km x 50km grid, summarized into 18 US watershed shows that a large share of the P-emission and eutrophying impacts occurs in four main watersheds both eats and wets of US. Overall, best management practices and gain in efficiency matter along the whole milk supply chain.

**TUPC07 Improvements and applicability of newly impact categories in the case study of greenhouse tomato production** A. Assumpcio, M. Torrellas, IRTA; M. Núñez, UR050, Laboratoire de Biotechnologie de l'Environnement. Within the LC-IMPACT project, different case studies were carried out to test the newly developed operational life cycle impact assessment (LCIA) methods. Here, we focus on the assessment of one agricultural system: a tomato crop grown in a *parral* greenhouse on the coast of Almeria, Spain. This case study was selected as representative of the current agricultural practices in the Mediterranean region. The main goal of this research was to apply and evaluate in detail the newly LCIA methods developed over the project in our case study. To conduct the study, a first life cycle assessment (LCA) in accordance to the ILCD guidelines was performed, using ReCiPe complemented with USEtox as LCIA methodologies. A second assessment was conducted applying the newly characterisation factors (CFs) developed in the LC-IMPACT project. Here, we present those methods with special significance in agricultural practices, which were selected among the new developed methods. From an agricultural perspective, the results show the importance of including topics such as pesticides and water in LCA. Focusing on the applicability of new CFs, we would like to highlight aspects such as the importance of including spatial differentiation and uncertainty assessment, and the need to reach a better standardization about what units should be used in order to allow the comparison of different impacts. An important subject for further research is the need of an agreement about what should be the border between the inventory flows and the fate factors, especially for pesticide and nutrient emissions. The comparison of the results with old and newly LCIA methodologies allowed us to improve the environmental assessment of our case study. We could as well judge the applicability of newly CFs in a real case study, providing the required feedback to LCA developers.

**TUPC09 Ecological risk assessment for the diagnosis of environmental impacts in tropical freshwater ecosystems** A.L. Sanchez, USP / Department of Hydraulic Engineering and Sanitation, Center for Water Resources and Applied Ecology; L.D. de Freitas, C. Fileto, E.L. Espindola, University of São Paulo (USP) / Department of Hydraulic Engineering and Sanitation, Center for Water Resources and Applied Ecology. Ecological risk assessment studies are important to assess environmental changes that have been caused by various anthropogenic activities, such as leaching of contaminated areas, input of domestic and industrial sewage, runoff of agricultural areas and other impacts, which are responsible for physical, chemical and biological alterations. In this context, the aim of this study was to evaluate the impact of the anthropogenic activities developed in the Lobo Hydrographic Basin (Itirapina and Brotas, state of São Paulo, Brazil) on the ecological dynamics of lotic and lentic tropical freshwater ecosystems. To attempt it, limnological studies and the evaluation of the potential impacts were carried out before the subsequent application of an ecological risk analysis, which were based on chemical, ecotoxicological and ecological evidence lines. For this purpose, samples were taken in different periods (rainy, dry and intermediate seasons), in 14 sampling stations (tributaries and reservoir), including biotic and abiotic variables in relation to water and sediment. The results of the ecological evidence lines were obtained from the fishes and zooplankton communities, reporting the risk potential species. The results obtained suggest that domestic sewage, deforestation and diffuse pollution (associated with the accumulation of sediments and agrochemicals used in different cultures established in the region) were the main impacts in the Lobo watershed. The Triad Analysis (based on chemical, ecotoxicological and ecological measures), in addition of the quantitative results from chemical evidence line, indicated that risks

were higher in the reservoir in comparison with the tributaries, where the risks were considered low or moderate in dry season in some sampling stations. Ecotoxicological risks were not identified at most of sampled sites, but the inverse situation was registered for ecological risks due to the influence of limnological variables. Seasonal differences between dry and rainy seasons were observed after the integration of all evidence lines, with higher risk values occurring during dry season, in addition to the risk associated with environmental degradation of each ecosystem. We concluded that the activities developed in the Lobo Hydrographic Basin have been responsible for several ecological impacts in this system, beside some alterations promoted by climatological events.

**TUPC10 Life Cycle Human Exposure and Risk Assessment of Pesticide Application on Agricultural Products in Colombia.** Juraske, ETH Zurich; C. Mosquera, Universidad Nacional; C. Lesmes, C. Binder, University of Munich; M. Berdugo, J. Diaz, Uni Boyaca; J. Guerrero, A. Erazo, Universidad Nacional; G. Garcia Santos, University of Zurich; S. Hellweg, ETH Zurich. Although adverse effects of pesticides have decreased significantly in developed countries, misuse of pesticides in developing countries is still problematic. Chronic health problems and environmental impacts have been poorly investigated and might be significant. Possible factors contributing to these effects include miss- and overuse of pesticides and missing or insufficient protection of workers during pesticide application and use. Here we examine the various exposure pathways of pesticide application, over the whole life cycle of a variety of agricultural products grown in Colombia. Colombia was chosen because a large variety of crops is grown there on various altitudes. This allows for a broad study which is indicative also for other countries in the region. In particular, we look at bananas, passion fruit, flowers, and potatoes. These crops cover a range of uses, from food to decoration. They are either consumed locally (potatoes), or exported in large quantities to industrialized countries (bananas, flowers). Regarding the latter products, the importing country acquires a "virtual burden" in terms of human exposure in the country of origin. Thus, it is one of the goals of this project to account for indirect exposure, such as workers' exposure during pesticide application or population exposure through groundwater contamination, in addition to direct consumer exposure by ingestion of the pesticide-treated crops or by dermal contact. In order to quantify exposure, field trials were conducted at selected sites and fate and exposure models were developed, adapted and applied to Colombian conditions. Model calculations and measurements were shown to correspond well. Application- and crop-specific models were able to predict the magnitude and temporal profile of experimentally derived pesticide concentrations. Human intakes of pesticides by inhalation, ingestion and dermal exposure were quantified for each crop, permitting the identification of "hot spots" and measures to mitigate exposure. When good agricultural practices were applied, it was shown that pesticide concentrations were below maximum residue limits and therefore do not violate against international regulatory thresholds. In addition to practical decision support, this work contributes to capacity building in Colombia. The results are intended to be disseminated among stakeholder groups, including authorities and producers in the "South" as well as retailers and consumers in the "North".

**TUPC11 Effects of the antibiotics enrofloxacin and ciprofloxacin on tropical freshwater organisms** M. Andrieu, Wageningen University and Research Centre; A. Rico, Wageningen University / Aquatic Ecology and Water Quality Management Group; T.M. Phu, N.L. Khoa, N.T. Thao, D.T. Huong, N.T. Phuong, Can Tho University; P.J. Van den Brink, Wageningen University and Research Centre. Several antibiotics are being intensively used in *Pangasius* catfish farms in the Mekong Delta region (Vietnam) for the treatment and prevention of bacterial disease outbreaks, posing a potential risk for surrounding tropical aquatic ecosystems. The objective of the present study was to assess the short-term effects of the antibiotic enrofloxacin and its main metabolite ciprofloxacin on tropical aquatic organisms of three different trophic levels: the green-algae *Chlorella sp.*, the invertebrate *Moina macrocopa*,

and the Nile tilapia (*Oreochromis niloticus*). Acute toxicity tests were performed with enrofloxacin and ciprofloxacin on *Chlorella sp.* (72h – growth inhibition test) and *M. macrocopa* (48h - immobilization test) according to standard test protocols adapted to tropical conditions. The toxic effects of the selected antibiotics on Nile tilapia were evaluated by assessing the cholinesterase (ChE) and catalase (CAT) activities in the fish brain and muscles, respectively. Two different routes of antibiotic exposure were considered: feed exposure and water exposure, for a period of 5 days. Samples of the fish brain and muscle were obtained on day 3 and 5 after the start of the experiment and on day 9 after the end of the exposure period. The results of the toxicity experiments showed that enrofloxacin had a higher toxicity for *M. macrocopa* than for *Chlorella sp.* with an EC50 of 69.1 and 111 mg/L, respectively, whereas the opposite was observed for ciprofloxacin (EC50 *Chlorella sp.* = 23.4 mg/L and EC50 *M. macrocopa* = 71.2 mg/L). Nile tilapia exposed to high concentrations of the studied antibiotics (5 g/kg feed) showed an increase in ChE activity three days after the start of the exposure period, and partially recovered to the control levels five days after the start of the exposure period. This trend potentially indicates a physiological adaptation to the antibiotic stress. CAT activity decreased significantly after 5 days of exposure in the water (0.4 and 50 mg/L) and in the feed (1 and 10 mg/kg of feed) exposure experiments. Since the measured and modelled environmental concentrations of these antibiotics are typically in the range of µg/L, the results of this study suggest that i) the use of antibiotics is not likely to result in short-term risks for non-target aquatic populations of *Chlorella sp.* and *M. macrocopa*, and ii) ChE and CAT biomarkers in fish are probably not the most suitable tool to monitor ecological effects of these antibiotics in the field.

**TUPC12 Initial results from high-volume air sampling of industrial organic contaminants at Cape Verde, Africa.** M. Nordum, University of Oslo / Department of Environmental Chemistry; K. Breivik, Norwegian Inst for Air Research; S. Eckhardt, Norwegian Institute for Air Research / Atmosphere and Climate Change Department; M. Schlabach, A. Backlund, Norwegian Institute for Air Research. Polychlorinated biphenyls (PCBs) and some halogenated flame retardants (HFRs), notably selected polybrominated diphenyl ethers (PBDEs) are industrial organic chemicals identified as toxic, bio-accumulative, persistent and subject to long-range atmospheric transport. After bans on production and use, reductions in atmospheric burdens of these chemicals are now typically observed in some industrialized countries in the northern hemisphere. However, elevated air concentrations of PCBs and HFRs have recently been reported off the coast of West Africa, with some of the measured air concentrations of PCBs simply being far too high to be rationalized by historical usage of these chemicals in this area. It has been hypothesized that the sources could be releases of PCBs and HFRs from contaminated wastes exported to African countries from former production and use regions like Europe and USA. The primary objective of this study is to track atmospheric sources and source regions of selected industrial organic contaminants in tropical regions implicated as recipient of relevant wastes, using West Africa as a case study. For this purpose, an active high-volume air sampler was recently installed on The Cape Verde Atmospheric Observatory (CVAO: 16.848°N, 24.871°W), a World Meteorological Organization (WMO) global station at Cape Verde outside the coast of West Africa. Weekly 24 hour sampling commenced in May, 2012 and data from the first 12 months will be used to establish a baseline without any *a priori* consideration of the origin of air masses. Here, we present initial results from the first samples which have been collected, and compare these with data from other background monitoring sites. Potential source regions affecting these measurements are next evaluated using the Lagrangian particle dispersion model FLEXPART. The FLEXPART model is additionally used to forecast episodes with transport from the West African continent to allow for a more rational, targeted sampling strategy towards suspected source regions. Finally, the model will be used to retrospectively evaluate the principal source regions leading to elevated observed concentrations of PCBs and HFRs in air at Cape Verde.

**TUPC13 Brominated and alternative flame retardants in precipitation and air samples from the Ugandan part of Lake Victoria watershed, East Africa** K. Arinaitwe, Makerere University / Chemistry Department; D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division; B.T. Kiremire, Makerere University / Department of Chemistry; P. Fellin, Airzone One Inc; C. Teixeira, Environment Canada; H. Li, Airzone One Inc. Precipitation (11 samples taken monthly) and high volume (24hr) air samples (56 samples taken weekly) were collected very close to the shore of Lake Victoria at Entebbe, Uganda, between October 2008 and July 2010 inclusive. The samples were extracted by accelerated solvent extraction and analyzed for PBDEs and alternative flame retardants (FRs) by GC-negative ion MS. BDEs 47, 99, and 209 were the predominant PBDEs with mean concentrations (in air) of 9.8, 8.2, 4.2 and 2.9 pg/m<sup>3</sup>, respectively and mean fluxes (in precipitation) of 3.24, 5.06 and 13.6 ng/m<sup>2</sup>/sample. Profiles of octa- and nona-BDEs supported atmospheric degradation of BDE-209 and indicated that elevated levels in 2010 were most likely due to fresh emissions. BTBPE, HBCD *anti*- and *syn*-Declorane plus were detected in both air and precipitation at levels comparable with those of penta? PBDEs. Generally, both PBDEs and AFRs in air increased from 2008 to 2010. Backtrajectory analysis showed that elevated PBDE levels were associated with slow moving low altitude air masses from the immediate watershed of the lake while low levels were mostly associated with fast moving westerly and south westerly air masses. BDE 183, 153, 154, 209 and 47 showed weak to moderate significant correlation with the PAHs measured in the same samples. BDE-183 had the strongest correlations with most PAHs, notably, pyrene, Benzo(b&j)fluoranthene and Benzo(e)pyrene whose correlation coefficients (r = 0.571, 0.667 and 0.603 respectively) with BDE-183 were very significant (Bonferroni adjusted probabilities p ? 0.001 at 95% confidence). This suggested combustion source influence on the profiles of Deca-BDE and Octa-BDE. The presence of octa- and nona-BDEs suggested atmospheric photo-debromination of Deca-BDE. This is the first study to report flame retardants in high volume air samples and precipitation in the East African region and constitutes a baseline reference dataset for future atmospheric monitoring of flame retardants in the region.

**TUPC14 Residues of DDT and its metabolites in food and environmental samples of Bangladesh** D.R. Mamun, University of Dhaka / Chemistry; M. Shueb, N. Nahar, University of Dhaka / Department of Chemistry. Considering the adverse effects on human health and environment, the use and production of DDT were banned in Bangladesh in 1993 and the factory producing DDT in Chittagong Chemical Complex (CCC) was closed down. However, several reports say that DDT is still being used illegally in the country and unused DDTs are deposited in few places without proper management for disposal. In addition, the management of pesticide during and after use is very poor and pesticide Ordinance and Regulations are very old in the country. In order to assess the level of DDTs, two popular small fishes (n=10), three large size fishes (n=30) in two different seasons, forty broiler chicken meats (from four different poultry farms) and soil samples (n=77) from the DDT factory area in CCC were analyzed. Fish and meat samples were extracted by solid dispersion method using ethyl acetate whereas the soil samples by solid-liquid extraction method using a mixture of cyclohexane and acetone(1:1). All the extracts were cleaned up with conc. sulphuric acid and were analyzed by GC-ECD and GC-MS. DDT and its metabolites were found in varying amounts in all the fish and meat samples. The amounts of DDTs in these samples were below the MRL value (5.0 ppm), however, regular consumption of DDT contaminated fish and meat can affect the human health. Amount of DDTs were found in the soil samples of the DDT factory area is alarming for the ecosystems and for the people living around. Results will help the government to take necessary measures of the CCC area and safe living condition of the former workers who are still living in the contaminated area.

**TUPC15 Chlorinated and brominated pollutants in African Penguin (*Spheniscus demersus*) eggs** H. Bouwman, NorthWest University;

D. Govender, SANParks; L. Underhill, University of Cape Town; A. Polder, The Norwegian School of Veterinary Sciences. Levels of pollutants in penguin eggs from the Antarctic are relatively well known. However, it has been about 30 years since an assessment has been made of pollutants in the African Penguin (*Spheniscus demersus*) (de Kock & Randall, 1984). We collected 10 eggs each from Robben Island near Cape Town and from Bird Island near Port Elizabeth, about 840 km to the east by coast. For HCB, ?HCH, ?chlordanes, ?DDT, mirex, PCBs, ?toxaphenes and PBDEs, the mean levels from Robben Island were always lower than from Bird Island. For ?HCH, ?DDT, mirex, ?PCBs and ?toxaphenes, the differences were significant (t-test; p< 0.05). Mean ?DDT were 310 ng/g lipid at Robben Island, and 630 lipid at Bird Island. PCBs were 520 ng/g lipid at Robben Island, and 800 ng/g lipid at Bird Island. ?PBDEs were 13 ng/g lipid at Robben Island, and 25 ng/g lipid at Bird Island. HBCD was detected in only one egg from Bird Island (2.5 ng/g lipid), and seven from Robben Island (mean 1.5 ng/g lipid). The reasons for the generally higher levels in eggs from Bird Island are unknown. Bird island is closer to areas where DDT is sprayed, but the %DDT at both were very low (1.1% at Robben Island and 1.8% at Bird Island) and significantly different (p< 0.001). At both islands, industry and agriculture are significant. Data from the 1980s showed that ?DDT in African Penguins from Bird Island had a mean of about 750 ng/g lipid (slightly higher than current levels), and ?PCBs were about 3 000 ng/g lipid, much higher than current levels (de Kock & Randall, 1984). Levels in African Penguin eggs were generally higher than in Rockhopper Penguins from the Falklands for ?DDT (22 ng/g lipid), PCBs (28 ng/g lipid), and ?PBDEs (0.98 ng/g lipid) (van den Steen et al., 2011). Halogenated pollutants are still quantifiable in African Penguin eggs and higher on the east coast compared to Robben Island. Emerging pollutants such as PFOS should be considered in future analyses. De Kock AC, Randall RM. 1984. Organochlorine insecticide and polychlorinated biphenyl residues in eggs of coastal birds from the Eastern Cape, South Africa. Environ. Pollut. (Series A) 35:193-201. van den Steen E, et al. 2011. Organohalogenated contaminants in eggs of rockhopper penguins (*eudyptes chrysocome*) and imperial shags (*Phalacrocorax atriceps*) from the Falkland Islands. Sci. Tot. Environ. 409:2838-2844.

**TUPC17 The effects of pH change upon signaling molecules- a new potential threat to marine life?** R. SAURABH, The University of Hull / Chemistry; M. Lorch, University of Hull; D. Benoit, University of Hull / Department of Chemistry; J. Hardege, Hull University / Biological Sciences; C. Hartley, The University of Hull; D. Lucas, University of Hull; P. Bell-Young, University of Hull / Biological Science; H. Bartels-Hardege, The University of Hull. Carbon dioxide emissions are predicted to decrease the world's oceans pH from currently 8.1-8.2 to 7.7-7.8, a rapid change that is unprecedented in evolutionary terms. There is now increasing evidence that marine organisms face significant problems in adapting to the changing environmental conditions brought about by the increase of dissolved carbon dioxide (CO<sub>2</sub>). In most marine organisms a range of behaviours are controlled via chemical cues, such as homing, predator detection and mating, and there is increasing evidence that lowering the seawater pH alters animal's responses to these cues. This may be due to physiological stress affecting signal reception as a result of changes in charge and structure of the signaling molecules. This scenario seems likely given that the acid dissociation constants (pKa) of many signal molecules fall within the pH range that is potentially affected by ocean acidification. Here we studied some of the few chemically characterized chemical cues from polychaetes and crustaceans to evaluate whether the signal molecules themselves are affected, and how this impacts upon signal detection and animal responses. Our data show changes in keto-enol tautomerization, partial charge distribution and pKa dependent conformational changes in the shape of these peptide and nucleotide signaling molecules. We predict that the majority of signaling molecules will be affected placing substantial ecological pressures upon marine life.

**TUPC18 Impacts of ocean acidification on chemical cue controlled reproduction in a marine polychaetes, *Platynereis dumerilii* and**

**Neanthes succinea.** L. Davidson; C. Hartley, The University of Hull; J. Hardege, Hull University / Biological Sciences; H. Bartels-Hardege, The University of Hull. Semelparous organisms with once in a lifetime reproductive events rely upon crucial timing. Nereidid worms use environmental and endocrine cues to ensure coordinated maturation and precise determination of the time and location of mass spawning events. Chemical signalling via sex pheromones then coordinates their reproductive behaviours that induce the 'nuptial dance' reproductive behaviour and the subsequent release of gametes. Pheromones in nereidids include diverse molecules such as volatile lipophilic organic acids and small peptides. In recent years, concern has arisen over changing ocean carbonate chemistry as a result of oceanic uptake of anthropogenic carbon dioxide (CO<sub>2</sub>). This study evaluates how successful development to maturity and the ability to successfully utilize sex pheromones of Nereidid polychaetes are potentially affected. When exposed to pH levels forecasted to occur at ca 2100 (pH 7.8) worms show reduced survival rates in addition to their ability to reach the sexually mature heteronereis stage. Where maturation occurs, individuals show a dramatically reduced response to natural as well as synthetic peptide sex pheromones with significant reduction in fertilisation and larval success rates. In the oceanic environment where olfactory cues are widespread, such results indicate disruption to the sensory ability of marine invertebrates may potentially impact upon essential life processes.

**TUPC19 Signal production versus detoxification- balancing homeostasis under climate change conditions** M. SHAFI, The University of Hull / Biology; R. SAURABH, The University of Hull / Chemistry; J. Hardege, Hull University / Biological Sciences. Detoxification of metals pose significant biochemical costs for individuals, but little is known about the physiological consequences of detoxification stress, and how cumulative stress such as climate change impacts upon animal fitness. The ability to produce and react upon chemical cues may form a potential endpoint biomarker for assessing such stressors. We used Nereidid polychaetes, *Neanthes succinea* to show how exposure to ZnCl<sub>2</sub> affects the tissue content of glutathione (GSH), the precursor of the worms sex pheromone Cysteinyl-glutathione (CSSG). Exposing clamworms for 30 days to ZnCl<sub>2</sub> we found the polychaetes capable of keeping the ZnCl<sub>2</sub> levels in the body similar independent of exposure levels highlighting their ability of homeostasis of metals. ZnCl<sub>2</sub> levels in the sediment and seawater increased significantly suggesting active excretion of ZnCl<sub>2</sub>. GSH levels in worms decreased with increasing toxicant exposure concentration and these effects exaggerated when worms were kept under climate change conditions (reduced pH, increased temperature). Oocytes from GSH depleted females show significantly decreased fertilization, and larval survival rate success leading to significantly decreased numbers of trochophora larvae within 24 hours of fertilization. Our data confirm a trade off for costly biochemical resources between detoxification and the upkeep of essential biological functions such as reproductive fitness such as pheromone production that may exist in many other stress situations in marine species, a balance that may well be under severe pressures from climate change.

**TUPC20 Interactive effects of xenobiotic, abiotic and biotic stressors on *Daphnia pulex* – results from a multiple stressor experiment** Scherer, Aquatic Ecotoxicology; A. Seeland, J. Oehlmann, R. Mueller, Goethe University Frankfurt/ Main. Pollutant effects on aquatic key species are confounded by multiple abiotic and biotic stressors. To better discriminate and understand the intrinsic and environmental correlates of changing aquatic ecosystems, we untangle in present study how the effects of a low-dosed fungicide on daphnids (via different exposure routes) becomes modified by increasing temperature and the presence of a predator. Using a fractional multifactorial test design, the individual growth, reproduction and population growth rate of *Daphnia pulex* were investigated under exposure to the fungicide pyrimethanil at an environmental relevant concentration - either directly (via the water phase), indirectly (via food), dually (via water and food) or for multiple generations (fungicide treated source population) - at three temperatures

and in presence/absence of the predator kairomones of *Chaoborus flavicans*. Our results clearly illustrate that multiple stress factors can modify the response of an aquatic key species to pollutants. The exposure route of the contaminant is of minor importance, while temperature and the presence of a predator are the dominant factors controlling the reproduction of *D. pulex*. We conclude that sublethal pyrimethanil pollution can disturb the zooplankton community at suboptimal temperature conditions, but the effects will become masked if chaoborid larvae are present. **Key words:** fungicide (pyrimethanil), climate change, daphnids, kairomones, multiple stressors

**TUPC21 Potential endpoints and test systems in screening for infochemical effects** E. von Elert, University of Cologne, Biocenter Cologne; G. Vella, C. Effertz, P. Fink, University of Cologne; R. Berghahn, Federal Environmental Agency. Three different test systems, which are so far unknown for use in aquatic toxicology, are most promising in screening of substances for potential infochemical effects, namely the tube system for testing effects of chemicals on the diel vertical migration of zooplankton in particular of daphnids (Loose et al. 1993), the snail test (Fink et al. 2006), and the carousel drift meter of Werth (2006, see Berghahn et al. 2012). After a brief introduction of the test methods and the endpoints first lessons learned with the test systems and selected chemicals, which are most likely to have infochemical effect features, will be presented.

**TUPC22 A critical comparative analysis of chemosensory effects of metals in fish** R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology; A. Fairbrother, Exponent Inc / EcoSciences. Many metals interfere with the fish olfactory system, however, there are important differences in the internal processing between metals so potential effects cannot be described by one common mechanism. In addition to binding to the olfactory epithelium and accumulation in the olfactory bulb, metals interfere with neuroendocrine and endocrine processes in the brain. Metals also affect the mechanosensory system of ciliated hair cells along the body axis of the fish that respond to physical displacement of water. Water chemistry including complexation and major ions alter metal effects on the olfactory system as could be expected from the free ion activity model, although acclimatisation and adaptation play also an important role. Most olfactory toxicity studies use short-term exposure regimes, while under natural conditions exposure is of a more permanent nature. Fish that spend multi-generations in contaminated environments do not seem to show the same negative olfactory responses to metals as laboratory-reared fish, suggesting that caution should be taken when extrapolating results from the laboratory to the field. A species sensitivity approach was adopted to compare the sensitivity of chemosensory responses with other acute and chronic responses in fish and other species to determine the relative sensitivity of the chemosensory responses to other ecologically relevant thresholds. Although some other aquatic species and processes included in setting water criteria appear to be more sensitive than fish olfaction to metal exposure, multispecies field tests may be needed to appropriately test behavioral thresholds.

**TUPC23 U.S. EPA biotic ligand model-based aquatic life criteria are protective against copper-caused impairment of olfaction in salmonid fishes** J.S. Meyer, ARCADIS; D.K. DeForest, Windward Environmental LLC; R.W. Gensemer, GEI Consultants / Ecological Division; B.K. Shephard, US EPA / Office of Env. Assessment (OEA-095); J. Zodrow, ARCADIS US Inc; J.W. Gorsuch, Copper Development Association Inc; W. Adams, Rio Tinto. Major concerns have recently been expressed by regulators of west coast states in the USA that short-term exposures to low Cu concentrations might impair olfaction and/or behavior in fish [especially migratory Pacific salmon and trout (*Oncorhynchus* spp.)], which might limit their ability to detect predators, reproduce, or migrate. However, water chemistry matters in determining lethal and sublethal effects (including olfaction) of metals to aquatic organisms. For example, although olfactory impairment can occur at low Cu concentrations in laboratory waters having low ionic



strength and low dissolved organic carbon concentrations, we demonstrate that the U.S. Environmental Protection Agency's (USEPA's) biotic ligand model (BLM)-based aquatic life criteria for Cu are protective against olfactory impairment in salmonid fishes across a wide range of fresh waters. In addition, even the USEPA's hardness-based freshwater Cu criteria would have been protective against olfactory and behavioral impairment in many of those waters. Similarly, the USEPA's current marine Cu criteria would have been protective against Cu-caused behavioral impairment in saltwater. Although no olfactory-impairment data have been published for saltwater fish exposed to Cu, a proposed unified freshwater-saltwater BLM developed to predict olfactory effects of Cu to salmonid fishes suggests that the threshold-effect concentrations of Cu will be even higher in salt water than in fresh water and that the USEPA's current marine Cu criteria will be protective across a wide range of saltwater chemistries. Limitations of study designs and interpretations of results will also be discussed.

**TUPC24 Disruption of info chemicals in marine organisms – a major threat to marine life?** J. Hardege, Hull University / Biological Sciences. The use of chemical signals to coordinate animal behaviour is widespread in the marine ecosystem, but very little is known on how chemical exchange of information between organisms is potentially affected by human activities. Disruption of essential behaviour such as predator – prey interactions or mating can potentially have dramatic effects on individuals and entire ecosystems. Successful utilisation of chemical cues can be affected at various levels from inhibited signal production, to transmission through the environment, signal detection and reception to reduced or altered responses by the receiver. Here I provide an overview of our recent studies that utilized *Nereidid* polychaetes and crustaceans as model systems. Using synthetically available peptide, thiol and nucleotide sex pheromones and feeding stimulants we examined environmental stressors such as endocrine disruption, heavy metal detoxification and reduced pH through ocean acidification. Our data show evidence for signal disruption at the level of cue production, structural changes of the signaling molecules, reduced and changed detection, and altered behavioural responses. We conclude that signal disruption associated with rapid climate change and ocean acidification is likely to become a major threat to marine life and much more studies are needed to evaluate marine organisms' ability to acclimatize and adapt to this threat.

**TUPC25 Effect of particle size and coating materials on silver nanoparticles (AgNPs) settling in model water** M. Jang, Future Environmental Research Center; S. Bae, Korea Institute of Toxicology; Y. Lee, Future Environmental Research Center; S. Lee, Korea Institute of Toxicology / Ecotoxicology Laboratory. Estimating the exposure of engineered nanomaterials in the environment is essential for their risk assessment. Because nanoparticles tend to aggregate, the main processes determining their fate and transport are aggregation and sedimentation. The aim of this study is to better understand the aggregation and sedimentation behaviors of nanoparticles in aquatic environment, with the goal of estimating the effect of particle size and type of coating materials on both behaviors. We measured the particle size distributions and time dependent concentrations of citrate coated silver nanoparticles (citrate coated AgNPs, average size of 10 nm) and PVP-coated silver nanoparticles (PVP coated AgNPs) in three different average size (50, 100, 160 nm). With the increase in ionic strength (CaCl<sub>2</sub>), the size of citrate coated AgNPs were rapidly increased due to aggregation, and thus the rate of sedimentation was also increased. On the other hand, the size of PVP-coated AgNPs were not changed with the increase in ionic strength. This is likely due to steric hindrance effects of PVP coating layer. However, concentrations of PVP-coated Ag NPs were decreased with time, depending on the particle size. A large fraction of PVP-coated Ag NPs with the smallest particle size (50 nm) remained suspended, but most of Ag NPs with the biggest particle size (160 nm) were settled after 7 day of settling. These results suggest that the size of nanoparticles and type of coating materials play an important role in determining their fate and transport.

**TUPC26 Analysis of nano-TiO<sub>2</sub> released from sunscreens in real-world samples: a feasibility study using spICPMS** R. Reed, Colorado School of Mines / Department of Chemistry and Geochemistry; A. Gondikas, University of Vienna / Department of Environmental Geosciences; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; F. Von der Kammer, Vienna University / Department for Environmental Geosciences; T. Hofmann, University of Vienna / Environmental Geosciences. Incorporation of nano-TiO<sub>2</sub> into commercially available sunscreens creates the potential for release<sup>2</sup> to the environment during typical use and exposure of these materials to organisms. Due to the low (ng/L) surface water TiO<sub>2</sub> concentrations predicted in the environment and the elevated background of natural particles, detection and quantification of these materials is not feasible by widely used methods such as electron microscopy or light scattering. The feasibility of single particle inductively coupled plasma mass spectrometry (spICPMS) was investigated for analysis of nano-TiO<sub>2</sub> in sunscreens and samples from a natural water body. Previous research has shown spICPMS to be a highly sensitive element-specific method capable of detecting ng/L concentrations for many metal nanoparticles, including TiO<sub>2</sub> in simple systems. Analysis of a reference nano-TiO<sub>2</sub> material (NM-104, OECD) by spICPMS was performed to optimize<sup>2</sup> a method for the size-dependent characterization of TiO<sub>2</sub> nanoparticles, with comparison of sizing results to sedimentation field-flow fractionation with light scattering detection. This method was then applied to analysis of TiO<sub>2</sub> in natural surface waters. To investigate the occurrence of nano-TiO<sub>2</sub> release from sunscreens in real-world systems, water samples were taken from bathing areas along the Old Danube in Vienna and analyzed by spICPMS for particle number concentrations and size distributions. This sampling site is a still-water lake which is used by thousands of bathers per day during the summer months, making it ideal for determining real-world exposure data for nano-TiO<sub>2</sub> released from sunscreens into natural waters due to bathing activities.<sup>2</sup> Water samples were collected and analyzed throughout the year to determine background levels of Ti due to natural inputs outside of the summer bathing season as well.

**TUPC27 Impact of silver nanoparticles on the microbial community of municipal wastewater treatment system** K. Kasemets, Nat Inst of Chemical Physics Biophysics / Laboratory of Molecular Genetics; S. Suppi, A. Kahru, National Institute of Chemical Physics and Biophysics; H. Nõlvak, M. Truu, J. Truu, University of Tartu. Nanotechnology is one of the fastest growing and most promising areas of the society. The number of consumer products on the market containing nanoparticles (NPs) already exceeds 1000 (www.nanotechproject.org). The NPs released from different nano-products used in the industry as well as in our household will end up in the wastewater treatment plants and wastewater sludge. The extent to which nanomaterials may influence the wastewater system microbial community and wastewater treatment efficiency is still unknown. Of the existing nanomaterials silver nanoparticles (AgNPs) have the highest degree of commercialization, especially in antimicrobial applications, but data on their potential hazards are still rare and mechanism of toxic action only partially understood. The current study focuses on the toxicity assessment of AgNPs to the microbial community of wastewater treatment system. Three different AgNPs were studied: non-coated (30–100 nm), polyvinylpyrrolidone (PVP; 11–30 nm) or protein (5–30 nm) coated AgNPs (collargol). AgNO<sub>3</sub> was used as ionic control in the toxicity studies. Wastewater was sampled from Nõo municipal wastewater treatment plant, Estonia. The toxic effect of the AgNPs/ions on the wastewater microbial community was evaluated by incubating the wastewater samples with AgNPs/ions at ambient temperature up to 11 days. The viability of exposed microbial community was evaluated by plating and counting of the colonies on the Plate Count Agar. From these data IC<sub>50</sub> (mg Ag/L) were calculated. The CFU (colony forming units) in non-exposed wastewater was 2.8–3.8 × 10<sup>7</sup>/mL. The IC<sub>50</sub>-values of the studied Ag-compounds ranged from 3–50 mg Ag/L and the order of the toxicity was as follows: AgNO<sub>3</sub> > PVP-AgNPs > non-coated AgNPs > collargol (protein-AgNPs). The agglomeration and solubility of the AgNPs in the wastewater will be studied by the dynamic light

scattering (Zetasizer Nano ZS, Malvern Instruments), metal-specific bioluminescent Ag-sensor bacteria and AAS, respectively. To study the modulating effect of the suspended solids on AgNPs physico-chemical properties non-filtered and filtered (0.22 µm) wastewater samples will be analysed in parallel. Changes in microbial community structure at sub-toxic and toxic level of AgNPs/ions will be assessed using quantitative PCR and next-generation sequencing approach. This work is supported by the State program „Aid for research and development in environmental technology“ grant 3.2.0801.11-0026, SF0690063s08, SF0180127s08, ETF9001 and EU 7<sup>th</sup> Framework Programme under grant agreement n°263147 (NanoValid).

**TUPC28 Considerations on the EU definition of a nanomaterial: science to support policy making** E.A. Bleeker, RIVM; W. de Jong, R. Geertsma, M. Groenewold, E. Heugens, M. Koers-Jacquemijns, D. van de Meent, J. Popma, A. Rietveld, S. Wijnhoven, F. Cassee, A. Oomen, National Institute for Public Health and the Environment (RIVM). In recent years, an increasing number of applications and products containing or using nanomaterials have become available. This has raised concerns that some of these materials may introduce new risks for humans or the environment. A clear definition to discriminate nanomaterials from other materials is prerequisite to include provisions for nanomaterials in legislation. In October 2011 the European Commission published the 'Recommendation on the definition of a nanomaterial', primarily intended to provide unambiguous criteria to identify materials for which special regulatory provisions might apply, but also to promote consistency on the interpretation of the term 'nanomaterial'. In this presentation, the current status of various regulatory frameworks of the European Union with regard to nanomaterials is described, and major issues relevant for regulation of nanomaterials are discussed. This will contribute to better understanding the implications of the choices policy makers have to make in further regulation of nanomaterials. Potential issues that need to be addressed and areas of research in which science can contribute are indicated. These issues include awareness on situations in which nano-related risks may occur for materials that fall outside the definition, guidance and further development of measurement techniques, and dealing with changes during the life cycle.

**TUPC29 Predicting the contribution of three types of nanoparticles (Zn, Ti and Ag) to the contamination of Rhine and Meuse in the Netherlands** A. Markus, University of Amsterdam / IBED-ESS; R. Laane, DELTARES; J. Parsons, University of Amsterdam / IBED-ESS; E. Roex, G. Kenter, DELTARES. While nanoparticles are used in many commercial products, the released amounts, the environmental concentrations and the risks they may pose to the environment and to human health remain largely unknown. One reason is that it is mostly unknown how much nanomaterials is used in these products. In addition there are currently no quantitative techniques to identify and measure the amount of nanomaterials under environmentally relevant circumstances. The work we report here is part of a project that aims at developing a mathematical model for the transport and fate of nanoparticles. We calculate the potential releases within the Netherlands of nanoparticles of ZnO, TiO<sub>2</sub> and Ag and compare them to the total loads in two major Dutch rivers. We were able to make at least some estimates of the total use and therefore the potential release into the environment from the available information. These results are compared to the annual averaged riverine load. The worst-case calculations (no retention) indicate that nanomaterial contributes a relatively small fraction (5 to 20%) to the total load of zinc and titanium. For silver the contribution is at most 3%. The contribution of the nanoparticles to the metal concentration is close to the minimum that can be detected, given the variability in the measured concentration.

**TUPC30 Biotransformation of <sup>14</sup>C radio-labelled carbon nanotubes via enzymatic oxidation** D.X. Flores-Cervantes, Forschungsanstalt Agroscope Reckenholz Tänikon ART; H.M. Maes, RWTH Aachen / Institute for Environmental Research; A. Schaeffer, RWTH Aachen

University / Chair of Environmental Biology and Chemodynamics; J. Hollender, Eawag / Dept Environmental Chemistry; H. Kohler, Eawag; Swiss Federal Institute of Aquatic Science and Technology. The worldwide production capacity of carbon nanotubes (CNT) is estimated to exceed 12,800 metric tons by 2016 (IRP, 2011 [1]). At such high production rates the presence of these materials in the environment is an eventuality. However, there is still much uncertainty regarding the fate and transport of CNTs once released into the environment, and we have even less information regarding the possible biotransformation pathways that CNTs might undergo due to biological processes. Due to the recalcitrant nature of CNTs, we expect these materials to be persistent in the environment and biotransform at very slow rates. Nevertheless, there has not been any previous quantification of the extent of CNT biotransformation. In this study we subjected <sup>14</sup>C-radiolabelled CNTs to oxidative degradation via horseradish peroxidase, and measured both mineralization rates via <sup>14</sup>C-CO<sub>2</sub> and increases in dissolved concentrations of <sup>14</sup>C (indicative of dissolved biotransformation products). Our results show linear mineralization rates during ten-day incubation experiments and indicate that only 0.01% of the initial material was mineralized. However, no dissolved <sup>14</sup>C carbon was detected. These results are consistent with observations from previous experiments in our lab where biotransformation rates of non-labeled CNTs subjected to HRP oxidation were below 0.03%, and where no dissolved biotransformation products were detected via HPLC-HRMS. Further tests with longer incubation periods and different enzymatic systems will provide additional information regarding the fate of CNTs in environmental settings. [1] IRP, Innovative Research Products. February 2011. Production and Applications of Carbon nanotubes, Carbon nanofibers, Fullerenes, Graphene and Nanodiamonds: A Global Technology Survey and Market Analysis. [http://www.innoresearch.net/report\\_summary.aspx?id=77&pg=531&rcd=ET113&pd=2/1/2011](http://www.innoresearch.net/report_summary.aspx?id=77&pg=531&rcd=ET113&pd=2/1/2011)

**WEPC01 LCSA for energy policy design: hype or hope?** M. Stefanova, A. Zamagni, O. Amerighi, P. Buttol, ENEA; P. Masoni, ENEA / Protezione e Sviluppo dell'Ambiente e del Territorio. The World Energy Outlook 2012 stresses, "Taking all new developments and policies into account, the world is still failing to put the global energy system onto a more sustainable path". At European level, the situation looks particularly complex, due to the dependency of Europe on imported fossil fuels of 55% (compared to 20% dependency of US), with 60% gas and 80% oil dependency. Within the current increasingly complex socio-economic and environmental context, the policy-makers are facing additional pressures in looking for simultaneous progress toward energy security, competitive growth, creation of new economic opportunities and environmental concerns and often need to consider contradictory choices. The existing tools provide substantial support for designing and evaluating energy-related policies aimed at cost-effective reduction of GHGs. Increasing concerns about policy options, which solve one problem while introducing others are leading to different model coupling approaches. Recent trends are toward an integration of the energy system modelling for policy makers with LCA-based approaches and methods. There are expectations that LCA-based methods could be turned into a new form of bottom-up integrated model assessment tools. In this paper we discuss current approaches in energy policy design in light of the Life Cycle Sustainability Framework. We found that despite several methodological progress in linking micro-meso-macro dimensions and in considering all three pillars of sustainability (environment, economy, and society), the existing approaches fail to accommodate aspects related to the organisational structure of local socio-economic contexts into which the energy technologies are inserted. This might be precluding policy makers from finding alternative sustainable configurations of the future energy systems.

**WEPC02 Application of Multiregional Input-output model to assess CO<sub>2</sub> emissions embodied in international trade: the case of Spain** E. Gemechu, Universitat Rovira i Virgili; I. Butnar, Universitat Rovira i Virgili / Chemical engineering department; S. Suh, University of

California / School of Environmental Science & Management; M. Llop, Universitat Rovira i Virgili / CREIP, Departament d'Economia; F. Castells, Universitat Rovira i Virgili / Chemical engineering department.

As a result of globalization and free trade agreements, international trade is enormously growing and inevitably putting more pressure on the environment over the last few decades. This has drawn the attention of both environmentalist and economist in response to the ever growing concerns of climate change and urgent need of international action for its mitigation. In this work, we aim at analyzing the implication of international trade in terms of CO<sub>2</sub> between Spain and its important partners using a multi-regional input-output (MRIO) model. A fully integrated 13-region MRIO model is constructed to examine the pollution responsibility of Spain both from production and consumption perspectives. The empirical results show that Spain is a net importer of CO<sub>2</sub> emissions, which is equivalent to 29% of its emission due to production. Even though the leading partner with regard to import values are countries such as Germany, France, Italy and Great Britain, the CO<sub>2</sub> embodied due to trade with China takes the largest share. This is mainly due to the importation of energy intensive products from China coupled with Chinese poor energy mix, which is dominated by coal-power plant. The largest portion (67%) of the global imported CO<sub>2</sub> emissions is due to intermediate demand requirements by production sectors. Products such as Motor vehicles, chemicals, a variety of machineries and equipments, textile and leather products, construction materials are the key imports that drive the emissions due to their production in the respective exporting countries. Being at its peak in 2005, the Construction sector is the most responsible activity behind both domestic and imported emissions.

#### **WEPC03 Technical framework for using partial and general equilibrium modelling results in the LCA of future energy scenarios**

**B. Rugani**, University of Siena / Resource Centre for Environmental Technologies (CRTE); E. Igos, S. Rege, E. Benetto, CRP Henri Tudor (CRPHT) / CRTE. At country level, energy production and supply is intrinsically related to political and socio-economical dimensions, with strong dependence on the availability and cost of fossil fuels. In the framework of the research project LUXEN, the CRP Henri Tudor, the Luxembourgish statistics institute STATEC and the Luxembourgish Ministry of Sustainable Development and Infrastructures (MDDI) are working on the integrated assessment of energy scenarios for Luxembourg. The focus is to assess economic and environmental consequences of possible future scenarios of energy supply and consumption. Indeed, Luxembourg is a highly developed country that faces an increasing energy demand, where energy sources and fuel provisions are mainly imported. The present communication is aimed at detailing the LUXEN integrated energy assessment framework, with special focus on the combination between process based lifecycle inventory and Input-Output datasets obtained from the combination of partial and computable general equilibrium models. First, we forecasted future energy demand and supply and the consequences on the Luxembourgish economy. This was performed by combining the LUXembourg General Equilibrium Model (LUXGEM) (used to derive economic drivers) and the Energy Technology Environment Model (ETEM) of Luxembourg (used to derive final energy consumptions satisfying the updated demands). Based on this coupling, the environmental impacts engendered on the national economy have then been evaluated. IO tables in time-series (from 2005 to 2009) were extended with environmental satellite accounts both at the domestic production and import levels. Specific EE-IO tables were thus created and further disaggregated within the energy sector to meet the purposes of LUXEN. Indeed, the energy sector (ie, production and use) within the use table for imports as well as the domestic IO was replaced by the results of ETEM. The corresponding technologies were integrated in the EE-IO and modeled via the Ecoinvent database, where adjustments performed according to ETEM assumptions (ie, efficiency ratios and combustion emissions). The model was then spanned up to the year 2030 by using the outputs from the equilibrium models. In this assessment, special attention was put to distinguish inputs and outputs which are either imported, domestically produced or exported, allowing

for spatial and time-scale modelling of present and future environmental impacts due to the Luxembourg's consumption.

**WEPC04 A solution to easily take into account the contribution of technical systems and utilities in LCA of buildings** **M. Sié**, Cycleco; T. Rieser, Enertech; **J. Payet**, Cycleco. In order to foster LCA application in building sector it is of main importance to tailor made method and database and make them easy to use in conception. With this objective in mind, Cycleco developed for Bourgogne District and ADEME a Product Category Rules (PCR)-like document and a database of processes related to building. An ergonomic tool – e-LICCO – was also provided to make LCA compliant with the PCR-like and using the database. Even if e-LICCO substantially shorten the study time from several months to a few days, it is still not enough to bring LCA into general use for building professionals and decision-makers. One of the issues is the big amount of primary data to know at the beginning of the project, especially for technical systems and utilities. Cycleco and Enertech work along to develop ratios in order to make easy for the user to take into account these packages. First Cycleco developed around 300 set-up datasets using ecoinvent background database (conduit, pipes, wires, battery, air handling unit, sensors, lights...). Meanwhile, Enertech divided technical systems and utilities perimeter in 7 subsections: Boiler room, Heating distribution, Terminal emission and regulation, Ventilation, Plumbing and sanitation, Solar collective Sanitary Hot Water and Electricity. For each subsection they compiled inventories for 5 to 12 case studies. Package specifications and purchase orders have been dissected for this purpose. Then, Cumulative Energy Demand model has been used to assess primary energy impact category of each case study. In each subsection, the 5 to 12 inventories were placed in 3 classification systems: building typology (office, habitation...), technology (materials, fuel...) and system (radiators, underfloor heating...). Linear regressions were applied to inventories of the same building typology, technology and system. The purpose was to model the relationship between energy impact and one variable to be identified among building characteristics. In each subsection, the classification system allowing the best linear regressions was selected. Eventually 35 to 40 ratios have been developed. They allow description of technical systems and utilities of buildings using parameters as simple as surface or number of habitations. Several in-conception buildings were assessed and the true share of these packages to the global impact was appraised. Up to 20% contribution has been observed depending on building characteristics.

**WEPC05 LCA of storage scenarios for future energy systems** **L. Goellner-Voelker**, TU Darmstadt / Industrial Material Cycles; L. Schebek, Technische Universität Darmstadt / Industrial Material Cycles. In order to mitigate climate change, governments worldwide are committed to transforming today's energy systems from fossil based energy carriers to renewable energies. In contrast to conventional energy production, energy from renewable resources like wind and solar power is volatile and fluctuating in time and space. Future energy systems will be crucially dependent on supply of sufficient storage capacities for energy, notably electricity. However, storage technologies need resources and energy which are adverse effects as to increasing the efficiency of energy supply. Life Cycle Assessment (LCA) can be used to identify most feasible options as to environmental impact. A multitude of storage technologies accrues, where each technology has different characteristics as integration in the energy system. Therefore it is important not to consider a single technology (for example lithium batteries) but to analyze different energy storage systems and strategies in a whole. For a LCA for different energy storage systems, first a classification of the different energy storage systems has been developed. Based on this, energy storage systems in context of different future energy systems are analyzed which are characterized based on scenarios for energy policies. The combination of LCA and scenario analysis is outlined as well as first results for selected storage systems.

**WEPC06 Guidelines for Life Cycle Assessment of photovoltaics system in France** **J. Payet**, B. Evon, Cycleco; I. Blanc, Mines

ParisTech / Centre for Energy and Processes; D. Beloin-Saint-Pierre, Mines ParisTech; E. Raison, N. Adra, Transénergie; Y. Durand, ADEME. Since 2004, the French Code of Public Procurements allow including environmental criteria in call for tenders. The increasing demand for photovoltaic (PV) systems in France has encouraged the French government to better consider the environmental impacts of the PV sector by introducing Life Cycle Assessment (LCA) results in call for tenders. The French Environment and Energy Management Agency (ADEME) has mandated a working group to develop a methodological framework, in accordance with ISO 14040, allowing industry PV players to conduct a Life Cycle Assessment (LCA) of their systems. These LCA must be used for the selection of the PV projects. The benefits of such harmonised methodology are to give access to a common framework enabling consistent and reliable LCA of PV systems following the ISO standards. The development of such methodological framework involved the following steps: A review of the present state of art of environmental assessment of PV systems and existing guidelines dealing with product environmental footprint declaration in order to identify the relevant technological processes and key factors to consider. The identification of reference data as a basis for LCA comparisons (functional unit, system boundaries, product categories, life time, etc.) following the recommendations of the ISO 14040 standards. The development of a specific LCA impact database to produce default impact values and the development of default technological parameters to perform LCA. If users do not have all the information about their PV system, the default values are to be used. These default data are mainly conservative data to encourage users to provide their own data. These data are classified according to product categories linked to the nominal power of the entire installation. Most of the background data for the Life Cycle Inventory step come from ecoinvent 2.2. The impact methods to be implemented for impact assessment are defined by setting a relevant short list in relation with the PV sector and based on the ELCD impact indicators recommendations. Validation and expert judgement with stakeholders at the national level. This framework has been available for PV electricity providers and PV system installers key players since the beginning of 2013 and will be used to assess the environmental impacts of PV systems to support decision in French public policy related to photovoltaics.

**WEPC07 The Role of Life Cycle Assessment in Evaluating Alternatives for Electrification of Roads and Long Haul Trucks in Sweden** A. Nordelof, A. Bjorkman, M. Ljunggren Soderman, A. Tillman, Chalmers University of Technology / Environmental Systems Analysis, Department of Energy and Environment. In September 2012, the Swedish Government presented a 1.3 billion SEK investment for the years 2013-2016 targeted on improving the infrastructure for transportation of iron ore powder between the mine in Kaunisvaara and Svappavaara, where it is transferred to train. This decision was preceded by an official report from the Swedish Transport Administration investigating necessary road reconstruction along with alternatives for electrification of the vehicles used. As a result, a new road of 140 kilometers will be constructed and at the same time serve as test project for electric road technologies in Sweden, with the initial focus set on heavy duty trucks. Scania CV AB is the supplier of the 90 ton long haul trucks which will transport the iron ore powder, and also an active part in the rapidly evolving area of electric roads in Sweden. In August 2012, the company's Hybrid Systems Development Department initiated a life cycle assessment (LCA) on this case, set up in the form of a master's thesis. The LCA study compares three different drivetrain alternatives for the heavy long haul trucks. In essence, the cradle to grave cycle impact of a set of additional components is compared with the effect of the reduced total energy use of fuel and electricity in the well-to-wheel phase, for a conventional truck, a hybrid (made more efficient by integrating electric propulsion) and a hybrid with external power supply from the road. The aim of the study is to evaluate the environmental impact of the three alternatives and to demonstrate how it changes over the different life cycle phases. The motive of Scania has been to increase their knowledge of the environmental impacts of drivetrain electrification, and also to provide support for internal decisions and

future strategies on how to meet energy efficiency targets. The idea is also to present the results within the test project working group and thereby contribute to the overall project evaluation. The aim of this presentation is to point out that the increased governmental focus on electric roads in Sweden and Scania's need to understand the effect of this technology in an environmental systems perspective, has given LCA a role in the evaluation. Hence, the results and conclusions of the LCA will be presented. Finally, the opportunities and limitations of LCA as a learning tool when applied on this type of emerging technology at a department with no prior LCA experience will be discussed.

**WEPC08 Applying a rigorous streamlined LCA approach to achieving low carbon vehicles through lightweighting and closed-loop recycling** M. Raugei, Oxford Brookes University / Mechanical Engineering and Mathematical Sciences; A.R. Hutchinson, D. Morrey, Oxford Brookes University / Department of Mechanical Engineering and Mathematical Sciences. The effects of climate change are becoming increasingly apparent globally, and the automotive industry's current position as one of the most significant contributors to greenhouse gas emissions is looking less and less sustainable and acceptable to an environmentally aware society. Besides turning to less carbon-intensive power train options, one of the most effective strategies to cut down on carbon emissions is, unquestionably, to reduce the weight of the vehicles. Lightweight metal alloys and composite materials are being introduced by OEMs at an increasingly fast pace; yet, it is important that the development of new materials and manufacturing processes always be guided by sound life cycle thinking principles, lest the advantages afforded by such technological advances are diminished or even annulled by unforeseen counter effects. Specifically, it is paramount to avoid or at least minimize impact shifting among different life cycle stages (e.g. material supply chain vs. vehicle manufacturing) or geographical regions, and to achieve the highest possible recycling rates for all energy-intensive materials (through the use of suitably reversible assembly and bonding practices). At the same time, due to the complex nature of the modern passenger vehicle, as well as the intrinsic uncertainty that underlies all medium-to-long term prospective studies, performing LCAs of future vehicle bodies using current levels of detail is both resource-intensive, but also the levels of certainty and accuracy might not justify such a degree of granularity. It is for these reasons that, in the on-going 'Towards Affordable, Closed-Loop recyclable Low Carbon Vehicle Structures (TARF-LCV)' research project, life cycle assessment has been envisaged from the very start as taking a pivotal role in guiding and iteratively analysing the research carried out by the consortium partners in terms of technological advancements, by means of an *ad hoc* streamlined and carefully standardised approach. The latter, presented here, includes first and foremost the unequivocal definition of the scope of the analysis: a starting benchmark vehicle and all the methodological assumptions on present and future background processes, as well as system expansion scenarios. The most environmentally relevant components and manufacturing processes are then to be singled out and analysed in order to arrive at sound and robust indications of the most advisable way ahead towards low carbon vehicles.

**WEPC09 Identification and risk assessment of environmental endocrine disruptors for EU regulatory purposes** S. Dungey, Environment Agency; S. Brescia, I. Dewhurst, S. Fairhurst, C. Pepper, Health & Safety Executive, Chemicals Regulation Directorate; M. Roberts, Department of Environment Food & Rural Affairs; T. Hutchinson, School of Biological Sciences Plymouth University. Current EU legislation covering the authorisation of pesticides, biocides and the regulation of chemicals in general make specific provisions for substances found to have endocrine disrupting (ED) effects. This focus on ED poses several implementation challenges that are not yet fully resolved: (i) what validated international test methods are available for evaluating the ED potential of a substance?; (2) what are the criteria that determine whether or not a substance is regarded as an ED for regulatory purposes?; (3) if the data already available on a substance are insufficient to determine whether or not it has ED potential, how much

further testing should be done, using which protocols?; (4) if it is concluded that a substance does possess ED properties (i.e. exhibits a relevant *hazard*), what is the appropriate approach to assessing the *risk* that the substance might pose? Given the current lack of definitions or criteria within these pieces of legislation by which to identify ED substances, we are developing interpretative criteria for the identification of ED substances of concern for which regulatory action can be taken within the provisions of the current legislative framework (including the EC Plant Protection Products Regulation (1107/2009), the Biocides Regulation (528/2012) and the REACH Regulation (1907/2006). Currently, our environmental proposal stipulates that in addition to the internationally recognized definition of an ED adopted by the WHO/IPCS in 2004, two additional criteria should be observed: (a) evidence of an endocrine mode of action and an adverse effect on end points relevant to population stability in intact organisms; and (b) the dominance of the endocrine-mediated effect relative to the mode-of-action of the lead toxic effect on the target organism. Also, under the REACH legislation, a substance shown to have ED hazardous potential becomes a candidate for identification as a Substance of Very High Concern (SVHC) and thereby a candidate for the Authorisation process. Authorisation involves assessing whether the applicant's use of the substance poses a level of risk that is acceptably low, such that use can continue under the 'adequate control' provision, or is unacceptably high such that the use cannot be allowed unless there is no suitable alternative available to provide the same socio-economic benefit. Case studies (octylphenol, nonylphenol and octylphenol ethoxylates) will be used to elaborate further on these issues.

**WEPC10 Magnifying Perceived Risk: Exposure to other EDCs dwarfs contribution of ethinyl estradiol (EE2) to the overall endocrine load in surface waters** D.J. Caldwell, Johnson Johnson; F. Mastrocco, Pfizer Inc / Department of Environment, Health & Safety; P.D. Anderson, ARCADIS US Inc. As we previously reported, inaccurate or snapshot field measurements used as 'environmentally-relevant' test concentrations in laboratory studies, biomarker detection (i.e., vitellogenin in male fish) incorrectly reported as an effect, and field experiments using confined exposure (i.e., lake) being inappropriately extrapolated to surface water (river) risk assessments have all contributed to the misconception that EE2 exposure is of great consequence to wildlife and humans. Monitoring or limiting individual substances and ignoring the multitude of other estrogenic substances will not eliminate responses in wildlife. A better approach is to establish a level of estrogenic activity that is without population impact and monitor waters for that endpoint. In this way, we identify 'hot spots' and correct them which is the ultimate intent of the Water Framework Directive—to bring river basins to "good" ecological status. In addition, the assessment of substances in groups is the topic of much discussion as a means to replace the current slow, inefficient substance-by-substance approach. Estrogen-active substances are the ideal test-case for this approach for several reasons. First, they act by a common mechanism of action that has been shown to demonstrate concentration-addition effects, i.e., additivity. Second, there are multiple categories of estrogen-active substances, naturally produced estrogens, naturally produced phytoestrogens, synthetic estrogens (e.g., EE2), and industrial chemicals (e.g., phthalates, BPA, octyl phenol, nonyl phenol) that have demonstrated estrogenic activity. Comparing the relative differences in occurrence/concentration and the relative differences in estrogenic potency among these categories will facilitate a science-based understanding of the relative importance of the individual categories of substances to the total estrogenic load to which ecosystems are exposed. We review monitoring data that show that exposures of fish to EDC in surface water are largely due to chemicals other than EE2 and that observed effects are likely due to the total estrogenic load, of which EE2 is a minor contributor. Unless estrogenic activity of surface water is addressed holistically we may miss important contributors to the total estrogenic exposure by focusing on individual EDCs.

**WEPC11 Environmental Risk Assessment of Finasteride** J.G. Tell, Merck Company Inc / Global Safety & the Environment; G.G.

Gagliano, Merck Co Inc / Global Safety & the Environment. Finasteride is approved in the EU for the treatment of benign prostatic hyperplasia (BPH) and male pattern baldness (MPB). It is a synthetic type II 5 $\alpha$ -reductase inhibitor, which prevents the conversion of testosterone to dihydrotestosterone (DHT) and, therefore, finasteride may be considered endocrine active. Recent testing on environmental fate and effects of finasteride has enabled a more complete characterization of environmental exposure and risks to the aquatic environment from patient use. This poster will summarize the environmental exposure including an assessment of metabolism and excretion in the patient and estimates of predicted environmental concentrations in Europe. Environmental effects analysis includes acute and chronic tests to algae and daphnids, as well as a partial life-cycle study with medaka under continuous exposure to evaluate endpoints more indicative of endocrine effects. Combined, the exposure and effects assessment will provide a realistic description of the environmental impact of finasteride.

**WEPC12 Indication of nonylphenol ethoxylates/nonylphenol status in environment in relation with chemical regulatory measures in Serbia** J. Milic, Serbian Chemicals Agency / Chemicals and Biocidal Risk Assessment; J. Randjelovic, S. Roglic, Ministry of energy, development and environmental protection; V. Mart, m. Nonylphenol ethoxylates (NPEs) are a group of man-made chemicals that occur in nature as a result of human activity. These chemicals are most widely used as surfactants in detergents and in textile, leather, paper and metal manufacturing. Once released to wastewater treatment plants, or directly into the environment, NPEs degrade to nonylphenol (NP) which is known to be bioaccumulative and very toxic to aquatic organisms, and is able to act as a hormone disruptor. NP has been included as a "priority hazardous substance" under the EU Water Framework Directive. Furthermore, within the EU, since 2003 preparations containing greater than 0.1% (w/w) of NP or NPEs may no longer be placed on the market and use, with some minor exceptions principally for closed loop industrial systems. This restriction has been also transposed and implemented in Serbia since 2010, as well as Prior Informed Consent (PIC) procedure since 2009. Annual monitoring of water quality in Serbia and the Second Joint Danube Survey (JDS2) Report showed few hot spots in Serbia concerning NP concentrations in surface water and suspended particulate matter. According to JDS2 Report (sampling was undertaken in 2007), 4-*iso*-nonylphenol is found in sediment samples from rivers Tisa (89  $\mu\text{g/kg}$  dry matter) and Velika Morava (74  $\mu\text{g/kg}$  dry matter), which indicates those rivers were obviously receiving untreated or insufficiently treated sewage. This may be caused by the use of alkylphenol-containing detergents in this region. The latest national annual report of monitoring quality of surface waters (2011) revealed almost all measuring points has NP and NPE concentrations under the detection limit, except Vrljovac point where concentration of NP was 0.004  $\mu\text{g/L}$ . Furthermore, annual import of NPEs in Serbia decreased in 2011 for almost 40% compared with the 2010, when the NPE restriction introduced. Based on data obtained from the Serbian chemicals register for 2011, related to composition of detergents, most of chemicals suppliers notified no NPEs—containing detergents. It is very important to continue with monitoring of NP level in surface water and sediment in Serbia in order to analyze the real impact of regulatory measure implementation.

**WEPC13 Progress of the Tier 1 testing under the Japanese program on endocrine disruption: EXTEND2010** K. Yamazaki, Env Health Dep Ministry of the Environment / Environmental Health Department. Under the Japanese Ministry of the Environment's program "EXTEND2010" (EXTEND: Extended Tasks on Endocrine Disruption) two-tiered testing framework for assessing endocrine disrupting effects to organisms in the environment is being developed. This framework is designed to effectively identify potential candidates for endocrine disruptors using available information and test results. Reliability evaluation of available information that might be relevant to endocrine disruption is being conducted to select candidate chemicals subject to testing to assess their endocrine disrupting effects to aquatic organisms.

Tier 1 consists of *in vitro* reporter gene assays and short-term *in vivo* tests. *In vitro* assays are also used for prioritization to select candidate chemicals for *in vivo* tests, such as fish short-term reproduction assay. In Tier 2, long-term “definitive” *in vivo* tests, such as medaka multi-generation test, should be conducted, although they are still under development. After reliability evaluation for the first two batches of chemicals, twenty-five of them were identified as candidates for testing. Reporter gene assays were conducted for eighteen chemicals in FY 2010 and 2011, and estrogenic and anti-estrogenic activities were detected in some of them. Fish short-term reproduction assay was conducted for estrone, cyanazine and phenytoin in FY 2011 and estrogenic activity was detected for estrone. Reliability evaluation for the third and fourth batches of chemicals was conducted in FY 2010 and 2011 and twelve of them were identified as candidates for testing. *In vitro* reporter gene assays are conducted for thirteen chemicals in FY 2012 and candidates for *in vivo* tests will be identified. Fish short-term reproduction assay will be conducted within the fiscal year. Fifth batch of chemicals are selected and reliability evaluation is conducted in FY 2012. Sixth batch of chemicals will be selected in FY 2013. This abstract summarizes the situation as of the end of November 2012. Updated progress of testing and assessment of chemicals under the EXTEND2010 is to be presented.

**WEPC14 Weight of the evidence (WoE) evaluation of Endocrine Disruptor Screening Program Tier 1 assays: lessons learned** B. Neal, Exponent, Inc.; J.P. Staveley, Exponent; E. Freeman, A. Williams, J. Lamb, J. DeSesso, Exponent, Inc.. Under the U.S. EPA's Endocrine Disruptor Screening Program (EDSP), Tier 1 testing has been performed on an initial list of chemicals, largely pesticides. Results of tests conducted according to the battery of 11 screening assays (*in vitro* binding, mammalian toxicity, and ecotoxicity tests) are discussed in this presentation for several chemicals. For each chemical, a Weight of the Evidence (WoE) evaluation was conducted to determine if potential endocrine pathway interactions (estrogen, androgen, steroidogenesis, hypothalamus-pituitary-thyroid (HPT) or hypothalamus-pituitary-gonad (HPG) axes) exist. Per EPA Guidance, the screening assay results were first assessed for quality and reliability then analyzed in a WoE evaluation to determine if they “provide relevant, robust and consistent evidence” that the subject chemical has the potential to interact with a specific hormonal pathway. Research suggests that direct potential interactions with the estrogen and androgen pathways may be most readily identified because of presumed correlations between *in vitro* and *in vivo* outcomes; evidence of such direct pathway interactions was not seen in the assays reviewed. Several compounds, showed findings suggesting potential endocrine pathway interactions (steroidogenesis, HPG or HPT axis interactions) only at high and otherwise systemically toxic dose levels, presumably related to changes in liver metabolism, or possibly to effects on membranes or hormone synthesis. Consideration of other scientifically relevant information (in addition to the results of the Tier 1 screening assays) may be useful in determining whether or not Tier 2 testing is needed, especially when marginal, weak or inconsistent findings are obtained in the Tier 1 assays. These additional data may support alternative explanations for study results (such as general toxicity or secondary consequences of non-endocrine mechanisms of action) or they may support a conclusion of a potential direct endocrine pathway interaction. In summary, it is imperative to consider the results of all 11 Tier 1 assays and other scientifically relevant data in concert, examining them for strength of evidence, biological relevance, mechanism of action, and consistency among assays.

**WEPC17 Analysis of the uptake of triclosan into plants grown in biosolids-amended soils** R.S. Prosser, University of Guelph / School of Environmental Sciences; L. Lissmore, University of Guelph Laboratory Services Division; L. Sabourin, E. Topp, Agriculture and Agri-Food Canada; P. Sibley, University of Guelph / School of Environmental Sciences. Triclosan (TCS) is an antimicrobial chemical that is present in a variety of personal care products. Due the relatively high persistence and hydrophobicity of TCS, the chemical partitions into the solid portion of municipal sewage. TCS continues to persist after the solid sewage is processed into biosolids. There is concern about whether

TCS is taken up in roots and translocated throughout plants that are grown in biosolids-amended soil. In order to answer this question, a modified method was developed to analyze triclosan residues in biosolids, soil, and plant tissue. Soxhlet extraction was used to extract TCS from matrices, followed by solid phase extraction with hydrophilic-lipophilic balance columns. TCS was quantified using LC-MS-MS. Soybean (*G. max*), radish (*R. sativus*), and carrot (*D. carota*) were then grown in soil amended with dewatered anaerobically-digested biosolids. Amendment rates followed the best management practices employed in the province of Ontario in Canada. Biosolids were spiked with varying quantities of TCS to produce a range of environmentally relevant exposures. Plants were grown under environmentally controlled conditions and harvested midway through their life cycle and at maturity. Root, stem, edible portion of the plant, and soil were analyzed using the modified analytical method. TCS was not present above the limit of detection (LOD: 2.8 ng/g) in the roots or shoots of radish plants. The concentration of TCS in soil corresponded with expected concentrations based on the level of spiking and amount of soil. Analysis of soybean and carrot tissue is in progress. In addition to the plants grown in growth chambers, carrot, green bell pepper (*C. annuum*), cucumber (*C. sativus*), and tomato (*S. lycopersicum*) were grown in a field amended with dewatered anaerobically-digested biosolids in 2011 and radish, carrot, and tomato were grown in 2012. The edible portion of these plants was harvested at maturity and analyzed for TCS residues. TCS was not present above the LOD in the edible portion of carrot, green bell pepper, cucumber and tomato plants sampled in 2011 and radish plants in 2012. The analysis of TCS residues in carrots and tomatoes sampled in 2012 is in progress. TCS did not accumulate in tissue of plants grown in biosolids-amended soils when the soil was amended according to best management practices prescribed by the province of Ontario.

**WEPC18 Development and validation of a method for the determination of plastic additives in sediments** F. Heydebreck, Institute of Coastal Research; H. Wolschke, R. Sturm, R. Ebinghaus, Helmholtz-Zentrum Geesthacht. Plastic products contain apart from the polymer up to 20% various additives which are responsible for their physicochemical properties. One major class are the UV stabilizers which protect polymers from the degradation by oxidative and photochemical reactions when plastics are exposed to ultraviolet light. Due to their weak non-covalent bonds in the polymer they can leach into the environment during the usage. Because of the hydrophobicity of these compounds they can adsorb to particles and accumulate in sediments. In the last few years there has been an increasing discussion about UV stabilizers as persistent environmental contaminants. In this study a method was developed and validated to determinate 20 commonly used plastic additives in sediments. The method is based on pressurised-liquid extraction (PLE) with an integrated sample clean-up using a mixture of acetone/hexane followed by clean-up on silica gel. The extracts were analysed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). In order to develop a sensitive, selective and robust method, three different ion sources were compared: electrospray ionisation (ESI), atmospheric pressure chemical ionisation (APCI) and atmospheric pressure photoionisation (APPI).

**WEPC19 Temperature effects to the biodegradation of biocides in sediments** M. Schluesener, Federal Institute of Hydrology; K. Broeder, Bundesanstalt für Gewässerkunde; T. Ternes, Federal Institute of Hydrology. In recent years, biocides and UV-filters have gained increasing interest as so called emerging contaminants since they are ingredients of various products used in every day life such as personal care products (PCPs), cleaning agents and paints and coatings. Biocides are biological active compounds applied to destroy or to inhibit the growth or “effects” of organisms, even low environmental concentrations might have negative impacts on aquatic organisms. For example, triclosan has been shown to induce changes in the thyroid hormone-mediated process of metamorphosis of the North American bullfrog *Rana catesbeiana* and to cause a significant shift in the community structure of a natural river algae community at

environmental relevant concentrations as low as 30 and 15 ng L<sup>-1</sup>, respectively. The fate of biocides in the aquatic environment, particularly in river and stream sediments, is poorly understood since biodegradation studies are mainly missing. The aim of the study was to examine the influence of the temperature on the biodegradation of biocides in sediments. Therefore, a comprehensive data set on the degradation kinetics of selected biocides in sediments and the sorption affinities are collected. Batch experiments according to OECD 308 at different temperatures are performed to determine the degradation kinetics of selected biocides, e.g. triclosan.

**WEPC21 Evaluation of selected chemicals used in personal care products with the model tool ETAPOS** C. Ehling, Institute of Environmental Systems Research / Institute of Environmental Systems Research; J. Klasmeier, University of Osnabrueck / Institute of Environmental Systems Research. Personal care products (PCPs) are substances of environmental concern because of their tendency to be persistent, bioaccumulative and toxic and to exhibit long-range transport potential. At the same time, annual production volumes of many PCPs are steadily increasing. Especially sun-screen agents are suspected to behave like classical POPs under environmental conditions. Emission independent indicators for persistence (P), long-range transport (LRT) and bioaccumulation (B) can be used to evaluate chemicals against a set of acknowledged reference substances [1]. Such an approach provides more insight into the environmental behaviour of a substance than evaluations based on single criteria such as half-lives in individual compartments or bioconcentration factors (BCF). The objective of the work is to identify those substances from a dataset that most likely will exhibit POP-like behavior in the environment. We used the existing chemical evaluation spread-sheet model tool ETAPOS (Environmental Transport and Accumulation of Persistent Organic Substances) that combines a steady state multimedia model with a dynamic bioaccumulation model. The model tool delivers relative indicators independent of emission quantities. This allows for simultaneous assessment of all three aspects using the indicators overall persistence (P), characteristic travel distance (CTD) and the body burden emission ratio (BER) under identical environmental conditions. For comparative evaluation we use a set of acknowledged POP-like and NON-POP-like reference substances [1]. We chose the data set of 254 PCPs recently evaluated by Gouin et al. [2] with the RAIDAR model. We used the same substance properties (based on EPI Suite) for evaluation of the compounds with ETAPOS. 38 substances exceeded the P boundary and five substances were above the CTD boundary. Eight of the investigated PCPs are identified bioaccumulative with respect to the endpoint fish, but two for the endpoint human. All in all we identified 44 substances that are above one indicator boundary and eight compounds that are suspected POP-like in two aspects. However, none of the PCPs in this study turned out to be critical in all three evaluated aspects. The multi-criteria approach of ETAPOS allows for advanced simultaneous evaluation and prioritization of chemicals according to P, LRT and B. [1] Klasmeier et al. (2006) Environ Sci Technol 40(1):53-60. [2] Gouin et al. (2012) Environ. Pollut. 165:208-214.

**WEPC22 Biodegradation of Non-Ionic Surfactants in Soil under Aerobic Conditions** N.C. Torres, University of Cadiz / Departamento de Química-Física, Facultad de Ciencias del Mar y Ambientales C. Corada-Fernández, Universidad de Cadiz / Departamento de Química Física; P. Lara Martín, Universidad de Cadiz / Physical Chemistry; E. Gonzalez-Mazo, University of Cadiz / Physical Chemistry; D. Alvarez Munoz, University of Cadiz / Physical Chemistry. Synthetic surfactants are among the most produced and used organic compounds worldwide. They are found today in a large variety of domestic and industrial detergents, this makes them one of the most relevant organic pollutants of anthropogenic origin with high potential of entering into the environment. After their use they are usually discharged into municipal sewer systems where they are removed in a high percentage by a combination of sorption and biodegradation processes. However there is a small fraction that remains and it will be released via waste waters to the natural media. In order to study their behaviors in the environment

two non ionic surfactants of very elevated consume have been chosen: Polyethylene glycols (PEG) and Alcohol polyethoxylates (AEOs). Their biodegradation under aerobic conditions in soil has been researched according to the OECD guideline 307. The soil sample was taken from an experimental plot located in an agricultural area close to a waste water treatment plant in Jerez de la Frontera (Spain). 50 g of soil was weighed into different glass jars and the moisture level was adjusted to 50% of the water holding capacity. The samples were spiked with PEGs-8 and C14EO8 at 1 ppm. Each glass jar was mixed well and incubated under darkness at 22 °C in a constant temperature room during one month. The samples were taken at 1, 3, 7, 14, 21 and 28 days of the experiment and analyzed. The target compounds were extracted using pressurized liquid extraction (PLE) followed by purification and preconcentration by solid-phase extraction (SPE). Ultra-performance liquid chromatography (UPLC) coupled to quadrupole time-of-flight (QTOF) has been used for the identification and quantification of PEG-8 and C14EO8. Preliminary data show that the biodegradation of both compounds is taken place on the soil and some of their degradation products have been identified. **Keywords:** Alcohol Polyethoxylates (AEOs), Polyethylene glycols (PEG-8), soil, Aerobic biodegradation.

**WEPC25 Deposition of Polycyclic Aromatic Hydrocarbons in the North Pacific and the Arctic** Y. Ma; Z. Xie, Centre for Materials and Coastal Research GmbH, Institute of Coastal Research; H. Yang, Tongji University / State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering; A. Möller, Helmholtz-Zentrum Geesthacht, Centre for Materials and Coastal Research GmbH, Institute of Coastal Research; C. Halsall, Lancaster Environment Centre, Lancaster University; M. Cai, Polar Research Institute of China / SOA Key Laboratory for Polar Science; R. Sturm, R. Ebinghaus, Helmholtz-Zentrum Geesthacht, Centre for Materials and Coastal Research GmbH, Institute of Coastal Research. Eighteen polycyclic aromatic hydrocarbons (PAHs) were simultaneously measured in surface seawater and boundary-layer air from the North Pacific toward the Arctic Ocean during the 4<sup>th</sup> Chinese National Arctic Research Expedition in the summer of 2010. Atmospheric PAH ranged from 910 to 7400 pg m<sup>-3</sup>, with the highest concentrations observed in the coastal regions of East Asia. Correlations of PAHs' partial pressures versus inverse temperature were not significant, indicating the importance of ongoing primary sources on ambient PAH levels in the remote marine atmosphere. The relatively high atmospheric concentrations observed in the most northerly latitudes of the Arctic Ocean suggest the influence of regional sources. For example, higher levels of particulate-bound PAHs were observed in the air of the Arctic Ocean than the North Pacific, indicating forest fire and/or within-Arctic sources. Concentrations of PAHs in surface seawater, within a range of 14-760 pg L<sup>-1</sup>, and generally decreased with increasing latitude. The observed air-sea gas exchange gradients strongly favored net deposition of PAHs along the entire cruise, with increasing deposition with increasing latitude, while the particle-bound dry deposition fluxes (particularly for the high molecular weight PAH) were highest at sample sites close to East Asia. Based on characteristic PAH ratios, atmospheric PAHs originated from the combustion of biomass or coal, while the ratios of seawater reflected a mixture of sources. Given the dominance of primary emissions to the atmosphere and the relatively fast removal of PAHs from the water column, then PAHs will continue to load into the surface waters of the remote marine environment via atmospheric deposition.

**WEPC26 Evaluating a Forecast System for Long-range Atmospheric Transport Episodes of POPs** A. Halse, NILU - Norwegian Institute for Air Research; S. Eckhardt, Norwegian Institute for Air Research / Atmosphere and Climate Change Department; M. Schlöbach, Norwegian Institute for Air Research; A. Stohl, NILU - Norwegian Institute for Air Research; K. Breivik, Norwegian Inst for Air Research. Background air measurements of persistent organic pollutants (POPs) within existing monitoring programs are typically conducted by use of active air samplers (AAS), but the high cost of AAS limits their spatial and temporal coverage. Sampling at many such sites



furthermore occurs at fixed intervals (e.g. one day per week) without any *a priori* consideration of air mass transport (i.e., whether the air is likely to be polluted or not). While the current strategy is appropriate for the purpose of assessing long-term trends (years, decades), the fixed interval non-continuous sampling approach is at risk of missing out key long-range atmospheric transport (LRAT) episodes. The objectives of this study have been to (i) develop a forecast system using the Lagrangian transport model FLEXPART to predict long-range atmospheric transport episodes of POPs using PCB-28 as a model compound, (ii) to evaluate the capability of the forecast system to capture specific LRAT events at a background site in southern Norway (Birkesnes) through targeted sampling (i.e. when LRAT events are predicted), (iii) to assess whether predicted LRAT events for PCB-28 coincide with elevated concentrations of additional PCBs and other POPs, and (iv) to identify source regions of POPs during individual episodes. The system has been initially evaluated by comparing targeted samples collected over 12 to 25 hours during individual LRAT episodes, with monitoring samples regularly collected over one day per week throughout 2011. The FLEXPART model was clearly successful in identifying LRAT episodes for both PCB-28 and other PCBs. The model fails to accurately reproduce the magnitude of PCB-28 concentrations during individual episodes, but this can be mainly attributed to uncertainties in the absolute emission rates of PCB-28 used to drive simulations. We conclude that forecasting of pollution episodes has the potential to add value to relevant monitoring efforts which are normally collecting active air samples at fixed intervals in a non-continuous manner. Observations targeted at strong pollution episodes (as in this study) or on transport from specific source regions with highly uncertain emissions (as could be done in a very similar forecasting framework) could significantly enhance our understanding of POP sources.

#### **WEPC27 Estimating uncertainties of different air monitoring techniques for Semivolatile Organic Compounds (SVOCs)**

**K. Pozo, RECETOX; J. Klanova, Masaryk University / RECETOX; J. Jarkovsky, Masaryk University; J. Kalina, L. Dusek, Masaryk University / IBA.** In this study we present the preliminary results of a field study conducted at Kosetice station during one year, in order to evaluate the variability and uncertainty of using high volume active air sampler (HVAAS) with two different sampling frequencies (daily and weekly) to determine ambient air concentrations of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs). Air sampling was conducted in Kosetice, a background site in the Czech Republic, during 1 year from April 2001 to April 2012, using HVAAS with two different sampling frequency: 1) weekly and 2) daily measurements resulting in 52 samples per year. HVAAS were taken using a Digital PM10 pre-separator sampler. Air samples were analyzed using GC-MS instrument for 7 indicator PCBs, 16 US EPA PAHs and 10 OCPs. Standard descriptive statistics (mean, median, percentiles) followed by autocorrelation analysis and trend identification using Mann-Kendall test were used. The statistical significance of differences was analyzed by means of a nonparametric Wilcoxon paired test. Results shows that PCBs, PAHs and OCPs air concentrations vary from season to season with air concentrations ranging from 3 to 38 (pg/m<sup>3</sup>) for total PCBs, from 0.6 to 22 (ng/m<sup>3</sup>) for total PAHs, from 7 to 166 (pg/m<sup>3</sup>) for HCB and from 2 to 23 (pg/m<sup>3</sup>) for g-HCH. The Mann/Kendall statistical test did not identify any trend during one year of sampling. The two different sampling frequencies exhibit similar pattern. More volatile compounds i.e., HCB and  $\gamma$ -HCH showed lower concentrations during weekly sampling than with daily sampling frequency which can be influenced by breakthrough effect. Further analysis of this results are required in order to identify the better air sampling strategies and to reduce the uncertainties of reporting data between different air sampling protocols.

#### **WEPC28 Additive models to quantify environmental POP exposure in marine sediments**

**G. Everaert, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; F. De Laender, Université de Namur ASBL / Lab of Env.Tox&Appl.Ecol.; K. Deneudt, Flanders Marine Institute VLIZ / InnovOcean Site; P. Roose, Royal**

**Belgian Institute of Natural Sciences / Management Unit; J. Mees, Flanders Marine Institute VLIZ / InnovOcean Site; P. Goethals, C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology.** Due to their ability to accumulate in fatty tissues, persistent organic pollutants (POPs) are an issue of growing environmental concern. Although long-term monitoring programs have been initiated to quantify the marine POP concentrations, the data collected are often scattered in space and time, hampering a thorough environmental risk assessment of these chemicals. In this paper, we used generalized additive mixed models (GAMMs) to predict spatiotemporal trends of POP concentrations from a fragmentary dataset. Using this method, trends were extracted from a large, but incomplete set ( $n = 4038$ ) of polychlorinated biphenyl (PCB) and polyaromatic hydrocarbon (PAH) concentrations measured between 1991 and 2010 in the sediment of the Belgian Continental Zone (BCZ) and the Western Scheldt estuary. The model that was developed was based on a time (year and month) and a zonal predictor variable, a periodicity term, the octanol-organic carbon partitioning coefficient (Koc) and the type of POP and explained 80% of the observed variability. The inferred spatiotemporal time trends indicated that, in the BCZ, POP sediment concentrations decreased with time. In the BCZ, PCB sediment concentrations decreased three-fold and currently vary around  $0.274 \pm 0.153$  ng/g dry sediment. Also the PAH sediment concentrations decreased three-fold in the BCZ (from  $0.0347 \pm 0.0126$   $\mu$ g/g dry sediment in 2001 to  $0.0135 \pm 0.0052$   $\mu$ g/g dry sediment in 2010). In the Scheldt estuary, PCB concentrations were ten times higher than in the BCZ and remained stable around  $2.41 \pm 2.79$  ng/g dry sediment in the same period. The PAH sediment concentrations slightly increased in the Scheldt estuary (from  $0.0579 \pm 0.0538$   $\mu$ g/g dry sediment in 2001 to  $0.0671 \pm 0.1013$   $\mu$ g/g dry sediment in 2010). Given the capacity of the proposed methodology to fill data gaps in fragmentary data sets, we propose that future monitoring efforts focus on reference substances from which educated extrapolations can be made to similar substances.

#### **WEPC29 European Emission Inventory and Environmental**

**Modelling of Decabromodiphenyl Ether** **M.R. Earnshaw, Centre for Chemicals Management / Centre for Chemicals Management; K.C. Jones, Lancaster University / Lancaster Environment Centre; A.J. Sweetman, Lancaster University.** 1. Introduction The production and use of pentabromodiphenyl ether (pentaBDE) and octaBDE was banned in Europe under the Stockholm Convention on Persistent Organic Pollutants, with their listing in Annex A in 2010. However, decaBDE was exempt from the ban and its production and use in Europe continued until a partial ban in electrical equipment came in to force under the EU's Restriction of Hazardous Substances directive in 2008. Mounting evidence shows that decaBDE can debrominate to form the lighter, banned congeners under a wide range of conditions [1]. This study presents a first European emission inventory of decaBDE for the period 1970-2020 using a substance flow analysis model in the first step towards understanding the importance of decaBDE as a source of the banned PBDE congeners. 2. Materials and methods We have estimated the historical Global and European consumption of decaBDE using reported data for the period 1970-2010. A discrete dynamic substance flow analysis (SFA) model has been developed to characterise the lifecycle of decaBDE and to quantify emissions of the main congener BDE-209. The consumption estimate is then used as input for the SFA model (Figure 1) to derive emission estimates. Figure 1: Substance Flow Analysis model for DecaBDE. 3. Results and discussion From 1970-2010, it was estimated that a total of 185–250 kilotons of decaBDE was consumed in Europe. Predicted BDE-209 atmospheric emissions peaked in 2012 at 18 tonnes/year and are currently declining. The waste management phase of the life cycle is responsible for the majority of atmospheric emissions. 4. Conclusions Estimated emissions to the atmosphere were found to be higher than to the hydrosphere and terrestrial environments. The major source of emissions to all environmental compartments is the waste management phase, in particular landfill sites, although more empirical evidence is needed to confirm this. References [1] Zeng X, Simonich SLM, et al., Application of a Congener-Specific Debromination Model to Study

Photodebromination, Anaerobic Microbial Debromination, and Fe0 Reduction of Polybrominated Diphenyl Ethers. 2010, *Env Tox Chem*; 29: 770-778. **Acknowledgement** - The authors acknowledge the Chemicals and Nanotechnologies Division of the Department for Environment, Food and Rural Affairs, UK for funding this work.

**WEPC30 Persistent bioaccumulative toxin (PBT) air-water concentrations and fluxes in Lake Superior** Z. Ruge, URI Graduate School of Oceanography; R. Lohmann, University of Rhode Island / Graduate School of Oceanography. Polyethylene passive samplers were deployed simultaneously in both near surface air and water at 19 coastal and mid-lake sites around Lake Superior from April-October, 2011. The deployments were separated into three time periods to determine distinctions in concentrations and flux rates at different times of the year. Overall, 24 organochlorine pesticides (OCPs), 18 polychlorinated biphenyl (PCB) congeners, and 12 polybrominated diphenyl ether (PBDE) congeners were analyzed. Results from Lake Superior deployments reveal higher spatial resolution of the relatively even distribution of long-banned pesticides, while highlighting the urban signals of PCBs and PBDEs, instead of relying upon one monitoring station as done in previous studies. Air-water flux directions indicate when the lake is acting as a PBT sink, as it is currently for PBDEs, and when it becomes a PBT source, as is evidenced by the volatilization of HCB. Such trends can have implications for Lake Superior air quality well into the future. Increased passive sampling coverage and frequency has the potential to significantly enhance our understanding of persistent bioaccumulative toxins in the Great Lakes region and can prove to be a critical component in monitoring the long-term trends of Stockholm Convention compounds.

**MO129 The influence of food dependent eco-physiological processes on the response of *Mesocyclops leuckarti* to triphenyltin exposure** D. Kulkarni, RWTH Aachen University / Institute for Environmental Research; B. Daniels, RWTH Aachen; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research. Population models have been recommended for extrapolating ecotoxicological test results to the relevant ecological effects in the field. To simulate effects on populations as realistically as possible, detailed information on life-history traits of species and their dependence on environmental factors needs to be incorporated into these models. In this study, we investigated the influence of food dependent eco-physiological processes i.e. development, reproduction and survival on population dynamics under exposure to triphenyltin. The cyclopoid copepod *Mesocyclops leuckarti* was used as a representative species for freshwater copepods. This species is known to exhibit herbivory during early copepodite stages, while the late copepodite stages and adults switch to omnivory. It has also been speculated that early naupliar stages do not feed but rather survive on fat reserves obtained from the egg. An individual-based model (IBM) was developed for *M. leuckarti* using data from laboratory experiments under different food regimes (pure algal diet of *Cryptomonas obovoidea* or a mixed diet of the algae and the rotifer *Brachionus calyciflorus*). This IBM was coupled with the General Unified Threshold model for Survival (GUTS), which was calibrated to 96 h laboratory acute toxicity data, to describe the toxicokinetics and toxicodynamics of TPT. We simulated a single-peak exposure scenario at 20°C for the two different food regimes and calculated extinction probabilities. We found that under a pure algal diet, the late copepodites and adults showed slower development and survival parameters were underestimated leading to a faster extinction of the population. However, assuming food switching from the late copepodite stage onwards and non-feeding nauplii, population resilience was higher and extinction was comparatively slower. We conclude that population models developed on copepod species in particular, and zooplankton in general, should consider the respective complex feeding behaviours in order to simulate realistic population dynamics under toxicant exposure.

**MO130 Organism-level mechanistic effect models: assessing combined effects of chemicals and environmental stressors for**

***Folsomia candida*** N.T. Hamda, Jagiellonian University / Institute of Environmental Sciences; T. Jager, Vrije Universiteit / Dept. of Theoretical Biology; R. Laskowski, Jagiellonian University / Ecotoxicology Stress Ecology Group. With the current focus on predicting population-level effects of chemicals, a tendency has developed to integrate population dynamics in risk assessment process. The intent of these models is to extrapolate organism-level effects observed in laboratory to population-level impacts. Currently, individual-level effects are assessed using dose-response relationships derived from standard ecotoxicological tests. However, extrapolating these effects to population is flawed because typical bioassays do not take into account the factor of time and other environmental stressors that can influence effects of chemical at individual-level and its consequence to the population. Formulating mechanistic effect models to interpret organism-level effects of chemicals helps to understand the interactions between individuals and the environment. It can also help to make an educated extrapolation to the population-level. Such mechanistic effect models can be derived from a general and comprehensive energetic theory: the Dynamic Energy Budget (DEB) model. DEB is an energy/mass balance based metabolic theory which provides a mechanistic interpretation of how organisms acquire and use energy. The development and implementation of this model framework for particular species require, however, reasonable model parameters for the organism of interest. As it is not possible to study and parameterize all species, this should be done at least for organisms used in standard ecotoxicological bioassays. One such organism is the springtail *Folsomia candida*. In this study we parameterized a simplified DEB model ("DEBkiss") for *F. candida* and we implemented the model to explore the combined effect of cadmium, food limitation and temperature on individual life history and population dynamics of the organism.

**MO131 Elevated temperature prolongs long-term effects of a pesticide on *Daphnia* spp. due to altered competition in zooplankton communities** S. Knillmann, Helmholtz Centre for Environmental Research UFZ; N. Stampfli, UFZ-Helmholtz-Centre for Environmental Research; Y. Noskov, Institute of Systematics and Ecology of Animals; M.A. Beketov, UFZ - Helmholtz Centre for Environmental Research / Department of System Ecotoxicology; M. Liess, UFZ Center for Environmental Research / Department of System Ecotoxicology. Considerable research efforts have been made to predict the influences of climate change on species composition in biological communities. However, little is known about how changing environmental conditions and anthropogenic pollution will affect aquatic communities in combination. We investigated the influence of three warming periods in combination with a pulse exposure to the insecticide esfenvalerate (0.03, 0.3, and 3 µg/L) in 55 outdoor pond microcosms. Warming periods increased the cumulative water temperature, but did not exceed the maximum temperature measured under ambient conditions. Under warming conditions alone the abundance of some zooplankton taxa increased selectively compared to ambient conditions. This resulted in a shift in the community composition that had not recovered by the end of the experiment, eight weeks after the last warming period. Short-term effects of the pesticide on the community structure and the sensitive taxa *Daphnia* spp. did not differ between the two temperature regimes. In contrast, the time until recovery of sensitive taxa under warming conditions was twice as long as than at ambient temperature. Under both temperature regimes, we identified interspecific competition as an underlying mechanism that determined the time until recovery. However, interspecific competition under warming conditions was prolonged and thus delayed recovery of *Daphnia* spp. from esfenvalerate. These results show that, for realistic prediction of the combined effects of temperature and toxicants on sensitive species, the impact of the two stressors on the competitive interactions within the community needs to be considered. For details see the upcoming publication: Knillmann S., Stampfli N.C., Noskov Yu.A., Beketov M.A., Liess M., 2012. Elevated temperature prolongs long-term effects of a pesticide on *Daphnia* spp. due to altered competition in zooplankton communities. *Global Change Biology*, accepted.

**MO132 Effects of chemical stressors on predator-induced defenses in snails: subtle effects with strong ecological implications** C.J. Salice, Texas Tech University / Environmental Toxicology; J.G. Suski, Texas Tech University / Department of Biological Sciences. There has been a long-recognized need to incorporate more ecology into ecotoxicology and ecological risk assessment. The mainstay of ecotoxicology has been to evaluate the effects of chemical contaminants on key life cycle traits related to survival, growth and reproduction. Ecological systems are, however, far more complex and species interactions are key drivers of community structure and function. Predator-prey interactions can have especially strong impacts at the community level. Because predator represent a significant stressor, many prey have evolved a suite of phenotypic response collectively referred to as predator induced defenses that reduce the risk of predation. In freshwater snails, for example, predator induced defenses manifest as alterations in behavior, shell shape and shell thickness. In the presence of predator threat, snails generally change habitat use to reduce risk, generate lower profile and thicker shells. Given the ability of prey to achieve predator defended phenotype is critical to reducing the risk of being consumed, we were interested in whether chemical contaminants might alter the ability of freshwater snails to achieve a defended phenotype. We evaluated the effects of environmentally relevant concentrations of malathion and a common road de-icing salt (NaCl) on the ability of *Physa pomelia* and *Helisoma trivolvis* to achieve defended phenotypes in the presence of cues from predator crayfish fed conspecifics. *P. pomelia* exposed to a single pulse of 0.25 ppm malathion showed a significant reduction in predator avoidance behavior two days after the cessation of exposure. In subsequent predator trials, snails exposed to 0.25 ppm malathion were significantly more susceptible to crayfish predators than control snails. Similarly, *H. trivolvis* snails in the presence of predator threat and exposed to 4000 micro S/cm NaCl produced thinner and taller shells, especially at higher temperatures, compared to control snails. Importantly, in both examples, effects on predator induced defenses occurred at stressor levels below what would be considered a significant effect on life cycle traits. We argue that these types of ecological interactions have potentially profound impacts on community structure and function and would likely be missed with traditional toxicity testing and especially molecular or model-based approaches to toxicity and risk.

**MO137 Combined effects of microplastics and other environmental contaminants on predation behaviour of juvenile *Pomatoschistus microps*** T. Miranda, University of Porto, ICBAS - Institute of Biomedical Sciences of Abel Salazar; CIIMAR - Interdisciplinary Centre of Marine and Environmental Research / Laboratory of Ecotoxicology and Ecology; R. Norberto, University of Porto, ICBAS - Institute of Biomedical Sciences of Abel Salazar; CIIMAR - Interdisciplinary Centre of Marine and Environmental Research; L. Sa, L. Luis, University of Porto, ICBAS - Institute of Biomedical Sciences of Abel Salazar; CIIMAR - Interdisciplinary Centre of Marine and Environmental Research / Laboratory of Ecotoxicology and Ecology; M. Oliveira, University of Porto, ICBAS - Institute of Biomedical Sciences of Abel Salazar; CIIMAR - Interdisciplinary Centre of Marine and Environmental Research; L. Guilhermino, Instituto de Ciências Biomédicas de Abel Salazar / Laboratory of Ecotoxicology. In this study, the combined effects of microplastics and other environmental contaminants on the predation behaviour of juveniles (0+) of the common goby (*Pomatoschistus microps*) were investigated. Fish from wild populations were collected in estuaries of the NW Iberian Peninsula. After a period of acclimatization to laboratory conditions, they were exposed for 96h to sub-lethal concentrations of different environmental contaminants (metals, polycyclic aromatic hydrocarbons, and pharmaceuticals) in the presence and absence of microplastics. At the end of the bioassays, the post-exposure fish predation behaviour was individually assessed using *Artemia* and microplastics (of different sizes and colours) as preys. The results suggest that fish may confound some microplastics with preys, and that the presence of microplastics is able to modify the toxicity of some of the other tested substances highlighting the need of more research on the subject.

**Acknowledgements:** This work done in the scope of the project *SIGNAL – Effects of pollution on estuarine ecological interactions zooplankton-zooplanktivorous fish in relation to climate changes*” (PTDC/AAC-AMB/110331/2009), funded by the COMPETE programme (FCOMP-01-24-FEDER-01387601) and the Portuguese Foundation for the Science and Technology (FCT) through the OE component. M. Oliveira had a post-doc grant of FCT and L. G. Luís had a grant in the scope of the project.

**MO138 Microplastics observations in wastewater treatment effluents – much ado about something** H.A. Leslie, Institute for Environmental Studies VU Amsterdam; D. Vethaak, DELTARES. A new type of pollution is surfacing in municipal wastewater treatment plants (WWTPs): they're known as microplastics. Tiny particles of synthetic polymers often invisible to the naked eye, these microplastics are finding their way into the world's oceans and have been recognized as part of the global marine litter problem – or as it is sometimes called, 'plastic soup'. Versatile, high-performance yet very cheap, plastics make up a relatively high percentage of litter overall. Plastics particularly dominate the floating fraction of freshwater and marine litter. Wastewater effluents and rivers are a source of land-based marine litter and are just beginning to be investigated as such. We examined wastewater influent, sewage sludge and effluent from local wastewater plants in the Netherlands and have detected a variety of synthetic plastic particles. Wastewater from household washing machines contain synthetic textile fibers which become concentrated with other microplastics in the sewage sludge, but which cannot be fully degraded and removed in the bioreactors. What makes microplastics peculiar compared to other pollutants entering wastewater treatment plants is their degradation half-lives are on the order of hundreds of years – many times longer than even the persistent organic pollutants (POPs). The emergence of microplastic pollution in municipal wastewater is a reflection of the city's metabolism. The main drivers are our massive consumption of plastic products, from clothing to single-use items such as plastic bags and microplastic particles in cosmetics (e.g. shower gel, toothpaste etc.) on the one hand, and our current limitations regarding resource efficiency on the other hand. Microplastics are incompatible with and detrimental to biological cycles. Therefore we suggest ways to close the plastics cycle as opposed to releasing the materials as waste to municipal waterways and ultimately the marine environment.

**MO139 Toxicity of micro polystyrene particle for marine copepod *Tigriopus japonicus*** K. Lee, Korea Institute of Ocean Science and Technology KIOST; J. Kang, Korea Institute of Ocean Science and Technology; W. Shim, Korea Institute of Ocean Science and Technology / Oil and POP Research Group. We investigated the influence of three polystyrene (PS) micro-beads (0.05, 0.5 and 6 µm diameter) which were selected to measure the sized effect of microplastic for survival, development and fecundity of the copepod *Tigriopus japonicus* using acute and chronic toxicity test. In this study, *T. japonicus* ingested and excreted all PS beads used in the study even at the condition with livefeed without food selection. The copepods (nauplius and adult female) could survive at all sized-beads and its various concentrations tested in the acute toxicity test for 96 h. In the two-generation chronic toxicity test, 0.05 µm PS beads with over 12.5 µg/mL concentration caused the mortality of nauplius or copepodite in the *F*<sub>0</sub> generation even at 1.25 µg/mL concentration in the next generation. In 0.5 µm PS beads treatment, although there was no significant effect in the *F*<sub>0</sub> generation, the highest concentration (25 µg/mL) induced a significant decrease in the survival compare with the control in the *F*<sub>0</sub> generation. 6 µm PS beads did not affect the survival of *T. japonicus* over two generations. The length of nauplius phase and generation time of the copepod were similar pattern with the result of survival. Sex ratio showed no significant difference at all treatments over two generations. The smallest sized-beads (0.05 µm) did not affect the fecundity of the copepod. However, 0.5 and 6 µm PS beads caused a significant decrease of the fecundity at all concentrations. These results suggest that the microplastic such as micro sized-PS bead may lead to negative effect to marine filter feeder.

**MO140 Microplastic ingestion by zooplankton** M. Cole; P. Lindeque, E. Fileman, T. Galloway, Plymouth Marine Laboratory. Small plastic detritus, or microplastics, are a widespread and ubiquitous contaminant of marine ecosystems. The ingestion of microplastics by a wide range of marine biota, including mussels, worms, fish and seabirds, has now been documented. However, despite the vital ecological role of zooplankton in the marine food web, the impact of microplastics on zooplankton has remained under-researched. My work explores the uptake and biological effects of microplastics on a range of zooplankton species. We have used an integrated approach, combining feeding rate studies and novel bio-imaging techniques to document ingestion and egestion for a range of plankton displaying different feeding behaviours. Our initial experiments, using fluorescence- and coherent anti-Stokes Raman scattering (CARS) microscopy, have established that many zooplankton species, including copepods and decapod larvae, can ingest a range of polystyrene microplastics (0.4 – 31 µm diameter). We have further demonstrated that the presence of 7 µm microplastics significantly decreases algal feeding in the copepod *Centropages typicus*. Suppressed feeding may have repercussions on zooplankton health (e.g. reduced egg production and growth), which is the focus of our on-going work. In our most recent study we consider how the ingestion of microplastics by zooplankton may have adverse effects on a range of biological scales.

**MO244 Life cycle inventory of Asian Aquaculture Feeds - including estimates for dispersion** P.J. Henriksson, Leiden University / Institute of Environmental Sciences; J. Guinee, University of Leiden / Institute of Environmental Sciences; R. Heijungs, Institute of Environmental Sciences - CML, Leiden University; A. De Koning, Leiden University / Institute of Environmental Sciences. Over the last decade, LCA has been implemented to evaluate several aquaculture production chains. A common conclusion amongst all these studies is that feed production is a major contributor to most environmental consequences. In the meantime, there is a shortage in current LCA databases of processes identifying most of the underlying raw materials used in Asian aquaculture feeds. Therefore, as part of the on-going EU FP7 SEAT project ([www.seatglobal.eu](http://www.seatglobal.eu)), we here have evaluated aquaculture feed production in Asia, including estimates of dispersion, defined as the sum of inherent uncertainty, spread and representativeness. Primary data was collection was collected from feed mills in Bangladesh, China, Thailand and Vietnam, during 2011 and 2012. From these a number of raw materials were identified as common ingredients. However, the data quantity and quality available in literature differed greatly, and was simply missing in some cases (e.g. Apple snails from Bangladesh). In other cases, where processes were well described in literature (e.g. soybeans, Brazil), large differences amongst datasets were experienced. In order to commonly meet these challenges, and produce dispersion estimates around point values, the methodology proposed by Henriksson et al. (in review) was adopted. This approach promotes subjective sourcing of data using a decision tree, a weighting procedure amongst values based upon representativeness, and the inclusion of inherent uncertainty, spread and unrepresentativeness in the overall dispersion parameter. The findings identified a highly globalised trade of resources, which posed clear challenges for consistent data sourcing. Measurement error and modelled uncertainty most strongly influenced inherent uncertainty, while spread was the result of horizontal averaging. Finally, representativeness captured the additional uncertainty introduced by decision stakes and data characteristics. Overall, the here presented research showed that overall dispersion estimates can be produced and implemented for all sources of data.

**MO245 Applying text mining to characterize the use of Computational Sustainability techniques in LCA** Marvuglia, CRP Henri Tudor / Cork Constraint Computation Centre; E. Benetto, CRP Henri Tudor / Resource Centre for Environmental Technologies (CRTE). Since its beginning in the late 60's LCA has evolved significantly both on the methodology side and the data availability side. While at that time the problem was mainly on data availability,

nowadays a considerable amount of data is available at the inventory level. Nonetheless, with the current evolution of LCA towards spatial differentiation, a new trend started in the direction of data hunting at an ever increasing level of spatio-temporal detail. Consequently, an additional problem lies now in the existence and mastering of efficient software to manage big amounts of data and computational tools able to fill the existing practical and conceptual gaps in LCA. Several computational techniques, spanning from spatial statistics to data mining, from economic modelling to mathematical optimization (to mention only a few) have been developed over the last decades and successfully applied to several case studies in the Computational Sustainability research field. Their application in the LCA domain is certainly promising and some instances already exist. Several issues remain however still open and a number of techniques could be proficiently applied to address them. For example, the utilization of data mining and ML algorithms could be highly beneficial to address the problem of data gaps with toxicity-related impact categories; the use of visual analytics and advanced processing of remotely sensed images could support the evaluation of the status of ecosystems goods and services. Text mining is an advanced analysis technique more and more applied in several disciplines. This study consists in the application of text mining to trace the evolution of several computationally advanced techniques in LCA. In particular, heterogeneous co-occurrence maps have been built using a corpus extracted from the ISI Web of Knowledge (WoK) website, thus unveiling clusters of closely related textual items for the period 1995 – 2012. Even though we recognize the set of selection criteria used to extract the corpus and the use of only one database are limitative factors for the analysis, it is still remarkable the fact that the number of applications detected is not very high (except for the increasing application of linear programming especially from 2010). This probably means that in the LCA field there is still significant a lack of cross-domain interaction especially with the areas of applied mathematics and artificial intelligence.

**MO246 LCA-GIS INTEGRATION FOR THE ENVIRONMENTAL ASSESSMENT OF WASTE MANAGEMENT SYSTEMS** D. Bernad-Beltran, Universitat Jaume I. Dpt. Mechanical Engineering & Construction / Dpt. Mechanical Engineering & Construction; M.D. Bovea, Universitat Jaume I Department of Mechanical Engineering Construction; V. Ibáñez Forés, Universitat Jaume I; V. Perez-Belis, Universitat Jaume I. Dpt. Mechanical Engineering & Construction. Life Cycle Assessment (LCA) is the most appropriate methodology for the environmental assessment of waste management systems. There are a number of computational tools in the market with the goal of performing this task. Nevertheless, these tools seldom allow the user to incorporate own inventory data, making it difficult to model close to reality scenarios. Besides, spatial or temporal aspects are rarely considered in the analysis. The capabilities offered by Geographic Information Systems (GIS) allow tackling efficiently these issues. In the context of an LCA-GIS integration, a computational tool has been developed using Model Builder application by ESRI. The theoretical framework of LCA has been taken into account to consider every stage of a waste management system: pre-collection, collection, transport, treatment and disposal. GIS provide the user-friendly workspace where the appropriate input data overlaying and final results presentation will be performed. This computational tool is easily shareable between GIS software users. The developed tool allows the user to assess a variety of waste management scenarios from an environmental perspective, taking into account primary inventory data to define the characteristics of waste fractions and facilities which form part of the system. Network analysis applications are used to increase the accuracy in the assessment of waste transport stage, considering optimal routes among facilities. Site-specific and temporal issues are incorporated to the analysis within the land use impact category. A proposal for the assessment of this controversial impact category is presented here, using aboveground biomass as an environmental indicator. The elaborated tool provides results for a variety of indicators, which consistently define the environmental performance of a waste management system. The value of these indicators can be numerically

presented or graphically represented in map format. In order to validate the functioning of the presented tool, an environmental assessment will be performed, being its aim to determine the environmental consequences of incorporating biowaste separate collection to a system where paper, glass and light packaging are already collected separately.

**MO247 The worth of assessing Ecosystem Services in Luxembourg from a biophysical perspective** B. Rugani, University of Siena / Resource Centre for Environmental Technologies (CRTE); M. Machado De Sousa, CRP Henri Tudor (CRPHT) / CRTE; L. Coscieme, F.M. Pulselli, University of Siena / Department of Physical, Earth and Environmental Sciences; E. Benetto, CRP Henri Tudor (CRPHT) / CRTE. The concept of ecosystem services (ES) is used to represent and measure the benefits humans derive from nature. Despite being one of the most relevant topics in environmental science nowadays, this concept is still lacking commonly accepted definition and framework. For example, The Economics of Ecosystems and Biodiversity (TEEB) project is spreading worldwide and becoming a standard. It intends to give economic valuation of the consequences of biodiversity loss and support to ES assessments. However, prior to assign an economic price to ES, it would be worthwhile assessing functions and properties of ecosystems. This gives ultimately the extensive- and ecological-oriented basis for a consistent analysis that does not neglect limits, complexity and constraints of the ecosystems. Further, ES are not yet comprehensively assessed in LCA, which usually inventories biophysical inputs and assesses impacts on the biophysical dimension of the cause-effect chain. This work aimed at performing a preliminary biophysical ES inventory in Luxembourg. Since this was the first national-scale attempt on this matter in the country, we also identified the availability, limitations and challenges for data collection. Moreover, a comparison and combination with an Emergy application was carried out. Indeed, Emergy is a valuable tool to synthesize the dynamics of complex territorial systems, as well as a common denominator for physical evaluations of ES in LCA. After a literature review, we selected 20 ES, which were regrouped into five categories (provisioning, regulating, supporting, cultural and other services), as well as related indicators. We found out that, while provisioning, regulating and other services are relatively well documented, supporting and cultural services are not. Data were then mapped using QGIS. In addition, we built a matrix for qualitative assessment with scores of 'service relevance'-by-'land cover type', including also those services showing data lacks and high uncertainties. These results were compared to the Emergy values calculated with an Emergy conventional perspective, which can also monetize physical flows, and the scores combined to observe the relationship between Emergy and the most relevant ES. We finally identified pertinent opportunities to improve the proposed methodology, which focused on the combination of very different but apparently complementary tools to assess the complexity and worth of ecosystems.

**MO249 A novel life cycle impact assessment methodology for assessing the direct and indirect impacts of fossil resources depletion**

F. Fatemi, Ecole Polytechnique de Montreal / CIRAIG, Department of Chemical Engineering; C. Bulle, CIRAIG Polytechnique Montreal / Chemical Engineering; M. Margni, Ecole Polytechnique de Montreal / Department of Mathematical and Industrial Engineering. Various life cycle impact assessment (LCIA) methods have characterized the impacts associated with the depletion of fossil resources. Methods vary widely in their approach, however, the ILCD handbook, which recently performed an analysis of existing LCIA methods, recommends ReCiPe as the most developed LCIA method for endpoint impact characterization of fossil resources depletion, but it is still classified as an interim method due to its limitations. In this work, a new LCIA methodology for the depletion of fossil resources is defined based on ReCiPe's definition as our starting point with the goal to make certain enhancements to it in line with the ILCD Handbook recommendations. The World Energy Projection System Plus (WEPS+), an energy modelling system used to produce the International Energy Outlooks by the US Energy Information Administration, was used to model the shifts in the energy market as a consequence of the marginal depletion of a

fossil resource. A change in the price of the resource as a consequence of depletion is applied to the price of the fossil resource in the model. The changes in the world energy market up to 2035 are calculated and compared to the reference scenario. The changes in the world energy market present themselves as 1) changes in demand and production of the fossil resource itself and 2) changes in the production and prices of other energy resources. The direct impacts are defined as the total difference in costs that the world competing users will have to pay in order to meet its energy needs as a consequence of the change in the price of the resource due to marginal depletion. Characterization factors are calculated for petroleum, natural gas and coal in \$/MJ and are integrated in IMPACT World+ LCIA methodology. \n

**MO250 Monetisation of fossil resource depletion by assessing the surplus cost** T. Ponsioen, PRe Consultants / Consultancy; M. Vieira, M. Goedkoop, PRe Consultants. For the life cycle impact category fossil resource depletion, monetisation can be applied by assessing the future consequences of resource extraction. This is then based on the concept that resources will be extracted under more and more challenging conditions and with alternative technologies in the future. The additional efforts can be described by surplus costs, which have an increased impact on society. Surplus cost is defined here as the global future cost increase due to marginal fossil resource use. This approach has been applied in the past for the ReCiPe method, but the data gaps for fossil resources in this method compromised its robustness. Here, we present an improved indicator for surplus cost of fossil resource depletion and its underlying assumptions. First, the marginal cost increase (MCI) is calculated as an intermediate parameter for crude oil, natural gas and coal separately. Its calculations are based on production cost and cumulative future production data per production technique or country. To calculate the surplus cost from the MCI, several value choices were made regarding fossil resource production scenarios and discount rates. We chose for three different societal perspectives with different production scenarios as published by the IPCC and fixed discount rates. The results show that the ratios between the indicators of the different types of fossil resources (crude oil: natural gas: coal) are rather constant in most cases, only in the egalitarian perspective the ratio deviates because only there zero discounting is applied (egalitarian: 100:47:21; hierarchist: 100:54:1.1; individualist: 100:39:0.7). The MCI gives a similar ratio (100:48:1.0); so, in general, the MCI gives a good indication of the impact indicator. However, there are large differences between the surplus cost indicators for each perspective in cost per gigajoule (egalitarian > hierarchist > individualist). From Monte Carlo simulations, we found that data uncertainty is low. However, the uncertainty of total available resources per production technique or country was not included, because there is no information available to quantify it. We also found that the results are extremely sensitive to the discount rate assumed. When applying the present approach to other resources, such as minerals, water, and land, consistent modelling of the discount rate for different perspectives is therefore recommended.

**MO251 Integrating Payment for Environmental Services into Life Cycle Analysis: Metal Removal from Run-off from Abandoned Mine Sites in North Yorkshire** H.A. Baxter, University of Hull / Centre for Environment and Marine Sciences joint with Department of Engineering; W. Mayes, University of Hull / Centre for Environment and Marine Sciences. Payment for Environmental Services (PES) schemes are becoming more widely used globally to encourage practices which actively improve local ecosystems, in order to improve/maintain environmental services (ES) which are of value to humanity. Through linking potential purchasers for the ES with those who maintain the land, negotiations about the value of benefits derived imposes a value upon the range of services which result from actions taken by the land custodian. Life cycle analysis techniques can determine the impacts of implementing land management practices, identify potential purchasers and provide information for negotiations between the parties about appropriate levels of monetary remuneration and realistic expectations of what can be achieved by the service provider. PES schemes in turn will provide LCA practitioners with real-world data relating to the value

that society places on different impact categories within an LCA framework. Conditionality is a requirement of the most PES schemes, this presents an opportunity for collection of monitoring data to improve future LCA models and determine the levels of uncertainty and error present by comparison with a predictive LCA used for a specific PES scheme. By using LCA techniques to determine the impacts of different remediation methods for specific sites in North Yorkshire integrating PES methodology, for the use phase of the LCA, to identify the potential monetary benefits that can be derived from remediation the specific size is being undertaken so that a determination can be made about; 1. whether the benefits derived from site remediation outweighed the impacts which result from the production, deployment, and use of different remediation techniques 2. the potential for recovery of the upfront investment and continuing costs related to the remediation method deployed 3. the additional economic value derived from metal removal provided by improvement in the four categories of environmental services (provisioning, regulating, auditing/habitat and cultural services)

**MO252 Socioeconomic analysis of the use of nickel sulphate in the manufacture of bathroom fixtures and fittings** R. Mistry, eft@; Simpson, WCA Environment Ltd; M. Holland, EMRC; A. Provins, eft@; E. Stutt, WCA Environment; M. Vander Straeten, Consultant; F. Capon, Nickel Institute. Most bathrooms in the EU have fittings (e.g. taps) made using a nickel sulphate (NiSO<sub>4</sub>) based manufacturing process. This poster describes a socio-economic analysis (SEA) for this use of NiSO<sub>4</sub> undertaken within the hypothetical context of REACH Authorisation. Nickel substances are not subject to Authorisation under the EU REACH regulation. Where possible, SEA impacts are monetised, enabling direct comparison of “continued use” versus “refused REACH Authorisation”. An analysis of alternatives found that the most likely outcome is that fittings made using NiSO<sub>4</sub> will be imported into the EU. Results were, as follows: **Economic costs:** Net loss of production value in the EU of €350-388 billion Net Present Value (NPV) over 20 years. This would be offset by production outside the EU. **Health benefits:** Based on conventional risk assessment, OELs and DNELs for NiSO<sub>4</sub> are not exceeded. Therefore, impacts on workers or the public should be negligible. An economic assessment based on a unit risk factor approach for carcinogenicity, estimates of the costs of allergic contact dermatitis and unit damage costs (€/kg) for airborne emissions of nickel suggests total avoided damage to health of €16-98 million NPV over 20 years. If nickel salts are assumed to have a practical threshold for carcinogenicity the lower bound for this estimate would be close to zero. **Environment benefits and costs:** Approximately 100-150 waterbodies in the EU-27 may improve (to some extent) as a result of a refused Authorisation. However, this conclusion requires site-specific data to confirm. This benefit should be balanced against the impacts of an increase in CO<sub>2</sub>, NO<sub>x</sub> and SO<sub>x</sub> emissions from transportation (partial estimate of €12 million over 20 years in Europe only) due to imports if Authorisation was not granted. **Social costs:** Up to 150,000 EU jobs could be at risk. **Wider economic costs:** Up to 0.22-0.24% of EU GDP may be lost. The costs of a refused authorisation are dominated by the loss in EU production of bathroom fittings. Potential health benefits are small relative to this (well below 0.1%) and improvements in water bodies are uncertain. These benefits are also partially offset by the health and environmental impacts of a refused authorisation (e.g. from transport emissions linked to additional imports). Given the scale of economic and social benefits relative to the risks of continued use, the benefits of Authorisation outweigh the risks to human health and the environment.

**TU063 Potential artifacts in nanoecotoxicology testing** E.J. Petersen, National Institute of Standards Technology. Engineered nanoparticles are a novel technology that are expected to be incorporated into increasing numbers of consumer products given the unique properties observed with particles that possess nano-scale (1 nm to 100 nm) dimensions. However, one potential barrier to the widespread commercialization of products containing nanoparticles is uncertainty regarding the potential toxic effects that these particles could

pose. Assessing such risks is hindered by uncertainty regarding the adequacy of current standard methods for assessing the ecotoxicological risks of these particles given substantial differences in behaviors between nanoparticles and traditional environmental pollutants. Public sensitivity to the potential effects of nanoparticles and the risk that miscommunication of these risks could bias the public against nanomaterials make it critical that robust nanoecotoxicology tests are developed and their results are reported accurately. One of the most important factors that can influence the accuracy of nanoecotoxicology measurements is the potential for nanoparticles to cause artifacts during testing. Artifacts due to nanoparticles have been identified during all stages of the test setup including preparation of the nanoparticle dispersion and quantification of changes in biomarkers after nanoparticle exposure. However, these artifacts have not yet been systematically reviewed. Some of the artifacts that will be discussed during this presentation include interferences with reagents during toxicity assays, toxic byproducts produced during nanoparticle synthesis or dispersion techniques such as sonication, misinterpretation of nanoparticle distributions in tissues during electron microscopy analysis, lack of a mechanism for nanoparticle transit to organs where toxic effects are reported, and endotoxin contamination in nanoparticles tested.

**TU064 Method development to visualize nanoparticle uptake in phytoplankton and higher plants** F. Schwab, Duke University / Civil Environmental Engineering CEINT; S. Marinakos, A. Baddireddy, Center for the Environmental Implication of NanoTechnology (CEINT); B. Colman, Duke University / CEINT; M.R. Wiesner, Duke University / Pratt School of Engineering. The study of ENM uptake mechanisms and modes of action into phytoplankton and plant tissue is often hampered by the lack of validated, easily accessible high-resolution visualization techniques [1]. The objective of this work was therefore to develop and set of complementary visualization techniques applicable to different phytoplankton and plant species exposed to nano-Au particles (nAu). Experiments were performed using axenic cultures of *Pseudokirchneriella subcapitata*, *Anabaena Flos-aquae*, and *Navicula pelliculosa*, and as a representative species for higher plants, *Egeria densa*. The phytoplankton and *E. densa* were exposed to citrate coated nano-Au particles (15 nm) at standard conditions [OECD]. Protocols for three complementary visualization techniques using TEM, confocal microscopy, and the novel technique hyperspectral imaging were developed and tested. TEM imaging carried out at the outer cells of *E. densa* revealed heavy accumulation of the nAu in the cuticle of the cell. The nAu particles were identified by highly resolved imaging of the cuticle tissue, and the X-ray pattern of Au. Preliminary results of the HSI and confocal microscopy imaging will be presented at the conference. The size and shape of the pores across the cell wall of *E. densa* was quantified. The implications on the currently discussed hypotheses on uptake mechanisms will be discussed. [1] Kahru, A, Dubourguier, HC. 2010. From ecotoxicology to nanoecotoxicology. Toxicology 269:105-119. [OECD] Organisation for Economic Co-operation and Development. 2006. Freshwater alga and cyanobacteria, growth inhibition test, Nr. 201. In: OECD Guidelines for the Testing of Chemicals. Paris, F. **Acknowledgement** - The authors thank the Swiss National Science Foundation, the National Science Foundation (NSF), the Environmental Protection Agency (EPA, EF-0830093), the Center for the Environmental Implications of NanoTechnology (CEINT), and TINE for the funding of the study. Any opinions, findings, conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF or the EPA. This work has not been subjected to EPA review and no official endorsement should be inferred.

**TU065 Effect of changing environmental conditions on silver nanoparticle aggregation and dissolution** C. Walters, CSIR / Natural Resources and the Environment; V. Somerset, Council for Scientific and Industrial Research; E.J. Pool, University of The Western Cape. The release of metal nanoparticles (NPs) into the environment is on an increase due to their increased use in several consumer products.

Once released into the aquatic environment, NPs tend to form aggregates, or are associated with suspended solids which may be accumulated by aquatic organisms thereby entering the aquatic food chain. These fate processes are largely dependent on both the characteristics of the NP and that of the environment. Of all the metal NPs, silver NPs (AgNPs) are the most widely used, making up approximately 55% of nano-containing consumer products. As such, there is a high potential for the release of AgNPs into the aquatic environment. To better understand their fate and toxicity in aquatic environments, this study investigated the aggregation and dissolution behaviour of AgNPs in freshwater microcosms under simulated environmental conditions. Specifically, this study aimed to assess whether AgNPs will be present as aggregates or isolated particles in aqueous solutions. Dry AgNPs, purchased from the supplier, was characterized via scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive x-ray (EDX), powder x-ray diffraction (PXRD), Brunauer, Emmet and Teller (BET) and ultraviolet-visible (UV-vis) spectroscopy methods. TEM was used to investigate the aggregation potential of aqueous AgNPs under various environmental conditions. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to investigate dissolution potential by assessing the concentrations of Ag in aqueous media. The results obtained showed that at the low temperature regime, higher Ag concentrations were measured when compared to the high temperature regime. The smaller aggregates and lower Ag concentrations measured in T3 when compared to T2 possibly reflects resorption of Ag to the particle surface. The TEM images obtained for primary AgNP suspensions in the flood regime showed micrometer-size aggregates of varying densities. This study proved necessary in order to predict the potential environmental fate, behaviour, bioavailability and toxicity of NPs in the natural environment.

**TU066 Considerations and Recommendations to Standard Testing with *Daphnia magna*.** D. Cupi, DTU Technical University of Denmark / Department of Environmental Engineering; A. Baun, Technical University of Denmark / Department of Environmental Engineering. Standard testing procedures for aquatic organisms, as developed by OECD and ISO, have been developed for chemicals that generally dissolve in aqueous solution. However, many nanomaterials will not dissolve in media and incubation durations used in standard ecotoxicity tests. Therefore, modifications of current testing procedures may be warranted. Here, we focus on highlighting limitations to *Daphnia magna* immobilization testing, and proposing modifications to ensure the procedures are fit to the type of testing being conducted. In the current study, six reference nanoparticles [TiO<sub>2</sub> (NM-104), Ag (NM-300K), CeO<sub>2</sub> (NM-212), ZnO (NM-110), ZnO (NM-111), and SiO<sub>2</sub> (NM-200)] were employed to test acute toxicity on freshwater crustacean *Daphnia magna*. It was seen from the results that the toxicity of nanoparticles to *Daphnia magna* ranked in the following order Ag > ZnO (NM-110) > ZnO (NM-111) > CeO<sub>2</sub> > TiO<sub>2</sub> > SiO<sub>2</sub>. In general, nanoparticles in the powdered form were more difficult to suspend in MilliQ water, especially those present in a hydrophobic state, which had a tendency to float. For the latter, 0.1% ethanol was used to create a wetting effect and achieve a paste form, prior to suspension. It was noticed that the physico-chemical properties/size distribution of the stock suspensions changed over time, therefore it is recommended that stock suspensions are prepared fresh shortly before testing. Another issue that affected size distribution was the presence of ions in media (ionic strength), and sonication procedure (bath vs. probe sonicator). Therefore, dispersion of different types of nanoparticles should be considered on case-by-case basis. Additions of environmentally relevant substances such as natural organic matter (NOM) to different concentrations of Ag NPs decreased toxicity. This effect was seen starting from 10 mg/L humic acid, and more efficiently at concentrations of 50 mg/L and 100 mg/L. While addition of NOM may assist in more controlled dispersions with less degree of agglomeration, it may also influence the sensitivity of the test systems. Hence, further research is needed to disclose the influence of allowing addition of NOM in standard *daphnia* tests.

**TU207 Respiratory uptake efficiency of perfluorooctane sulfonate (PFOS) in fish** T. Sakurai, National Institute for Environmental Studies / Center for Environmental Risk Research; J. Kobayashi, Prefectural University of Kumamoto; K. Kinoshita, N. Ito, S. Serizawa, H. Shiraishi, National Institute for Environmental Studies; K. Mizukawa, Tokyo University of Agriculture and Technology; Y. Imaizumi, T. Kawai, N. Suzuki, National Institute for Environmental Studies. Understanding the bioaccumulation process is a key component of environmental risk evaluation of chemicals. Uptake efficiency is an important parameter in the models describing the bioaccumulation kinetics. This study examined the uptake efficiency of PFOS in fish at the respiratory surfaces based on a laboratory experiment and analysis of literature data. PFOS is persistent in the environment and is expected to accumulate in surface water, in which it is ionized. Respiratory uptake efficiency of PFOS in a marine fish, the marbled flounder (*Pseudopleuronectes yokohamae*), was investigated in our laboratory. Two-year-old flounders were exposed to dissolved PFOS at an average concentration of 74 ng/L for 28 days, and then depurated for 84 days (water temperature averaged 17.3 °C). The respiratory uptake rate constant for fish whole body was estimated at 22 L/(kg-wet-fish d). This value corresponded to a respiratory uptake efficiency of PFOS 3.2% that of oxygen, based on the ventilation rate of the fish estimated from a respiration measurement of marbled flounders, and assuming that the respiratory uptake efficiency of PFOS was proportional to that of oxygen. Few respiratory uptake efficiencies have been reported for PFOS in fish. We therefore analyzed reported respiratory uptake rate constants of PFOS in a similar way by using allometric estimation of oxygen demand by the fish. The experimental result and the data analysis resulted in the respiratory uptake efficiency of PFOS 0.8%–10% that of oxygen, for marbled flounder, rainbow trout, common carp, and bluegill. By taking into account the uptake efficiency of oxygen, this range corresponded to the respiratory uptake efficiency of PFOS of roughly 0.6%–7%, which was lower than those typically reported for neutral hydrophobic compounds. We note, however, that higher values of respiratory uptake rate constant were reported with increasing hydrophobicity for surfactants and perfluoro acids in the literature, and that these values correspond to uptake efficiencies up to about 100% based on an analysis as described above. Investigation on the mechanisms responsible for the uptake of ionized chemicals at the respiratory surfaces of fish has been limited, and further investigation would contribute to a better prediction of the uptake efficiency of ionizing compounds.

**TU208 Environmental risk assessment: which pH-range is relevant in the environment?** R. Wess, Harlan Laboratories Ltd / Global Registration and Strategic consulting; T.W. Schmidt, Harlan Laboratories Ltd / Ecotoxicology & Registration. The term "environmental pH" is widely used in the environmental risk assessment of chemicals, but the ranges used differ even between guidance documents, e.g. pH 4-9 in OPPTS 835.0001 (2008) or pH 5-9 in ECHA Chapter R.7A – Endpoint Specific Guidance (2012). The Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market considered the same range for the determination of the partition coefficient, obviously considering this range environmentally relevant. The range was extended to pH 4-10 by Commission Directive 94/37/EC of 22 July 1994. These obvious inconsistencies regarding the range of the environmental pH are problematic since the fate of some ionisable chemicals in the environment (e.g. water, sediment, soil) strongly depends on the pH within the respective medium: its solubility and bioavailability may vary at different environmental pH values since the unionised form of the compound tends to be more hydrophobic than the ionised form. In a first step, a synopsis of different regulatory sources is shown which are relevant for regulatory assessment regarding the different finalities (e.g. PPP, MHP, VMP, Biocide, Industrial Chemical) of chemicals. In the second step, pH values of relevant media for the entry of chemicals in the environment are collated in a literature survey. Depending on the results of the comparison between the pH-ranges of the survey and the



recently used guidance documents, a justification for the selection of one general environmentally relevant pH range may be given.

**TU209 Thermodynamics Study of Ferrous Ion Binding to Keratin from Sheep Wool** Y. Zhao; L. Chen, L. Han, China Agricultural University; G. Lian, Unilever Research Colworth. Ferrous ion has been shown to play a critical role as a mordant in dyeing hair, thus prompting intense investigations into the formation of metal–hair protein complexes. Keratin is the key structural component of hair and accounts for more than 90% of hair dry mass. In this study, Isothermal titration calorimetry (ITC) measurement has been used to study the associated thermodynamic properties of ferrous gluconate and keratin solution from sheep wool. ITC has been widely used to quantify metal-protein binding affinity and associated thermodynamics. By fitting the enthalpy data with the identical independent binding sites model, the association constant (K), stoichiometry (n), free energy (G) and enthalpy (H) associated with the interaction were obtained. 1 mole keratin can bind with 9 mole ferrous gluconate. The large positive entropy value obtained shows that  $\text{Fe}^{2+}$  binding to keratin is entropically driven. A systematic investigation of the binding of ferrous gluconate and keratin has been carried out by ITC at temperatures of 298 K, 308 K and 318 K. It turns out that increasing temperature results in an increase in the binding affinity, further suggesting that the interaction is entropy-driven. Positive entropy observed also indicates the changes in the hydration of the keratin upon  $\text{Fe}^{2+}$  ion binding to the protein.

**TU248 Keeping USEtox up-to-date: What is coming and how you can contribute** R.K. Rosenbaum, Technical University of Denmark / Management Engineering; P. Fantke, Technical University of Denmark; M.Z. Hauschild, Technical University of Denmark (DTU) / Dept. of Management Engineering; M.A. Huijbregts, Department of Environmental Science; O. Jolliet, University of Michigan / School of Public Health; M. Margni, Ecole Polytechnique de Montreal / Department of Mathematical and Industrial Engineering; T.E. McKone, University of California / Sustainable Energy Systems Group; D. van de Meent, RIVM / Institute of Wetland and Water Research. It is essential that USEtox keeps up with scientific developments and user needs, while adhering to its development principles, remaining an interface between science and application. The USEtox team intends to publish updates to the model algorithms and inputs balancing frequency and stability. Therefore, updates not affecting published characterization factors (CFs) can be done anytime. Updates affecting published CFs may be published yearly at the maximum. The USEtox team has developed an update procedure aiming for an optimal mix of transparency, stability, and scientific quality of model and CFs. Everybody can submit an update proposal via <http://update.usetox.org> and all (worthwhile) proposals – including those coming from the USEtox team itself – have to go through this procedure. Three kinds of updates are distinguished: 1) Corrective updates affecting existing CFs found erroneous (e.g. algorithms, parameters). Correction proposals will be decided and implemented by the USEtox team without consulting external experts. 2) Updates based on data, scientific, and technical progress, further distinguished into updates of a) substance-specific data (affecting one or very few CFs) and b) model structure, parameters, and algorithms (affecting many CFs). Updates of type 2 are differentiated respectively into those affecting existing CFs, promoting “interim” CFs to “recommended”, and those adding new CFs. For any type 2 update judged worthwhile, the review chair of the UNEP/SETAC Life Cycle Initiative invites external experts to review the proposal and advice on implementation with respect to the USEtox development criteria (e.g. scientific quality and consensus, parsimony, evaluation, transparency). Based on that the USEtox team decides whether and how to implement the proposal. If the USEtox team refuses a proposal recommended for implementation by the review panel, the reasoning shall be published. The USEtox team documents the entire updating process, making all documents and decisions publicly accessible (website). The update procedure assures transparency, quality, and independence, avoiding biased influences. Several updates have been submitted for the 2013 USEtox update, pending peer review and final approval, e.g. improved

modelling of freshwater metal toxicity, ionizing substances, indoor exposure, sub-continental-specific landscape parameter sets, exposure to pesticide residues in food and new substance input data.

**TU249 Biodiversity damage assessment for global warming considering extinction risk of species** L. Tang; M. Higa, N. Tanaka, Forestry and Forest Products Research Institute; N. Itsubo, Tokyo City University. According to Millennium Ecosystem Assessment (2005), climate change is recognized as one of the main factors of biodiversity damage. Though several damage assessment methods used in LCIA have been developed to quantify the damage of biodiversity caused by land use and chemical exposure, there are still few methods for climate change. Therefore the aim of this study is to develop a new method which can quantify the extinction risk per a unit of  $\text{CO}_2$  emission for each species using EINES (expected increase in number of species). Procedures are shown below. Firstly, the current and future potential distribution area (up to 2100 year, with two  $\text{CO}_2$  emission scenarios) of each species was predicted based on a niche based species distribution model. Secondly, extinction years of two emission scenarios were estimated by assuming that the decrease ratio of the potential distribution area every 100 years is constant. Finally, extinction risk of each species per a unit of  $\text{CO}_2$  emission was obtained by dividing the difference of EINES namely the change of inverse of life expectancy between two emission scenarios by additional  $\text{CO}_2$  emissions. Based on this method, representative values of extinction risk of vascular plants in the temperate zone in Japan was obtained using climatic data and distribution data of 216 plant species. The annual damage of plant species diversity caused by global warming is bigger than that caused by toxic substance pollution, and smaller than that caused by land use change.

**TU267 Demonstration of a modular computational tool for human health risk assessment due to contaminated sites or emissions from facilities** R. Bonnard, INERIS / Chronic Risks. In France, risk assessment studies have to be conducted before the implementation or the enlargement of some new facilities and before the reuse of some contaminated sites. These studies have to be performed in accordance to four principles : precautionary, specificity, proportionality, transparency. Until now, no devoted software has been developed in France to assess future exposure and risks for such studies. Because of that, many problems have been reported in exposure assessment studies leading to inconsistency between risk assessment studies and a lack of confidence in results provided. To improve the practices and the transparency of the estimates obtained in these studies, INERIS develops and diffuses modeling tools in the framework of one's missions for the Ministry in charge of the Environment. A peer-reviewed handbook, entitled “Sets of equations for modeling exposure linked to soil contamination or emissions from an industrial facility”, has been published and is available on INERIS' website. This document presents the equations used at INERIS for estimating the media concentrations, the exposure and risk levels. It also describes the origin of these equations and underlines the hypotheses on which they are built and their limits. A modeling and simulation platform (MODUL'ERS) based on the equations presented in this handbook has also been developed. Our main objectives during its conception were to provide a tool (1) suited to different site conditions and tier studies, (2) transparent for any stakeholders and helpful to perform uncertainties analysis. MODUL'ERS consists in a library of preset modules enabling the users to build models in accordance with the site conceptual model (that is to say the pathways from the source to the receptor), by downloading modules and connecting them, to create an exposure matrix. Many options are also available to create a customized application. To improve transparency of studies all the equations, parameters can be viewed by the users, as well all the intermediate calculations performed. Especially, hyperlinks enable to browse among variables and their equations. The first version of the tool will be available at soon. Coupling with a GIS is forecast. Some reports will be also published to make more transparent the assignment of values to input parameters, by describing the values collected and the choices made (best-estimate, ranges, probabilistic distributions).

**TU268 Developing and evaluating exposure models for use in chemical risk assessment in China** O. Price, Unilever / Colworth Science Park; A. Franco, Unilever; K. Jones, A. Sweetman, Lancaster University; S. Tao, Peking University; N. Xu, Peking University / Shenzhen Graduate School; Z. Liu, H. Wang, Chinese Research Academy of Environmental Sciences; G. Ying, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences; H. Li, Y. Zhu, S. Zhao, W. Chen, Lancaster University. As a developing industrialized country, China is evolving a progressive approach in the development of chemicals risk assessment regulations. The Ministry of Environmental Protection of China released in 2010 the revised version of the Provisions on Environmental Administration of New Chemical Substances. This new regulation, which replaced the previous hazard based approach issued in 2003, describes a risk based approach to chemicals management. As part of this process a new version of the existing chemical substances inventory, the Inventory of Existing Chemical Substances Produced or Imported in China (IECSC) was released, which records approximately 45,000 chemicals. The systematic prioritisation of chemicals on the IECSC is a complex task and requires a clear definition of the protection goals. However, to date no exposure models have been developed to enable a systematic prioritisation and currently the Authorities are using models that have been developed in Europe and the US, the relevance of which remains unknown (Liu et al., 2012). This project is tasked with developing a risk assessment framework, specific for the Chinese environment and population. Four work streams were initiated to develop: a regionalised multimedia fate model for China to provide an assessment of key source, transport and fate pathways; an understanding of the key human exposure pathways to determine the appropriate algorithms for consumption of key food groups to estimate indirect exposure to humans via the environment; methods to quantify exposure through sewage treatment plants (STPs) using a model that is representative of the technology, performance and connectivity in China; passive sampling technologies to enable the wide scale evaluation of surface water quality and STP performance in China. Here we provide an update on the research activities and future integration of the modules into a framework that could be used (1) to prioritise chemicals of concern in China, (2) in risk-based assessments and (3) to guide future National monitoring programmes.

**TU269 Apportionment of sources of PCDD/Fs in Baltic Sea sediment cores** A. Assefa, Umea University / Department of Chemistry; K. Sundqvist, NIRAS Environment; M. Tysklind, Umea University / Department of Chemistry; P. Geladi, Swedish University of Agricultural Sciences / Unit of Biomass Technology and Chemistry; K. Wiberg, Swedish University of Agricultural Sciences (SLU) / Department of Aquatic Sciences and Assessment. The Baltic Sea is one of the water bodies where high levels of dioxins are observed. In this study, historical contributions of responsible sources to offshore and coastal sediment cores of the Baltic Sea are presented. The source tracing was conducted using positive matrix factorization (PMF). Five factors (F) (potential source types) were obtained, then the chemical fingerprints of these were compared with real source fingerprints. The identification of the sources is summarized in Table 1. **Factor Source Remark on model fingerprint** F1 Atmospheric background or pentachlorophenol Complete dominance of Hp- and O- CDDs F2 Incinerator or High temperature processes Higher fractions of PCDFs F3 Tetrachlorophenol Dominance of 1234678-, 1234689- HpCDFs and OCDF F4 HxCDD / kraft pulp Dominance of 123679- and other HxCDDs F5 Chlorine (e.g. chlorine bleach, chlor-alkali prod.) Higher fraction of 2378- and other TCDFs Table 1: The five source profiles (Factors) identified by receptor modelling Generally, both in coastal and offshore areas the contributions of all sources have been decreasing from peak years (PkY) (Table 2). **Coastal Periods PkY F1 F2 F3 F4 F5 Residual** Seskarö Bay 1944-2009 1966 5-14 0-9 21-50 26-36 0-2 7-41 Gussö Bay 1972-2008 1992 17-18 20-21 20-24 28-31 1-2 8-9 Kallholm Bay 1928-2008 1984 12-15 61-63 4-7 3-6 3-4 10-15 Nordmaling Bay 1969-2008 1982 64-83 2-13 12-18 1-7 0-1 0-4 Nätra Bay 1961-2009

1976 17-28 3-15 15-23 2-11 18-28 9-35 Svartvik Bay 1960-2009 1970 30-43 0-15 19-35 4-11 9-24 5-11 Sandarne Bay 1925-2009 1967 8-20 0-3 4-19 49-70 0-2 7-19 Lövsta Bay 01 0cm-40cm 40cm 54-65 0 24-32 0 0 9-19 Lövsta Bay 02 0cm-47cm 40cm 52-67 0 26-44 0-3 0-1 0-11 Table 2: Percentage contributions of sources to coastal sediment cores after peak year

**WE094 Lethal and sublethal toxicity of six nanoparticles to freshwater primary producers and consumers.** T. Galindo, University of Aveiro; R. Pereira, University of Aveiro / CESAM, Center of Environmental and Marine Studies, University of Aveiro; F. Antunes, Department of Chemistry, University of Coimbra; T. Rocha-Santos, ISEIT, Instituto Piaget Viseu & Cesam; M. Rasteiro, University of Coimbra / Department of Chemical Engineering; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM - Centre for Environmental and Marine Studies; A.d. Duarte, University of Aveiro & CESAM / Chemistry; I. Lopes, University of Aveiro / CESAM. Engineered nanoparticles (NP) possess unique properties that enable their use for a wide variety of applications. Consequently, within the last years the use of NP in consumer products increased considerably. This widespread use of NP in the society foresees their inevitable release into the aquatic environment, where they can constitute a risk to aquatic biota. Accordingly, this study aimed at investigating the lethal and sublethal effects of six NP (vesicles of sodium dodecylsulfate-dodecyltrimethylammonium bromide SDS/DDAB, vesicles of monoline/sodium oleate Mo/NaO, gold rods NM-Au, quantum dot Lumidot™ CdSe/ZnS 530, TiO<sub>2</sub>, and TiSiO<sub>2</sub>) to the green algae *Pseudokirchneriella subcapitata* (primary producer) and to the rotifer *Brachionus calyciflorus* (primary consumer). These two species constitute important model organism that have long been used in ecotoxicology studies, as they are very sensitive to chemical contamination and are representatives of different trophic and functional levels. To attain the main goal of this work, each test species was exposed to serial dilutions of the six NP plus the corresponding control (media without NP) for 72h (algae), 24h (lethal effects; rotifer) and 48h (sublethal effects; rotifer). At the end of the assays the population growth rate was calculated for both species and percentage of mortality was computed for the rotifers. In order to characterize NP exposure, zeta potential, hydrodynamic diameter and surface charge were determined by light scattering techniques and the NP size was determined by transmission electron microscopy. All NP formed aggregates when suspended in the aqueous media. Except for Mo/NaO, the size of the NP-aggregates was higher in the rotifer media, comparatively with the medium used for algae assays. The NP of TiO<sub>2</sub> and TiSiO<sub>2</sub> did not exert toxic effects to the rotifer or to the green algae, even at high concentrations (49 mg/L), which is in agreement with data already reported in the literature for Ti based NP. For the other four NP, *B. calyciflorus* exhibited a higher sensitivity than *P. subcapitata*, as in general rotifers mortality occurred at concentrations below those that induced sublethal effects in algae: LC<sub>50</sub> for rotifers were 0.39 mg/L (SDS/DDAB), 17.27 mg/L (Mo/NaO), 50.24 μg/L (NP-Au), and 3.14 mg/L (QD-CdSe/ZnS), and EC<sub>50</sub> for *P. subcapitata* were 4.54 mg/L (SDS/DDAB), 42.54 mg/L (Mo/NaO), 59.56 μg/L (NP-Au), and 7.86 mg/L (QD-CdSe/ZnS). No association between toxicity and NP size was observed for both tested species.

**WE095 Investigation of toxicity of MWCNT of different functional groups and sizes using in vitro and in vivo (C.elegans) tests.** L. Choi, School of Environmental Engineering; C.P. Roca, Universitat Rovira i Virgili; H. Eom, J. Jung, University of Seoul; F. Giralte, Universitat Rovira i Virgili. Carbon nanotubes (CNTs) have attracted a great deal of attention due to their unique structural, physical and chemical properties that lend their use to a variety of industrial and biomedical applications. However, there are concerns that the properties of the CNTs might lead to adverse health effects and various factors, such as, tube size, state, functional groups and impurities may contribute to varying toxicological effects of CNTs. Therefore, in this study, toxicity of multiwall carbon nanotube (MWCNT) with functional groups and sizes was compared using HepG2 cells cytotoxicity test and the

nematode *Caenorhabditis elegans* survival test, subsequently their mechanism of toxicity was investigated using proteomics and functional mutant analyses. To identify the effect of functional group on toxicity of MWCNT, toxicity of pristine, OH- and COOH-MWCNT was compared. To test the effect of sizes on toxicity, MWCNT with small outer diameter (OD) was compared to that with big OD similarly, MWCNT with short tube length was compared to that with long tube. It was found that functionalized MWCNT was more toxic than pristine MWCNT in *C. elegans* survival test and OH-MWCNT was more toxic than COOH-MWCNT in HepG2 cells cytotoxicity test. MWCNT with small OD is more toxic than that with big OD and short MWCNT is more toxic than long tube in both cytotoxicity and survival tests. Toxic intensity of MWCNT in HepG2 cells was found to be correlated with the hydrodynamic diameters of MWCNT in test media measured with DLS. In the next step, to understand the intrinsic toxicity of MWCNT, proteomics followed by pathway analysis was conducted on pristine MWCNT exposed *C. elegans*. Differentially expressed proteins (DEPs) and pathway analyses revealed endocytosis, phagocytosis and ER stress being involved in MWCNT toxicity. To further confirm this, survival test was conducted on *C. elegans* mutants of the genes detected in stress response pathways raised in proteomics-pathway analyses. Mutants were exposed to MWCNTs with different functional groups and sizes. The results suggest small OD with short OH-MWCNT is the most toxic among tested MWCNTs and ER stress and endocytosis being involved in MWCNT toxicity. Overall results suggest that toxicity of MWCNT is dependent on its functional groups and sizes. The study also suggests integrated approach seems to provide comprehensive insight to understand toxicity of chemicals, of which toxicity is poorly understood, such as, MWCNT.

**WE096 Operationalization and application of “early warning signs” to screen nanomaterials for harmful properties** S.F. Foss Hansen, Technical University of Denmark / DTU Environment; K.N. Nielsen, GenØk, Centre for Biosafety; N. Knudsen, LEO Pharma A/S; K.D. Grieger, Technical University of Denmark / Department of Management Engineering; A. Baun, Technical University of Denmark / Department of Environmental Engineering. In 2001 the European Environment Agency (EEA) published a report that analyzed 14 cases of technological developments that later on turned out to have negative side-effects and they identified 12 “late lessons” for current and future policy-makers to have in mind when initiating new technological endeavors. This paper explores how the first lesson - “Acknowledge and respond to ignorance, uncertainty and risk in technology appraisal” could be applied to screen nanomaterials. In cases of ignorance, uncertainty and risk, the EEA recommends paying particular attention to important warning signs such as novelty, persistency, whether materials are readily dispersed in the environment, whether they bioaccumulate or lead to potentially irreversible action. Through an analysis of these criteria using five well-known nanomaterials (titanium dioxide, carbon nanotubes, liposomes, poly(lactic-co-glycolic acid) and nanoscale zero-valent iron, and carbon nanotubes), it was found that only nanoTiO<sub>2</sub> fulfills all the five criteria. Dependent on the length of the nanotubes, carbon nanotubes fulfills 3 or 4 criteria whereas liposomes, poly(lactic-co-glycolic acid), nanoscale zero-valent iron fulfills only one criteria. Finally, we discuss how these warning signs can be used by different stakeholders such as nanomaterial researchers and developers, companies and regulators to design benign nanomaterials, communicate what is known about nano-risks and decide on whether to implement precautionary regulatory measures. \n

**WE097 Species sensitivity distributions for silver and zinc oxide nanoparticles: using taxonomic and trait-based risk assessment approaches** S. Loureiro, Universidade de Aveiro / Biology; P.V. Silva, department of Biology & CESAM; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science; D. Spurgeon, Centre for Ecology Hydrology; T. Backhaus, University of Gothenburg / Dep. of Biological and Environmental Sciences; M. Matzke, Centre for Ecology Hydrology NERC / Hails Section; C. Svendsen, CEH Wallingford / Pollution and Ecotoxicology. Nanotechnology industry has been

developing potential materials and applications for the welfare and human benefit, resulting in the large scale production of nanoparticles (NPs). The proliferation of manufactured NPs and their consequent entrance in the environment has led to concerns about their potential effects on organisms. Silver and zinc oxide nanoparticles (Ag-NPs and ZnO-NPs) are two metal-based NPs forms that are currently in widespread use (e.g. as antimicrobial agent and in personal care products). This work aims to investigate how well and with what modifications species sensitivity distribution (SSD) methodology can be applied to address the ecological risk of silver and zinc oxide NPs towards aquatic and terrestrial organisms and the estimation of protective environmental limits. Toxicity data was gathered from the published literature and from results obtained within the EU-funded NanoFATE project. Applying SSDs the hazardous concentrations at 5% (HC) were estimated through two different approaches, using the geometric mean effect concentrations (EC50, EC10) for the Ag-NPs and ZnO-NPs for 1) each taxonomic group and also 2) considering the relevant functional role or physiological traits of the test species. The results analysis also took into account how possible patterns resulting from effects of size and charge of the NPs, media properties, amongst others can be taken into account.

**WE240 Predictions gone wrong: mixture toxicity of a repellent and a pyrethroid on *Daphnia magna*** R. Wolf, Goethe University / Aquatic Ecotoxicology; M. Wagner, Goethe University Frankfurt / Dept. Aquatic Ecotoxicology; J. Oehlmann, Johann Wolfgang Goethe-Universität Frankfurt am / Aquatic Ecotoxicology. In the past decades, combinatory effects of substances have been of growing interest. Still, knowledge on the “cocktail-effect” of compounds is limited. For the prediction of mixture toxicity, the models of concentration addition (CA) and independent action (IA) are well established. CA’s main assumption is a common mode of action (MoA) of substances, while IA assumes different molecular targets. Repellents and insecticides are both regularly detected in aquatic ecosystems. In addition to the most common and effective repellent, N,N-diethyl-m-toluamide (DEET), pyrethroids (e.g. permethrin) are often used to impregnate cloth. DEET’s MoA is a competitive inhibition of acetylcholine esterase, whereas permethrin irreversibly opens pre-synaptic, voltage dependent sodium channels. Both result in elevated levels of acetylcholine within the synaptic cleft and hence increase nervous excitation, ultimately killing target organisms. The water flea *Daphnia magna* was chosen as pelagic non-target organism to evaluate the effects of a combined exposure to DEET and permethrin. Mixture toxicity was predicted using single substance data and applying CA and IA models. As expected, the toxicity of the mixture is more severe than those of DEET and permethrin alone. With increasing exposure time, the observations diverge from the predictions provided by the CA and IA models. The failure of the models’ predictions could be due to indirect effects of the chemicals. The iso-effect plot results in a predicted “no effect concentration” (NEC) equalling 50 µM of the mixture. Interestingly, testing for 48 hours would have been insufficient for an assessment on long term effects. With regard to future assessments of mixture toxicity, it should be considered that *Daphnia magna* acute toxicity assays may not provide ideal terminal effects for testing ecotoxicological relevant mixtures. Biomarkers, however, would be a welcome alternative. Additionally, we think it is necessary to extend testing periods. In the future, a more detailed mixture toxicity testing, including biomarkers, and an appropriate test duration would be desirable

**WE241 Mixture toxicity assessment within the biocidal products authorisation process** A. Kehrer, Federal Environment Agency; D. Frein, Umweltbundesamt / IV; C. Riedhammer, B. Schwarz-Schulz, Federal Environment Agency (Umweltbundesamt, UBA). Biocidal products are usually multi-component mixtures of one or more active substances and a range of co-formulants that serve different purposes e.g. anti-foaming agents, stabilisers, pigments, emulsifiers, solvents or diluents. Additionally, metabolites and degradation products might be formed during and after use of a biocidal product. Therefore the overall ecotoxicity of a biocidal product might be significantly different from

that of each individual ingredient(s) alone and hence, needs to be assessed during the product authorisation. In fact, the assessment of the mixture (eco-) toxicity is foreseen by the new Biocidal Products Regulation (BPR; Regulation (EU) 528/2012) which will replace the current Biocidal Products Directive (BPD, Directive 98/8/EC) in September 2013. The article 19(2) states that “*the evaluation [...] shall take into account the following factors: [...] (d) cumulative effects, (e) synergistic effects.*” This is further elaborated in Annex VI (common principles for the evaluation of biocidal products) which states that the risks associated with the relevant individual components of the biocidal product shall be assessed, taking into account any cumulative and synergistic effects. However, only very limited details on how mixture effects should be considered during the authorisation of a biocidal product are provided in the current Guidance Documents and no specific guidance is at hand on how potential combination effects of active substance(s) and other ingredients should be accounted for during the environmental risk assessment of biocidal products. Therefore a tiered assessment scheme for an adequate consideration of mixture effects during the environmental risk assessment (ERA) of biocidal products was developed to assess the mixture toxicity of the products and/or, where relevant, also of the environmental relevant mixtures derived from the use of the products as well as synergistic effects as required by BPR and BPD. The scheme presented was discussed and finally agreed among the European Member states at the Biocides Technical Meetings III/2012 and I/2013. More detailed information on the tiered assessment approach can be found in the Guidance Document on mixture toxicity assessment during biocidal products authorisation prepared by the German Federal Environment Agency (Umweltbundesamt, UBA), which will be made available after final discussion at member state level in summer 2013.

#### **WE242 Risk assessment of chemical mixtures on the Swedish west coast using biotic and water quality monitoring data**

**M. Gustavsson**; T. Backhaus, University of Gothenburg / Dep. of Biological and Environmental Sciences. Currently chemical monitoring on the Swedish west coast is carried out in a slightly fragmented manner. The monitoring is carried out in a large number of different programs. For instance a national monitoring program exist which primarily look for persistent organic pollutants and heavy metals in biotic as well as sediment samples. Also, a number of regional programs with differentiated focuses between programs, as well as years, survey for compounds with everything from agricultural to consumer product origins. These regional programs deal with a number of different sampling matrices such as biota, sediments and water. But also sewage treatment effluent and sludge is sampled in some campaigns. Finally, so called screening programs are carried out yearly in a multitude of different sampling matrices. These screening programs generally focus on a different set of compounds between each campaign, trying to act as early warning systems for emerging contaminants. Thus, all of these campaigns are in essence geared towards the single compound, single risk paradigm. Mixture toxicity and risk assessment of chemical mixtures has been receiving increasing attention over the last few years and the need to get an overview of the combined exposure has become even more apparent. By merging all of the available data from the monitoring campaigns mentioned, a more in depth view of the exposure scenario on the Swedish west coast becomes possible. Combining this combined database with knowledge of the sensitivity of algae, crustaceans and fish enables the risk assessment of the combined exposure scenario on the Swedish west coast. A number of suggestions on how monitoring can be performed in the mixture toxicity paradigm is also provided; hopefully enhancing the power of each individual sample taken from a risk assessment point of view.

#### **WE243 Use and interpretation of bioanalytical tools in the drinking water production chain – casus Ames fluctuation test**

**A. van Wezel**, KIWA Water Research; M. Schriks, L. Puijker, KWR Watercycle Research Institute. Over 100.000 chemical compounds are in daily use. Many compounds are found in low concentrations in sewage effluents, groundwater and surface waters. Only for some official drinking water

quality guidelines do exist. Consistently it is concluded that there is a large margin of safety and human health risks are unlikely. Nevertheless, the possible presence of chemical contaminants in drinking water is a major concern for citizens. Bioanalytical tools can give a clue on possible mixture effects, without knowing all individual compounds in the mixture. Within the drinking water sector, concentrations of chemicals occurring in (sources for) drinking water are very low and hence the focus is thus upon hormonal disruption and reactive toxicity, both possibly relevant modes of toxicological action for human health at chronic exposure to mixtures of compounds in low concentrations. As the use of bioanalytical tools within the context of drinking water production is relatively recent, these are not yet embedded within any legislation. Guidelines for the interpretation of results gathered with help of bioanalytical tools have not been developed yet within this context, neither decision support schemes for subsequent steps. Here, we describe a first attempt for such interpretation and subsequent steps, based on an extensive discussion within the Dutch drinking water sector. The ‘*Threshold of Toxicological Concern*’ (TTC) is helpful for risk assessment of data-poor chemicals. For drinking water, conservative TTC-values are deduced of 0,1 µg/L for non-genotoxic (sum 1 µg/L) and 0,01 µg/L for genotoxic compounds (sum 0,05 µg/L). The response in the bioanalytical tests expressed in term of equivalents can be compared with the TTC-values, to conclude on potential relevance of the response. After a repeated relevant response: -on the drinking water production site evaluate and possibly adapt treatment and sources used -discover the cause of the effect; is it a natural or anthropogenic cause, can it be induced during treatment? -combine bioanalytical and analytical-chemical tools to identify responsible (mixture of) compounds, followed by human toxicological risk assessment -in case that forementioned approaches do not yield enough information and the effects are measured in the final product, a risk assessment based on in vivo experiments is possible

#### **TH073 Linking algal toxicity with freely dissolved concentrations of organic contaminants in the Firth of Forth Scotland**

**S. Emelogu**, The Robert Gordon University Aberdeen / Engineering; P. Pollard, The Robert Gordon University Aberdeen / Institute for Innovation, Design and Sustainability in research (IDEAS); C.D. Robinson, L. Webster, Marine Scotland Science; C. McKenzie, The Robert Gordon University Aberdeen / Institute for Innovation, Design and Sustainability in research (IDEAS); J. Dobson, Scottish Environment Protection Agency / Marine; P. Dymond, E. Bresnan, C.F. Moffat, Marine Scotland Science. The Firth of Forth in the east coast of Scotland receives inputs of a diverse range of trace organic contaminants from petrochemical, agro-chemical and pharmaceutical industries and discharges of urban waste water. Diffuse input through agricultural, urban and riverine run-off further adds to the contaminant pressure in the estuary. Due to recent legislation, direct discharges of organic materials into the Firth of Forth are in the decline. As a result, in addition to the low aqueous solubility of organic contaminants and dilution factors, the concentrations of most organic compounds monitored in the water phase using conventional sampling techniques are often very low. This, however, does not exclude their potential to cause toxicological effects on sensitive organisms, owing to their persistent, bioaccumulative and toxic potentials. Further, chemical analysis cannot assess the bioavailability or the combined toxicity of all the identified and unknown compounds to which organisms are exposed. In this study, silicone rubber passive sampling devices (SR-PSDs) were deployed in water at five sites along the estuary for ~2 months. Extracts from the deployed SR-PSDs were assessed for both algal growth inhibition and environmental exposure of wide range of organic contaminants including PAHs, PCBs and pesticides. Algal toxicity of the complex mixtures of freely dissolved organic contaminants were assessed on a native algal strain *Pavlova lutheri* in 24 well multititre plates for 72 hr. Time weighted average concentrations ( $C_{TWA}$ ) of freely dissolved (e.g. bioavailable) fractions of 40 PAHs and 32 PCBs were calculated using performance reference compounds (PRCs). The algal toxicity tests exhibited varied effects at the five sites suggesting the presence of algal growth inhibiting compounds in the estuary. Dissolved concentrations of the individual contaminants

measured did not differ significantly at the five sites. However, slightly higher total dissolved concentrations were measured from the upper estuary to the inner Firth of Forth suggesting the influence of dilution. Further, pesticides of diverse polarities were identified in the extracts indicating sources from agricultural and riverine run-off or discharges from the agro-chemical industries. This study thus illustrates the value of combining bioassays and chemical analysis (with an effective sampling technique) for a realistic and rapid assessment of organic contaminants in the aquatic environment.

#### **TH074 Assessment of different passive samplers to predict bioaccumulation of polycyclic aromatic hydrocarbons by carrot roots**

**L. Kokovic**, University of Reading / Geography and Environmental Science; C.D. Collins, Reading University / Department of Geography and Environmental Science. Polycyclic aromatic hydrocarbons (PAHs) belong to a class of hydrophobic organic pollutants, produced during the incomplete combustion or pyrolysis of organic materials from primarily anthropogenic sources such as fossil fuels and agricultural waste. The fate of PAHs in the nature is of great concern to human health as these contaminants are widely distributed in the environment and are known to be toxic, mutagenic and carcinogenic. In the past decades, various passive sampler devices (PSDs) have been developed to concentrate hydrophobic organic compounds (HOCs) from environmental matrixes and to mimic bioconcentration. Previous research has indicated that triolein embedded cellulose acetate membrane (TECAM) and petroselinic acid embedded cellulose acetate membrane (PECAM), types of lipid containing membranes have potential to accumulate certain polycyclic aromatic hydrocarbons (PAHs) from water and soil and to estimate the bioavailability of these pollutants to living organisms. The present study compares the ability of these passive samplers to mimic bioaccumulation of 15 PAH prioritised by the European Scientific Committee on Food (ESCF) in carrot roots grown in acid washed sand.

#### **TH075 Using performance reference compounds to evaluate freely dissolved concentrations of DDT and its degradates in contaminated sediments**

**A. Tcaciuc**, Massachusetts Institute of Technology / Department of Civil and Environmental Engineering; R. Borrelli, ENI S.p.A. / Corporate; P. Cesti, ENI S.p.A. / Direzione Ricerca ed Innovazione Tecnologica, Centro Ricerche per le Energie non Convenzionali; J. MacFarlane, P. Gschwend, Massachusetts Institute of Technology / Department of Civil and Environmental Engineering. Despite its ban in the 1970's, DDT and its degradates remain pollutants of concern at various sites in the world. Passive samplers have been used successfully for determining freely dissolved concentrations of various hydrophobic organic contaminants such as PCBs and PAHs, in pore waters of contaminated sediments. The losses of performance reference compounds (PRCs), which are preloaded into the sampler, are used to assess how close to equilibrium the sampler is after the deployment time. However, in our recent work involving *in situ* deployment of passive samplers in DDT-contaminated sediments, where the <sup>13</sup>C labeled DDE, DDD and DDT were used as performance reference compounds, we observed a loss of the DDT PRC that was inconsistent with expectations based on its mass transfer properties and the behavior of the other PRCs. This was observed both in *in situ* and in lab exposures of PE to contaminated sediments. We believe that this loss is due to a reaction which is consuming the DDT PRC, and which has implications for the behavior of the DDE and DDD PRCs, the potential products of the reaction. Also this substantially complicates estimation of the correction factors needed to calculate the equilibrium concentration of the compounds of interest in the passive sampler. We have developed a reaction-diffusion model for understanding the behavior of the PRCs and for calculating the equilibrium concentrations in the sampler of the compounds of interest. To validate our model, we compare the results of this model against concentrations of these compounds from tumbling experiments done in the laboratory with the sediments collected from the deployment sites.

#### **TH076 Passive dosing in aquatic toxicity testing with the nematode**

**Caenorhabditis elegans** **S. Schaefer**, Federal Institute of Hydrology; P. Zurek, University of Applied Sciences Bingen; C. Moehlenkamp, Federal Institute of Hydrology; B. Becker, University of Applied Sciences Emden Leer; S. Hoess, Ecossa; E. Bluebaum-Gronau, G. Reifferscheid, E. Claus, Federal Institute of Hydrology. Nematodes are the most abundant multi-cellular organisms in sediment and soils. The bacterivorous nematode *Caenorhabditis elegans* - a well-studied model organism - is frequently used to assess the toxicity of sediments, soils and aqueous solutions (ISO 10872). As in many aquatic bioassays, toxicity testing of hydrophobic compounds is challenging due to their low aqueous solubility and the difficulties in maintaining constant freely dissolved concentrations. In order to control freely dissolved concentrations of analytes the standard nematode bioassay was modified by passive dosing of test compounds. Four chlorobenzenes (1,2,3-TCB, 1,2,3,4-TeCB, PeCB and HCB) as well as the disinfectant triclosan and its degradation product methyltriclosan were loaded on silicone O-rings as passive dosing reservoir. Since preliminary experiments with chlorobenzenes have shown that equilibration between medium and O-ring is not achieved in micro plates that are frequently used for this bioassay, tests were run in 10 ml-headspace vials. The effect of the type of test vial - micro plate vs. headspace vials - on *C. elegans* was previously assessed. O-rings were loaded by partitioning from a methanolic solution containing the test compounds. The release of analytes from O-rings into nematode test medium was assessed for determining required equilibration times before start of nematode toxicity testing. Freely dissolved concentrations were measured with SPME fibres simultaneously exposed in the nematode medium inside the test vessels and compared to liquid-liquid extraction of aqueous samples. Analytes were quantified by GC with MS/MS detection. After determining the loading and release kinetics of the selected analytes, their toxicity on growth and reproduction of *C. elegans* was assessed after exposure through passive dosing. The data were compared to toxicity data obtained after active spiking of test compounds into the test using acetone as solvent.

**TH117 Bioenergy – A Review of Environmental Impacts** **S. Muench, E. Guenther, R. Rieckhof**, TU Dresden. On a global scale, bioenergy plays a dominant role among renewable-energy options. Unlike fossil fuels, bioenergy can be carbon-neutral and will play an important role in the reduction of greenhouse gas emissions. While an assortment of reviews of biofuel Life Cycle Assessments (LCAs) is already available, only few of them are concerned with electricity and heat generation from biomass. Furthermore, the latter studies only include energy consumption and global warming potential as impact categories. We come to the conclusion that the insufficient consideration of impact categories in bioenergy LCA reviews constitutes a research gap. The goal of this study is to synthesise state of the art bioenergy LCAs and deduct further research needs. A systematic review is chosen as research method to gain a comprehensive and unbiased overview of state of the art bioenergy LCAs. We find that global warming potential, abiotic depletion potential, eutrophication potential and photo-oxidant creation potential are the impact categories most frequently addressed in bioenergy LCAs. The results of bioenergy LCAs show significant variations. Three main sources of variations are choices regarding allocation procedures, the system boundary and whether to include environmental impacts from capital equipment and transportation. There are still multifaceted discussions regarding methodological issues in bioenergy LCAs. No general consensus has been reached regarding the optimal functional unit for the environmental analysis of bioenergy, the ideal allocation of environmental impacts when more than one product is produced as well as how to treat alternative biomass fuel pathways such as decomposition if it is not used for energy purposes. The impact categories land use, biotic depletion, respiratory organics and respiratory inorganics are not often considered in bioenergy LCAs. A few LCAs already include an economic assessment whereas social factors have been widely ignored so far. This review is a first step in identifying sources of deviating results in bioenergy LCAs due to choices. We conclude that a stricter standardisation of environmental evaluations of bioenergy is recommended to improve their comparability. Furthermore,

considering economic and social factors in LCAs will be an important step towards a holistic sustainability assessment of bioenergy pathways.

**TH121 Spanish biofuels sustainability assessment, by means of life cycle analysis**

**I. Herrera**, CIEMAT / Energy Dpt. - Energy Systems Analysis Unit; **C. LAGO**, **Y. LECHON**, **R. SAEZ**, CIEMAT. Sustainability criteria have been set to biofuels to be consumed in Europe. Following these criteria, only biofuels that can prove greenhouse gas (GHG) emissions savings of at least 35% (and 50% from 2017 onwards) can be taken into account in order to (a) measure compliance with the requirements of Renewable Energy Directive (RED); (b) measure compliance with renewable energy obligations and (c) be eligible for financial support. In order to implement these sustainability criteria, the purpose of this study is to carry out updated and developed life cycle assessments of biofuels produced and used in Spain during 2010. This study included the current characteristics of the biofuels consumed in Spain. Furthermore, some default and typical GHG emission values for many biofuel pathways are provided disaggregated in the different stages of the cycle namely: raw materials cultivation, processing and transport & distribution. The study includes sensitivity analyses showing the impact on changed raw material and origin. Results show the best sustainable pathway for sugarcane in the case of imported ethanol and the barley for internal production. For biodiesel, soybean would be the best raw material for imported, and in the case of domestic production is the waste vegetable or animal oil the raw material with the best environmental behaviour.

**TH122 Sustainability assessment to guide the development of new biosolar technologies: defining the system first**

**C. Van der Giesen**, Leiden University / Institute of Environmental Science (CML); **R. Kleijn**, **G. Kramer**, Leiden University. The Dutch research program Toward Bio Solar Cells (TBSC) aims to develop technologies that make better use of solar energy by artificially improving the process of photosynthesis. To guide the development of these technologies, performance criteria for contributing to a sustainable energy future are identified and analyzed by performing a sustainability assessment. For this assessment the LCSA framework as presented in the CALCAS project ([www.calcas.net](http://www.calcas.net)) is used. This framework covers the assessing environmental, social and economic performance of technologies on micro, meso and macro scale levels and therefore a comparison of biosolar technologies with their competitors on the relevant sustainable performance criteria. Since little practical experience with LCSA exist, in this research a stepwise approach is applied. Here we focus on the first step: defining the broad system in which the technologies are expected to be implemented. This broad system description consists of a technical description of the specific technology under assessment, its intended use or function, alternatives or competing (existing) technologies and the societal context in which the technology will be implemented. Next steps will be (2) scenario building, (3) defining indicators and tools, (4) applying tools to calculate indicators and (5) interpreting results. TBSC technologies are still in their earliest stage of development which makes that the availability of data is limited. Therefore first the technical context and possible application of the technologies are defined. It is assumed that the theoretical concept of solar fuels provides an appropriate system for analysis. Solar fuels can be described as liquid hydrocarbon fuels produced from solar energy, carbon dioxide and water. A first comparative LCA study focused on a comparison of different routes to produce solar fuels and existing (renewable) fuels. It provides a technical description and an assessment of the environmental performance of relevant alternatives to biosolar technologies on a micro scale. Additionally, stakeholder consultations are organized in which the LCA results are discussed and social-economic drivers for a sustainable energy future are identified through workshops. The outcomes are used to develop scenario's and to define environmental and socio-economic indicators that are relevant to assess the performance of biosolar technologies in the future and on meso/macro scales.

**TH123 Development of Social LCA database considering**

**employment and labor accident** **N. Matsunaga**; **N. Itsubo**, Tokyo City University; **Y. Ono**; **H. Yamaguchi**, Tokyo City University; **K. Horiguchi**, Tokyo City University / Research Division in Env and Infor Studies. There are many study focus on evaluation of sustainability have been conduct in LCA. These sustainability assessment are based on Life Cycle Initiative proposed the method for sustainability assessment based on environmental aspects LCA, the economic aspects LCC, and the social aspects SLCA. International standards and guidelines for sustainability assessment have been created by ISO. On the other hand, ISO also mentioned that it is important to take into account the triple bottom line of environmental, economic and social aspects. However, there is no detail item or method was determined in the social aspects of sustainability assessment. Therefore, some method on LCA and LCC have been developed, but SLCA is still under development. In this study, a social LCA database considering employment and labor accident was developed. Finally, the database was verified by some case study.