Investigation of Immunotoxicity and Thyroid Disrupting Effects after Developmental Exposure to Perfluorohexane Sulfonate (PFHxS) in Rats

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Creating a Safer and Healthier World by Advancing the Science of and Increasing the Impact of Toxicology

2016 Annual Meeting Abstract Supplement

Late-Breaking Abstract Submissions

All Late-Breaking Abstracts are presented on Thursday, March 17, from 9:30 am–12:45 pm.

These abstracts are available via the Mobile Event App, Online Planner, and a downloadable PDF from the SOT website.

55th Annual Meeting and ToxExpo™

March 13–17, 2016

New Orleans, Louisiana

SOT

THURSDAY POSTER SESSION MAP

March 17, 2016—9:30 AM to 12:45 PM—Great Hall A

Poster Set Up—8:30 AM to 9:30 AM

Late Breaking Poster #s: P251-P646 (highlighted in gray)

P646	*P645*	*P644*	*P643*	*P642*	*P641*	*P640*	*P639*	*P638*	*P637*	*P636*	*P635*	*P634*	*P633*	*P632*	*P631*
P615	*P616*	*P617*	*P618*	*P619*	*P620*	*P621*	*P622*	*P623*	*P624*	*P625*	*P626*	*P627*	*P628*	*P629*	*P630*
P614	*P613*	*P612*	*P611*	*P610*	*P609*	*P608*	*P607*	*P606*	*P605*	*P604*	*P603*	*P602*	*P601*	*P600*	*P599*
P583	*P584*	*P585*	*P586*	*P587*	*P588*	*P589*	*P590*	*P591*	*P592*	*P593*	*P594*	*P595*	*P596*	*P597*	*P598*
P582	*P581*	*P580*	*P579*	*P578*	*P577*	*P576*	*P575*	*P574*	*P573*	*P572*	*P571*	*P570*	*P569*	*P568*	*P567*
P551	*P552*	*P553*	*P554*	*P555*	*P556*	*P557*	*P558*	*P559*	*P560*	*P561*	*P562*	*P563*	*P564*	*P565*	*P566*
P550	*P549*	*P548*	*P547*	*P546*	*P545*	*P544*	*P543*	*P542*	*P541*	*P540*	*P539*	*P538*	*P537*	*P536*	*P535*
P519	*P520*	*P521*	*P522*	*P523*	*P524*	*P525*	*P526*	*P527*	*P528*	*P529*	*P530*	*P531*	*P532*	*P533*	*P534*
P518	*P517*	*P516*	*P515*	*P514*	*P513*	*P512*	*P511*	*P510*	*P509*	*P508*	*P507*	*P506*	*P505*	*P504*	*P503*
P487	*P488*	*P489*	*P490*	*P491*	*P492*	*P493*	*P494*	*P495*	*P496*	*P497*	*P498*	*P499*	*P500*	*P501*	*P502*
P486	*P485*	*P484*	*P483*	*P482*	*P481*	*P480*	*P479*	*P478*	*P477*	*P476*	*P475*	*P474*	*P473*	*P472*	*P471*
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P454	*P453*	*P452*	*P451*	*P450*	*P449*	*P448*	*P447*	*P446*	*P445*	*P444*	*P443*	*P442*	*P441*	*P440*	*P439*
P423	*P424*	*P425*	*P426*	*P427*	*P428*	*P429*	*P430*	*P431*	*P432*	*P433*	*P434*	*P435*	*P436*	*P437*	*P438*
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P391	*P392*	*P393*	*P394*	*P395*	*P396*	*P397*	*P398*	*P399*	*P400*	*P401*	*P402*	*P403*	*P404*	*P405*	*P406*
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P359	*P360*	*P361*	*P362*	*P363*	*P364*	*P365*	*P366*	*P367*	*P368*	*P369*	*P370*	*P371*	*P372*	*P373*	*P374*
P358	*P357*	*P356*	*P355*	*P354*	*P353*	*P352*	*P351*	*P350*	*P349*	*P348*	*P347*	*P346*	*P345*	*P344*	*P343*
P327	*P328*	*P329*	*P330*	*P331*	*P332*	*P333*	*P334*	*P335*	*P336*	*P337*	*P338*	*P339*	*P340*	*P341*	*P342*
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P295	*P296*	*P297*	*P298*	*P299*	*P300*	*P301*	*P302*	*P303*	*P304*	*P305*	*P306*	*P307*	*P308*	*P309*	*P310*
P294	*P293*	*P292*	*P291*	*P290*	*P289*	*P288*	*P287*	*P286*	*P285*	*P284*	*P283*	*P282*	*P281*	*P280*	*P279*
P263	*P264*	*P265*	*P266*	*P267*	*P268*	*P269*	*P270*	*P271*	*P272*	*P273*	*P274*	*P275*	*P276*	*P277*	*P278*
P262	*P261*	*P260*	*P259*	*P258*	*P257*	*P256*	*P255*	*P254*	*P253*	*P252*	*P251*	P250	P249	P248	P247
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P165	P166	P167	P168	P169	P170	P171	P172	P173	P174	P175	P176	P177	P178	P179	P180
P164	P163	P162	P161	P160	P159	P158	P157	P156	P155	P154	P153	P152	P151	P150	P149
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Entrance

Photography and video in all poster sessions is prohibited without the consent of the poster presenter(s)/author(s). Please respect your colleagues' rights to privacy.

Thursday, March 17, Poster Session by Location

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Emerging Technologies	3559-3562	P251-P254
Nanotoxicology	3563-3578	P255-P270
Systems Biology and Toxicology, and Chemical and Biological Weapons	3579-3586	P271-P278
Respiratory Toxicology	3587-3601	P279-P293
Oxidative Injury and Redox Biology, POPs, and Regulation/Policy	3602-3615	P294-P307
Late-Breaking Poster Session 2		
Biological Modeling	3616-3621	P308-P313
Risk Assessment, and Exposure Assessment/Biomonitoring	3622-3651	P314-P343
Safety Assessment: Non-Pharmaceutical	3652-3656	P344-P348
Safety Assessment: Drug Development and Discovery	3657-3671	P349-P363
Mixtures	3672-3673	P364-P365
Late-Breaking Poster Session 3		
Animal Models	3674-3678	P366-P370
Disposition/Pharmacokinetics	3679-3681	P371-P373
Developmental/Juvenile Toxicology and Developmental Basis of Adult Disease	3682-3690	P374-P382
Reproductive Toxicology and Endocrine Toxicology	3691-3711	P383-P403
Epidemiology and Public Health	3712-3719	P404-P411
Pharmacogenomics/Genetic Polymorphisms, and Receptors	3720-3724	P412-P416
Stem Cell Biology and Toxicology	3725-3727	P417-P419
Clinical and Translational Toxicology	3728-3730	P420-P422
Late-Breaking Poster Session 4		
Computational Toxicology and Data Integration, and Bioinformatics	3731-3743	P423-P435
Biomarkers	3744-3753	P436-P445
Biotransformation/Cytochrome P450	3754-3758	P446-P450
Gene Regulation/Signal Transduction/Genotoxicity and DNA Repair	3759-3770	P451-P462
Immunotoxicity, and Inflammation	3771-3781	P463-P473
Cell Death/Apoptosis	3782-3784	P474-P476
Natural Products	3785-3787	P477-P479
Late-Breaking Poster Session 5		
Food Safety/Nutrition	3788-3797	P480-P489
Pesticides	3798-3806	P490-P498
Neurotoxicity, and Neurodegenerative Disease	3807-3835	P499-P527
Epigenetics	3836-3843	P528-P535
Skin	3844-3846	P536-P538
Late-Breaking Poster Session 6		
Metals	3847-3861	P539-P553
Ecotoxicology	3862-3866	P554-P558
Carcinogenesis	3867-3872	P559-P564
Cardiovascular Toxicology/Hemodynamics	3873-3883	P565-P575
Kidney and Liver	3884-3892	P576-P584
Medical Devices	3893-3896	P585-P588
Alternatives to Mammalian Models	3897-3911	P589-P603

Thursday, March 17, Poster Session by Topic

TOPIC	ABSTRACT #s	POSTER BOARD #s	Session Title
Alternatives to Mammalian Models	3897-3911	P589-P603	Late-Breaking Poster Session 6
Animal Models	3674-3678	P366-P370	Late-Breaking Poster Session 3
Bioinformatics	3739-3743	P431-P435	Late-Breaking Poster Session 4
Biological Modeling	3616-3621	P308-P313	Late-Breaking Poster Session 2
Biomarkers	3744-3753	P436-P445	Late-Breaking Poster Session 4
Biotransformation/Cytochrome P450	3754-3758	P446-P450	Late-Breaking Poster Session 4
Carcinogenesis	3867-3872	P559-P564	Late-Breaking Poster Session 6
Cardiovascular Toxicology/Hemodynamics	3873-3883	P565-P575	Late-Breaking Poster Session 6
Cell Death/Apoptosis	3782-3784	P474-P476	Late-Breaking Poster Session 4
Chemical and Biological Weapons	3585-3856	P277-P278	Late-Breaking Poster Session 1
Clinical and Translational Toxicology	3728-3730	P420-P422	Late-Breaking Poster Session 3
Computational Toxicology and Data Integration	3731-3738	P423-P430	Late-Breaking Poster Session 4
Developmental and Juvenile Toxicology/Developmental Basis of Adult Disease	3682-3690	P374-P382	Late-Breaking Poster Session 3
Disposition/Pharmacokinetics	3679-3681	P371-P373	Late-Breaking Poster Session 3
Ecotoxicology	3862-3866	P554-P558	Late-Breaking Poster Session 6
Emerging Technologies	3559-3562	P251-P254	Late-Breaking Poster Session 1
Endocrine Toxicology	3701-3711	P393-P403	Late-Breaking Poster Session 3
Epidemiology and Public Health	3712-3719	P404-P411	Late-Breaking Poster Session 3
Epigenetics	3836-3843	P528-P535	Late-Breaking Poster Session 5
Exposure Assessment/Biomonitoring	3624-3632	P316-P324	Late-Breaking Poster Session 2
Food Safety/Nutrition	3788-3797	P480-P489	Late-Breaking Poster Session 5
Gene Regulation/Signal Transduction	3759-3761	P451-P453	Late-Breaking Poster Session 4

Thursday, March 17, Poster Session by Topic

TOPIC	ABSTRACT #s	POSTER BOARD #s	Session Title
Genotoxicity/DNA Repair	3762-3770	P454-P462	Late-Breaking Poster Session 4
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Kidney and Liver	3884-3892	P576-P584	Late-Breaking Poster Session 6
Medical Devices	3893-3896	P585-P588	Late-Breaking Poster Session 6
Metals	3847-3861	P539-P553	Late-Breaking Poster Session 6
Mixtures	3672-3673	P364-P365	Late-Breaking Poster Session 2
Nanotoxicology	3565-3578	P257-P270	Late-Breaking Poster Session 1
Natural Products	3785-3787	P477-P479	Late-Breaking Poster Session 4
Neurodegenerative Disease	3830-3835	P522-P527	Late-Breaking Poster Session 5
Neurotoxicity	3807-3829	P499-P521	Late-Breaking Poster Session 5
Oxidative Injury and Redox Biology, and POPs	3602-3613	P294-P305	Late-Breaking Poster Session 1
Pesticides	3798-3806	P490-P498	Late-Breaking Poster Session 5
Pharmacogenomics/Genetic Polymorphisms	3720-3721	P412-P413	Late-Breaking Poster Session 3
Receptors	3722-3724	P414-P416	Late-Breaking Poster Session 3
Regulation/Policy	3614-3615	P306-P307	Late-Breaking Poster Session 1
Reproductive Toxicology	3691-3700	P383-P392	Late-Breaking Poster Session 3
Respiratory Toxicology	3587-3601	P279-P293	Late-Breaking Poster Session 1
Risk Assessment	3622-3651	P314-P343	Late-Breaking Poster Session 2
Safety Assessment: Non-Pharmaceutical	3652-3656	P344-P348	Late-Breaking Poster Session 2
Safety Assessment: Pharmaceutical-Drug Development and Drug Discovery	3657-3671	P349-P363	Late-Breaking Poster Session 2
Skin	3844-3846	P536-P538	Late-Breaking Poster Session 5
Stem Cell Biology and Toxicology	3725-3727	P417-P419	Late-Breaking Poster Session 3
Systems Biology and Toxicology	3579-3584	P271-P276	Late-Breaking Poster Session 1

ABSTRACT FINAL ID: 3559 Poster Board: P251
TITLE: Development of a Gene Expression Analysis Platform Suitable for High Throughput Transcriptomics

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J.M. Yeakley[1], P. Shepard[1], D. Goyena[1], H. VanSteenhouse[1], J. McComb[1], S.S. Auerbach[2], R. S. Paules[2], B. Seligmann[1]. *[1]BioSpyder Technologies, Inc., Carlsbad, CA; [2]Division of the National Toxicology Program, NIEHS, Research Triangle Park, NC.*
KEYWORDS: Genomics; Computational Toxicology

ABSTRACT BODY: High-throughput gene expression data collection requires a readily automatable biochemistry with a quantitative read out. We have developed TempO-Seq, a highly multiplexed RNA monitoring technology that is amenable to automated processing, yet maintains high input sensitivity, single-base specificity, and highly accurate fold difference detection. TempO-Seq is based on detector oligos targeting known RNA sequences in cell lysates or purified RNAs, with no need for cDNA synthesis or specialized equipment, in a single-day process with a sequencing read out and simplified data analysis. Efficiency is further maximized by quantitative and predictable attenuation of highly expressed genes. Assay performance was characterized using the Tox21 S1500+ surrogate whole transcriptome gene panel to monitor >2200 genes in MAQC reference RNAs and lysates of cultured cells. Reproducibility among replicates was high, with R2 values routinely greater than 0.97, dependent on sequencing depth. Using RNA mixtures, a lower limit of 1.2-fold difference between samples could be confidently measured for sample-specific transcripts. Cross-platform comparison between TempO-Seq and RNA-Seq results for MAQC RNAs showed highly correlated fold differences in expressed transcripts, with an R2 of 0.89, despite the substantial differences in these two methods. Sensitivity to changes in expression due to compound exposure was tested by monitoring 2,284 genes in rat liver RNAs, with good correlation to RNA-Seq results, suggesting that sub-transcriptome content is sufficient for sample classification. To test this idea, the assay was scaled up to target the whole human transcriptome (20,580 probes monitoring 38,247 RefSeq IDs). The whole transcriptome results showed good reproducibility and differential expression among samples. In both the surrogate and the whole transcriptome, a dynamic range of about 4 logs was measured. We conclude that the TempO-Seq assay meets all the performance requirements of a highly useful expression analysis tool suitable for toxicological analyses.

ABSTRACT FINAL ID: 3560 Poster Board: P252
TITLE: Non-Invasive Fecal Analysis to Evaluate Stress in Vulnerable Marine Mammals

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: C.B. Rolsky, J. Senko. *Arizona State University, Tempe, AZ.*
KEYWORDS: Endocrine; Androgens; Exposure, Environmental; Endocrine Disruptors; Endangered Marine Mammals

ABSTRACT BODY: A growing number of anthropogenic stressors have been found to impact marine mammals, leading to deleterious effects at both the individual and sometimes population-level. Hormones are an important facet of wildlife health, and can be used to quantify a range of anthropogenic stressors such as vessel noise, habitat disturbance, and pollution. Fecal analysis provides a non-invasive form of stress assessment that allows for evaluation of hormone levels, which can be utilized for marine mammal species whose feces float. Fecal analysis, mostly targeting the glucocorticoid hormone cortisol and its metabolites, has been widely employed in the terrestrial animal realm and is now being extended to marine systems. Here, we reviewed all known studies that have employed fecal analysis on threatened or endangered marine mammals. We found that large whales, both baleen and toothed, have been sampled comparatively more than other vulnerable marine mammal species, with no dolphin and few pinniped species being sampled. Non-invasive fecal analysis remains largely underutilized on vulnerable marine mammals due to logistical difficulties associated with obtaining marine fecal matter, coupled with logistical challenges associated with fecal processing. We opportunistically interviewed experts working with threatened or endangered marine mammal species that have not been targeted for fecal analysis to obtain information on whether or not those species would be potential candidates for fecal stress research. We then draw on various anthropogenic interactions that have been documented on vulnerable marine mammals that would warrant additional hormonal scat research to gain a better understanding of animal physiology and behavior. We chronicle the evolution of fecal analysis for cortisol as a viable technique for stress evaluation, and highlight future challenges and potential opportunities to expand this approach to studying other vulnerable marine mammals.

ABSTRACT FINAL ID: 3561 Poster Board: P253
TITLE: TempO-Seq Surrogate Whole Transcriptome Gene Expression Profiling of Archived Rat FFPE

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: B. Seligmann[1], M. Babic[1], S. Auerbach[2], A. Merrick[2], D. Mav[3], R. Shah[4], R. Thomas[5], R. Paules[2], J. Yeakley[1]. *[1]BioSpyder Technologies, Inc., Tucson, AZ; [2]Division of the National Toxicology Program, NIEHS, Research Triangle Park, NC; [3]Sciom LLC, Research Triangle Park, NC; [4]Sciome LLC, Research Triangle Park, NC; [5]National Center for Computational Toxicology, US EPA, Research Triangle Park, NC.*
KEYWORDS: Gene Expression/Regulation; Risk Assessment; Non-Mammalian Species; FFPE

ABSTRACT BODY: Whole transcriptome profiling of formalin fixed paraffin embedded (FFPE) tissue samples with existing methods is problematic for several reasons; i) high cost to profile many samples; ii) quality of extracted RNA; iii) often a portion of FFPE samples fail to provide sufficient quality or amount of RNA; iv) large amount of FFPE required for extraction; v) lack of sensitivity to reliably quantify low expressed genes, with required deep sequencing further increasing cost; and vi) lower quality/reproducibility of whole transcriptome data. We demonstrate that the targeted sequencing TempO-Seq assay, which measures gene expression from lysates of FFPE without extraction and can be performed using any PCR instrument overcomes all these limitations. Using a rat surrogate whole transcriptome assay of 2282 genes to test matched archived frozen and FFPE samples from a study of hepatotoxic alkenylbenzene flavoring agents, and sequencing up to 60 samples/MiSeq run, we demonstrate that TempO-Seq provides highly sensitive and correlated results from as little as 0.1 fg or 0.01 mm3 FFPE tissue. Sensitivity from lysates was greater than using RNA extracted from matched frozen tissue (0.5 ng yielded 1 pg RNA gave equivalent sensitivity). We speculate that TempO-Seq measures total RNA from lysates, cross-linked plus extractable. Replicates within and between animals for (the 1,525) genes expressed at a level of >20 counts gave R2 values of 0.98, and avg 8.7% CVs. Measurements from FFPE correlated to matched frozen, R2=0.947. *In silico* analysis predicted changes in genes across the whole transcriptome and identified active and regulated molecular pathways. Thus, TempO-Seq provides an accurate and highly reproducible and sensitive method to obtain whole transcriptome data from small amounts of archived FFPE at a low sequencing cost/sample.

ABSTRACT FINAL ID: 3562 Poster Board: P254
TITLE: Monocrotaline Toxicity in 3D-Bioprinted Human Liver Tissue

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: U.M. Hanumegowda[1], Y. Wu[1], T.R. Smith[2], L. Lehman-McKeeman[1]. *[1]Bristol-Myers Squibb, Wallingford, CT; [2]Organovo, San Diego, CA.*
KEYWORDS: Alternatives To Animal Testing; Methods/Mechanism; Hepatic

ABSTRACT BODY: Monocrotaline (MCT), a pyrrolizidine alkaloid causes liver injury in animals similar to that of hepatic venoocclusive disorder in humans. MCT induced liver injury occurs through a complex set of cellular insults involving multiple cell types which can ultimately lead to fibrotic changes. In this study, we evaluated the effects of MCT in 3D-bioprinted human liver tissue comprising of primary hepatocytes, hepatic stellate cells, and endothelial cells (exVive3D™; Organovo, San Diego CA). The bioprinted tissues were treated with MCT at concentrations of 0.02, 0.2 or 2 mM for seven days. MCT treatment led to time- and dose-dependent decreases in tissue health as measured by LDH leakage and albumin synthesis and by histopathologic changes in the tissues. A dose-dependent increase in soluble LDH and a decrease in albumin production was observed as early as 3 days of treatment with MCT. Additionally, on treatment day 3, increases in the production of the pro-inflammatory cytokines IL-1b, IL-4, IL-8 and IL-10 were observed. Histologic assessment of formalin-fixed, paraffin-embedded tissue sections on treatment day 7 revealed early signs of tissue damage, including dissociation of the network of hepatocytes and reduced cellularity within the tissues. Immunohistochemical analyses revealed a dose-dependent increase in $CD31^+$ cells and a marked increase in the appearance of large, $CD31^+$ bright cells that co-expressed smooth muscle actin (SMA), often forming clusters or complex multi-cellular structures. Changes in organization of CD31 expressing endothelial cells and appearance of SMA expressing cells are indicative of remodeling and initiation of fibrotic events. These observations capture the spectrum of changes induced by MCT ranging from reduced hepatocellular function and vascular remodeling, which may involve endothelial cell migration, organization, proliferation, apoptosis, and endothelial-to-mesenchymal transformation to early fibrotic events.

ABSTRACT FINAL ID: 3563 Poster Board: P255
TITLE: Genome-Wide Transcriptional Analysis of Silica Nanoparticles-induced Toxicity in Zebrafish Embryos

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: Z. Sun. *Capital Medical University, Beijing, China.*
KEYWORDS: Gene Expression/Regulation; Genetic Toxicology; Bioinformatics

ABSTRACT BODY: Although silica nanoparticles (SiNPs) have a promising application in biomedical fields, there is still a lack of comprehensive understand on genome-wide transcriptional analysis. This study is aimed to clarify the toxic effect and molecular mechanisms of SiNPs in zebrafish embryos based on microarray analysis and bioinformatics analysis. Microarray data analysis demonstrated that SiNPs-induced toxicity in zebrafish embryos affected expression of 2515 genes, including 1107 genes were up-regulated and 1408 genes were down-regulated. These differentially expressed genes were subjected to bioinformatic analysis for exploring biological processes triggered by SiNPs in zebrafish embryos. Gene ontology analysis showed that SiNPs caused significant changes in gene expression patterns related to many important functions, including response to stimuli, immune response, cellular process, and embryonic development. In addition, Pathway analysis and the Signal-net analysis indicated that the Gap junction, Vascular smooth muscle contraction, Metabolic pathways, Apoptosis, MAPK signaling pathway, Calcium signaling pathway and JAK-STAT signaling pathway were the most prominent significant pathways in SiNPs-induced toxicity in zebrafish embryos. In addition, results from qRT-PCR and western blot analysis showed that the IL-6 dependent JAK1/STAT3 signaling pathway was activated by SiNPs in zebrafish embryos. In summary, our data will provide compelling clues for further exploration of SiNPs-induced toxicity in zebrafish embryos.

ABSTRACT FINAL ID: 3564 Poster Board: P256
TITLE: Significant Revisions to OECD Inhalation Test Guidelines to Accommodate the Testing of Nanomaterials

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J.E. Whalan[1], I. Camacho[2], A. Gourmelon[3], M. Gonzalez[3], K. Dreher[4], W. Hall[5]. *[1]NCEA, US EPA, Washington, DC; [2]OPPT, US EPA, Washington, DC; [3]Environment Directorate, OECD, Paris, France; [4]NHEERL, US EPA, Durham, NC; [5]FEAD, US EPA, Washington, DC.*
KEYWORDS: Respiratory Toxicology; Nanoparticles; Inhalation Toxicology; OECD

ABSTRACT BODY: In response to a need for inhalation test guidelines for evaluating the potential health hazard of nanomaterials, the OECD is revising two existing (2009) Test Guidelines (TGs)—TG 412 (28-day inhalation) and TG 413 (90-day inhalation). Enhancements are also being made to reflect the state of the science for the inhalation testing of gases, vapours, and aerosols (both fines and nanomaterials). Notable features of these revised TGs include enhanced emphasis on toxicokinetic assessment and/or systemic toxicity evaluations, determination of particle biopersistence in the lung, and measurements of bronchoalveolar lavage (BAL) parameters and lung burden. A study may include a post-exposure period when testing a biopersistent aerosol and/or a test chemical that induces prolonged adverse lung effects. Two particularly notable changes from the previous TGs are testing in only the more sensitive sex, as determined during a range-finding study, and a new aerosol particle size criterion: MMAD <2µm with a geometric standard deviation of <3. Revisions are also underway for the acute test guidelines and for two inhalation Guidance Documents (GDs)—GD 39 (Inhalation Toxicity Testing) and GD 125 (Histopathology for Inhalation Toxicity Studies). The views expressed are those of the authors, and do not necessarily represent the views or policies of the US Environmental Protection Agency.

ABSTRACT FINAL ID: 3565 Poster Board: P257
TITLE: Stromelysin-2 (MMP-10) Facilitates Clearance and Moderates Inflammation and Cell Death following Lung Exposure to Long Multi-Walled Carbon Nanotubes (L-MWCNT)

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KEYWORDS: Nanotechnology; Inhalation Toxicology; Immunotoxicology

ABSTRACT BODY: Engineered carbon nanotubes (CNT) are a class of graphene cylinders, of one or more layers, with at least one external dimension in the 1-100 nanometer range. These characteristics impart unique electrical, mechanical, and thermal properties that make CNTs exceedingly valuable for a range of biomedical and commercial applications. However, rising global production has led to concerns regarding potential occupational exposures to raw materials during handling. This is especially true for long fibers, whose aspect ratio has been posited to initiate pathology similar to that of asbestos. Stromelysin-2 or matrix metalloproteinase 10 (MMP-10) is an extracellular endopeptidase that has been shown to mediate the response of various tissues to injury through its expression in activated macrophages and epithelial cells. Here we report, for the first, a role for MMP-10 in modulating the murine acute lung response to a type of CNT known as long multi-walled carbon nanotubes (L-MWCNTs). Oropharyngeal aspiration of 80 μg/mouse of L-MWCNT induced expression of Mmp10 mRNA in lung at 24 hours post-exposure, and was accompanied by a robust inflammatory response (i.e. Tnfa, Il6, and Il1b). However, in Mmp10-/- mice we found that the absence of MMP-10 led to impaired pulmonary clearance of MWCNT and reduced macrophage cell survival during this time. Complementary *In vitro* work with bone marrow derived macrophages (BMDMs), a primary mouse line, demonstrated that Mmp10 up-regulation is also associated with the inflammatory response to MWCNT in culture. Furthermore, BMDMs from Mmp10-/- mice exhibited enhanced expression of pro-inflammatory products, including IL-1β, which is associated with reduced viability in macrophages, and a corresponding increase in caspase-3 dependent cell death. Ultimately, a better understanding of the protective role that pulmonary MMP-10 serves in this model may yield invaluable information for understanding the physiological basis of MWCNT-induced lung disease.

ABSTRACT FINAL ID: 3566 Poster Board: P258
TITLE: The Effects of LPS, MWNCT, and Combination on MMP-9 and MMP-12 in Lung Alveolar Epithelial Cells

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KEYWORDS: Gene Expression/Regulation; Lung; Pulmonary Or Respiratory System; Nanoparticles; MWCNT

ABSTRACT BODY: Multi-walled carbon nanotubes (MWCNT) have been shown to induce lung fibrosis in animal models, however the underlying molecular factors/mechanisms are still unclear. In this study, we investigated the effects of LPS, MWCNT and combination on the expression of matrix metalloproteinase-9 and metalloproteinase-12 (MMP-9, MMP-12) in alveolar epithelial A549 cells. A549 cells were exposed to LPS (1 ng/ml), MWCNT (20 μg/ml), and combination and analyzed for paracellular permeability, MMP-9, MMP-12. LPS, the combination of LPS and MWCNT, and MWCNT only at the highest tested dose induced blue dextran extravation. LPS, and MWCNT increased the expression MMP-9 and MMP-12 mRNA. M Our results indicate that MWCNT activated alveolar epithelial cells to promote fibrogenesis, and that LPS differentially primes molecular factors involved in lung remodeling. These findings suggest a role of alveolar epithelial cells in fibrogenesis and also may aid in the design and development of tests for screening of fibrogenic agents.

ABSTRACT FINAL ID: 3567 Poster Board: P259
TITLE: Genome Wide Association Study of Mast Cell Degranulation by Nanoparticles

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M. Johnson[1], L. Saba[1], A. Bauer[1], R. Podila[2], J. Brown[1]. *[1]Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO; [2]Department of Physics and Astronomy, Clemson University, Clemson, SC.*
KEYWORDS: Nanoparticles; Bioinformatics; Inflammation; Mast Cells; Silver Nanoparticle

ABSTRACT BODY: Silver nanoparticles (AgNP) are the most widely manufactured engineered nanomaterial (ENM) due to their antimicrobial properties. However, with their increased use, there is concern that human and environmental exposures may lead to adverse outcomes. Our laboratory previously determined that mast cells are activated following AgNP exposure that is dependent upon key physicochemical properties. Mast cells, central to the innate immune response, are one of the earliest sensors of environmental insult. Genetics are a major contributing factor in many toxicological outcomes, however to date, few studies have examined the contribution of genetics to ENM toxicity. We hypothesized that in addition to ENM physicochemical properties, genetics contribute to regulation of mast cell degranulation following AgNP exposure. We grew bone marrow-derived mast cells from genetically diverse mouse strains, exposed them to AgNP (25µg/mL, 1h) and assessed degranulation. Quantitative trait loci (QTL) mapping was preformed to identify single nucleotide polymorphisms (SNPs) associated with variation in degranulation following AgNP exposure. Mast cells grown from 14 genetically different strains of mice displayed a range of degranulation patterns following AgNP exposure. This suggests that multiple genes are likely regulating the different responses leading to mast cell activation. QTL mapping identified 2 suggestive loci associated with mast cell degranulation; rs32615733 located on chromosome 1 and rs29103777 located on the X chromosome. These regions are currently undergoing annotation. These results provide evidence that a complex set of genes regulate mast cell responses to ENM exposure. Overall, the proposed research will contribute to the field of nanotoxicity by identifying genetic targets that play a role in adverse immune responses to further understand underlying mechanisms of toxicity. Funding: NIEHS R01 ES019311(JMB).

ABSTRACT FINAL ID: 3568 Poster Board: P260
TITLE: Applicability of *In Vitro* Methodologies to Assess Cytotoxicity and Genotoxicity of Gold Nanoparticles

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KEYWORDS: *In Vitro* and Alternatives; Cell Culture; Nanotechnology; Gold Nanoparticles

ABSTRACT BODY: Reliable *in vitro* toxicity testing is essential prior to the commencement of *in vivo* testing necessary for hazard identification and risk assessment of nanoparticles. In our laboratory, investigations into the potential of 14 nm citrate-stabilised gold nanoparticles (AuNPs) to interfere with *in vitro* cytotoxicity and genotoxicity methodologies have demonstrated that thorough and effective validation of testing systems is essential. The cytotoxicity XTT and ATP assays were shown to be the most susceptible to interference, which was attributed to optical interference with the detection system and direct interference with the assay components. AuNPs were also shown to quench the fluorescence of the DCF, propidium iodide, and JC-1, dyes commonly used to assess reactive oxygen species, cell viability, and mitochondrial membrane potential, respectively. Data from the luminescence-based lactate dehydrogenase assay showed no interference, and toxicity measurements correlated with label-free, cell impedance testing. Additional properties of gold nanoparticles may influence the outcome of *in vitro* assays, for example, gold nanoparticles inability to enter bacterial cells used in the Ames test could potentially result in a false negative in this mutagenicity assay. Steps in the individual assay procedures may also introduce points at which interference may occur. In the comet assay, lysis of the cells allowed for direct contact of the AuNPs with nuclear DNA which resulted in an overestimation of genotoxicity. However the procedure of the chromosome aberration assay was not susceptible to interference from AuNPs, and therefore provides a valid measure of genotoxicity. In conclusion, interpretation of results from *in vitro* toxicity assays should be preformed with caution, and interference of nanoparticles with the assays should be individually assessed. The use of assays that do not require optical detection methods of an endproduct may help in eliminating at least one aspect of interference, since simply subtracting particle controls may not be sufficient to overcome interference.

ABSTRACT FINAL ID: 3569 Poster Board: P261
TITLE: Interference of Gold Nanoparticles in the Assessment of Endotoxin Contamination *In Vitro*

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M. Vetten[1,2], M. Gulumian[1,2] . [1]*National Institute for Occupational Health, Johannesburg, South Africa;* [2]*University of the Witwatersrand, Johannesburg, South Africa.*
KEYWORDS: *In Vitro* and Alternatives; Nanotechnology; Endotoxins; Gold Nanoparticles

ABSTRACT BODY: The potential use of gold nanoparticles in medical applications necessitates the need for any nanoparticle-drug based formulation to be sterile and apyrogenic. The *in vitro* limulus amebocyte lysate (LAL) test, in various formats, is the assay of choice for the determination of endotoxin contamination. However, gold nanoparticles (AuNPs) have been shown to interfere with other *in vitro* test methodologies. In this study, we aimed to assess potential interference of gold nanoparticles with three assays, namely, the traditional LAL absorbance-based chromogenic assay, the LAL gel-clot assay, and the fluorescence-based recombinant Factor C (rFC) assay. The traditional chromogenic and gel-clot assays are dependent on the protease cascade which occurs in LAL, whilst the rFC assay is dependent only on the endotoxin-specific Factor C protein of the cascade. Testing for interference involved assessment of the AuNPs to influence the detection of the reaction products due to their optical properties; and the ability of the AuNPs to either inhibit or enhance the reactions of the assays. For the chromogenic assay, AuNPs were shown to increase the absorbance readings of the final product of the assay; and also appeared to exhibit catalytic activity. Subtraction of the absorbance values of "nanoparticle-only" controls was unable to account for all observed interference. AuNPs also interfered with the gel-clot assay where enhancement of the assay was observed, potentially resulting in false positives. In contrast, data from the rFC assay suggested inhibitory effect. Contradictory results from these assays question their applicability for detection of endotoxin contamination in gold nanoparticles. Alternative tests should be investigated; however these often rely on direct detection of endotoxin instead of the biological activity of endotoxin, or the use of *in vivo* testing.

ABSTRACT FINAL ID: 3570 Poster Board: P262
TITLE: Characterization of the Oxidative Potential of Three Manufactured Nanomaterials (MNM)

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: V. Mersch-Sundermann, T. Tang, A. Arif, M. Garcia-Käufer, R. Gminski. *Institute of Environmental Health Sciences, Medical Center, University of Freiburg, Freiburg, Germany.*
KEYWORDS: *In Vitro* and Alternatives; Predictive Toxicology; Mutagenesis; Oxidative Potential; Manufactured Nanomaterials (MNM)

ABSTRACT BODY: The toxicological and ecotoxicological relevance of nanoparticle emissions occurring during recycling and thermal utilization of nanocomposites (NC) were investigated within the scope of the German project ProCycle. Toxicological studies conducted in recent years have shown that correct prediction of the toxic properties of manufactured nanomaterials (MNM) is not possible per se; rather, much more knowledge of the nanomaterial itself is required and its behavior in human cells. Certain characteristics of MNM, e.g. size, surface properties and their ability to form reactive oxygen species (ROS) were used as promising parameters to predict their toxicity to humans. In the ProCycle project, the physical-chemical characteristics, especially the surface reactivity of nano TiO2, nano CeO2 and nano CuO were analyzed in comparison to their effects in in vitro cellular toxicity tests. This was done to identify potential hazards associated with these MNM. The physical-chemical characteristics of the suspended nanomaterials were determined as hydrodynamic diameter and zeta potential by Nanoparticle Tracking Analysis (NTA). In bioassays with human cells, a number of different endpoints related to extra- and intracellular oxidative stress, cytotoxicity and mutagenicity of the investigated MNM were compared. The results support the high oxidative potential and toxicity of nano CuO and the missing oxidative potential and toxicity of nano TiO2 and nano CeO2 under the conditions applied.

ABSTRACT FINAL ID: 3571 Poster Board: P263
TITLE: Primary Mouse Airway Epithelial Cells are Resistant to Silver Nanoparticles

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KEYWORDS: Lung; Pulmonary or Respiratory System; Nanoparticles; Cell Culture

ABSTRACT BODY: Engineered silver nanoparticles (AgNPs) are a widely used nanomaterial with anti-microbial properties. AgNPs are present in a variety of commercial products, including cosmetics, wound dressings, textiles, and paints. Given their small size and prevalence, occupational and environmental exposure by inhalational, ingestion, and trans-dermal routes are of concern. Prior mouse studies reported neutrophilic inflammation following acute inhalational exposures. Because the airway epithelium functions as an important barrier and innate immune cell to control inhalational exposures, we evaluated the effects of acute and sub-acute AgNP exposure on mouse tracheal epithelial cell (MTEC) organotypic culture. MTECs were isolated from C57BL/6 and A/J mice via enzymatic digestion and propagated in submerged culture. Confluent cells were differentiated at an air-liquid interface to form a pseudostratified, ciliated epithelial layer. Fully differentiated cells were exposed at the apical surface to citrate-coated 20 nm AgNPs for 24 hours, after which basolateral medium was collected to determine LDH as a measure of cell death, and RNA was collected to assess cellular responses. In a second experiment, cells were exposed to AgNPs daily for 4 hr over 5 days followed 2 days later by exposure to house dust mite (HDM) extract or PBS control. Acute AgNP exposure did not result in significant cell death for epithelial cells isolated from either C57BL/6 or A/J mice. AgNPs did not induce expression of the neutrophil chemokine, Cxcl1. However, AgNPs did induce expression of both Mt1 and Mt2, confirming exposure of cells to AgNPs. Similar results were observed for subacute exposure of C57BL/6 MTECs. HDM exposure increased chemokine expression; however, this effect was not modulated by prior AgNP exposure. Differentiated mouse primary tracheal epithelial cells are highly resistant to both cytotoxicity and inflammatory activation by AgNPs. AgNP exposure does not modulate epithelial inflammatory response to HDM. Supported by NIH U19ES019545 and USEPA 83573801.

ABSTRACT FINAL ID: 3572 Poster Board: P264
TITLE: Evaluation of Vascular Tone and Cardiac Contractility in Response to Silver Nanoparticles, Using Langendorff Rat Heart Preparation

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KEYWORDS: Systems and Integrative Toxicology; Cardiovascular System; Nanoparticles; Oxidative Stress; Nitric Oxide

ABSTRACT BODY: Silver nanoparticles (AgNPs) have been widely used in an increasing number of applications because of their antimicrobial properties. However, AgNPs exposure can induce adverse responses in major organs, including the heart. Several reports suggest that AgNPs-induced cardiac effects involve nitric oxide (NO) generation, derived from NO synthases (NOS), as well as oxidative stress generation either by reducing antioxidant enzymes activity such superoxide dismutase (SOD) and catalase (CAT) or increasing reactive oxygen species (ROS) levels. Nevertheless, no studies about the AgNPs-induced effects in the cardiac physiology have been performed. The aim of this study was to evaluate the direct actions of AgNPs on coronary vascular tone and cardiac contractility using the isolated and perfused rat heart Langendorff preparation. AgNPs (15±4 nm) at 0.1 and 1 µg/mL induced a slight vasodilation and decreased cardiac contractility dependent on NO production. Meanwhile, high concentrations of AgNPs (10 and 100 µg/mL) promoted vasoconstriction and cardiac contractility related to the inhibition of endothelial NOS phosphorylation and inducible NOS expression as well as increased oxidative stress, without modifying SOD or CAT expression. Furthermore, AgNPs inhibited classic actions induced by phenylephrine (Phe) and acetylcholine (ACh), indicating that AgNPs may influence the effects of vasoactive and inotropic agents. These data suggest that AgNPs affect cardiac physiology through generation of NO, NOS expression and oxidative stress generation. Further investigations are required to elucidate the mechanism of action and signaling pathway involved in these events.

ABSTRACT FINAL ID: 3573 Poster Board: P265
TITLE: Comprehensive *In Vitro* Evaluation of Cytotoxicity of TiO2 Nanomaterials

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KEYWORDS: Cytotoxicity; TiO2, Nanomaterials

ABSTRACT BODY: NanoTiO2 represents a wide group of materials with different physico-chemical properties rendering them desired industrial characteristics as well as influencing their behavior in a biological environment and possible toxic effects. No consensus on the safety of nanoTiO2 has been reached yet as the available toxicity data are highly inconsistent and of a limited comparability due to variability in experimental conditions. In this study, we evaluated cytotoxic potential of 17 variants of nanoTiO2 differing in their crystal structure (anatase or rutile), size (5-150 nm), shape (particles, tubes or wires) and hydrophobic coating (uncoated, SiO2 or Al2O3 coated). Samples were thoroughly characterized by XRD, TEM, XRF, BET surface area analysis, thermogravimetric analysis and DLS. Cytotoxicity was examined in three human cell lines (alveolar epithelial A549 cells, bronchial epithelial BEAS-2B cells and PMA-differentiated THP-1 cells) using MTS, WST-1 and LDH assays after 24-hour exposure in six concentrations ranging from 8 to 256 μg/ml. Overall, nanoTiO2 exhibited no or low cytotoxicity (i.e. more than 70% of viable cells compared to the negative control) in the selected cell lines and assays up to the highest tested concentration of 256 μg/ml. Slight differences in the cytotoxic potential of the nanoTiO2 samples were observed, however no single characteristic (crystal structure, size, shape, coating) could be directly linked to the increased cytotoxicity. When comparing sensitivity of the employed assays and cell lines, it was observed that THP-1 cells were more sensitive towards cytotoxic effects of the tested nanoTiO2 samples than the lung cell lines. LDH assay was less sensitive that the metabolic activity based MTS and WST-1 assays. The obtained data will be shared in nanotoxicological databases to contribute to development of computational models of nanomaterial toxicity.
Support: Czech Science Foundation (P503/12/G147), Nanotechnology Centre VSB-TUO (SP2015/172)

ABSTRACT FINAL ID: 3574 Poster Board: P266
TITLE: Disturbance of Ion Environment and Immune Regulation following Biodistribution of Magnetic Iron Oxide Nanoparticles Injected Intravenously

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KEYWORDS: Nanotechnology; Toxicokinetics; Antigen-Presenting; Ion Environment; Iron Oxide Nanoparticles

ABSTRACT BODY: Although it is expected that accumulation of metal oxide nanoparticles that can induce redox reaction in the biological system may influence ion homeostasis and immune regulation through generation of free radicals, the relationship is still unclear. In this study, mice received magnetic iron oxide nanoparticles (M-FeNPs, 2 and 4 mg/kg) a single via the tail vein, and their distribution in tissues was investigated over time (1, 4, and 13 weeks). In addition, we evaluated the effects on homeostasis of redox reaction-related elements, the ion environment and immune regulation. The iron level in tissues reached at the maximum on 4 weeks after injection and M-FeNPs the most distributed in the spleen at 13 weeks. Additionally, levels of redox reaction-related elements in tissues were notably altered since 1 week post-injection. While levels of K^+ and Na^+ in tissue tended to decrease with time, Ca^{2+} levels reached to the maximum at 4 weeks post-injection. On 13 weeks post-injection, the increased percentages of neutrophils and eosinophils, the enhanced release of LDH, and the elevated secretion of IL-8 and IL-6 were clearly observed in the blood of M-FeNP-treated mice compared to the control. While expression of antigen presentation related-proteins and the maturation of dendritic cells were markedly inhibited following distribution of M-FeNPs, the expression of several chemokines, including CXCR2, CCR5, and CD123, was enhanced on the splenocytes of the treated groups. Taken together, we suggest that accumulation of M-FeNPs may induce adverse health effects by disturbing homeostasis of the immune regulation and ion environment.

ABSTRACT FINAL ID: 3575 Poster Board: P267
TITLE: Silver Nanoparticles Induce Mitochondrial Toxicity in Caenorhabditis elegans

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KEYWORDS: Nanotechnology; Exposure, Environmental

ABSTRACT BODY: Silver nanoparticles (AgNPs) are added as an antibacterial agent to consumer products such as textiles, children's toys, and wound dressings. AgNPs are one of the more toxic nanoparticles studied to date, and the underlying mechanisms of toxicity remain elusive. Because AgNPs are widely used in consumer products as an antimicrobial agent, the mitochondrion is a plausible intracellular target of AgNPs, based on the endosymbiotic theory. The relationship between AgNP exposure and mitochondrial toxicity as a primary mode of cellular toxicity is largely unexplored. This study utilized *C. elegans* mutants deficient in mitochondrial homeostasis (*drp-1* (fission mutant); *eat-3*, *fzo-1* (fusion mutants) and mitophagy (*pink-1*, *pdr-1*)) to screen AgNPs for associated mitochondrial toxicity, with follow-up experiments specifically determining mitochondrial morphological changes and respiratory function. The experimental design allowed for systematic analysis of a particle-size effect and particle-coating effect. Up to 2.0mg/L of 10nm and 30nm polyvinylpyrrolidone-coated (PVP_{10} and PVP_{30}, respectively) and 6nm and 25nm gum arabic-coated (GA_6 and GA_{25}, respectively) AgNPs were assessed. Of the AgNPs tested, the smaller AgNPs (GA_6 and PVP_{10}) caused significant growth delay in wild-type (N2) and mitochondrial mutant strains of *C. elegans* at 48h, whereas at the same concentrations, the larger AgNPs did not. Exposure to smaller AgNPs caused an increase in mitochondrial aspect ratio in N2 worms, suggesting that mitochondrial fission may mediate this effect. Seahorse instrumentation used to analyze mitochondrial respiration in N2 and *drp-1* showed that exposure to PVP_{30} AgNPs caused increased basal oxygen consumption in N2 and *drp-1*. All other particles and the silver ion control caused a significant decrease in the maximal respiratory capacity and spare respiratory capacity (SRC) in N2s, but caused no further decreases in *drp-1*, which have low constitutive SRC. Exposure to PVP_{10} AgNPs caused decreased ATP-linked respiration in *drp-1*, whereas exposure to GA_6 AgNPs caused decreased ATP-linked respiration in N2. Exposure to PVP_{10} AgNPs induced proton leak in both *drp-1* and N2. Together, these data suggest that mitochondrial fission is important for mediating mitochondrial toxicity caused by AgNPs of different sizes and coatings.

ABSTRACT FINAL ID: 3576 Poster Board: P268
TITLE: Distribution, Cellular Localization, and Metabolomics of Multi-Walled Carbon Nanotubes in Female Rats

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KEYWORDS: Nanoparticles; Metabolomics

ABSTRACT BODY: The emerging use of multi-walled carbon nanotubes (MWCNTs) has increased the possibility of exposure, and studying the cellular localization of nanomaterials is an important aspect in safety assessment. The distribution of MWCNTs in mammals has not been comprehensively studied. We investigated the distribution of three sizes of MWCNTs functionalized with carboxylic acid (12-2: length [L]:0.5-2µm, outer diameter [OD] 10-20nm, 13-2: L:0.5-2µm, OD 30-50nm, and 14-2: L:10-30µm, OD 10-20nm) in tissues of female Sprague Dawley rats. Rats were administered MWCNTs suspended in water at 1 mg/kg by tail vein injection. Blood, liver, lung, spleen, and lymph nodes were collected 1 and 7 days post-dose. Urine and Feces were collected at 24 hour intervals. Tissues were stained with hematoxylin and eosin and then analyzed using a CytoViva Enhanced Darkfield and Hyperspectral Imaging System. MWCNTs were found in the lung, lymph node, spleen and liver in a length and diameter-dependent manner. At day 1 and 7 MWCNT were found as aggregates in lung tissues for 13-2, but only at day 1 for 14-2, and at neither time point for 12-2. In liver at 7 days after dosing the presence of MWCNT were likewise size-dependent such that 12-2 were more frequently found in Kuppfer cells than 13-2, and with 14-2 hardly present. Also, a significant area fraction of the phagocytosis cells in lymph node and spleen were occupied by MWCNT. By combining classic histopathology with hyperspectral imaging we investigated the distribution of MWCNT on a cellular level. In addition, 1 day and 7 day urine were analyzed for metabolomics by NMR to examine differences in endogenous metabolites. 7 day samples exhibited separation from 1 day samples and metabolites driving those separations were from pathways for the citric acid cycle (e.g., citrate, 2-oxoglutarate), starch and sugar metabolism (e.g., maltose, glucose), and amino acid metabolism (e.g., hippurate, succinate). There were similar effects for each size of MWCNT. (Supported by NIEHS ES019525 and NIDDK 1U24DK097193-01

ABSTRACT FINAL ID: 3577 Poster Board: P269
TITLE: A 104-Week Pulmonary Toxicity Assessment of Single-Wall Carbon Nanotubes after a Single Intratracheal Instillation in Rats: Comparison Between Short Tubes and Long Tubes

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KEYWORDS: Lung; Pulmonary or Respiratory System; Nanoparticles; Non-Genotoxic; Intratraceal Instillation; Single-Wall Carbon Nanotubes

ABSTRACT BODY: This study was conducted to compare the chronic pulmonary toxicity between two types of dispersed SWCNTs, namely relatively long linear shapes and short linear shapes with averaged (SD) lengths of 8.6 (4.3) um and 0.55 (0.36) um, respectively. They were instilled intratracheally at 0.2 and 1.0 mg/kg for the long tubes and 1.0 mg/kg for the short tubes to male F344 rats. The pulmonary response was characterized by analysis of lung weight and histopathological evaluation of lung tissue at 26, 52 and 104 weeks after a single instillation. Fifty rats were used in each group for the 104 weeks observation. Inflammatory changes along with deposition of the test substance, macrophages engulfing the test substance, alveolar epithelialization, cell debris, and alveolar wall fibrosis were observed in the lungs of almost all the test rats at 52 and 104 weeks after the instillation of the short tubes. Incidences of these changes were much less in the long tube treated groups. In almost all the rats of the long tube treated groups, fibrosis and loss of the epithelium in the terminal bronchiole with deposition of the test substance were observed. These bronchiole changes were not observed after the short tube treatment. Bronchiolo-alveolar adenoma and carcinoma were found in respectively 2, 1, and 9 cases in the vehicle control, the higher dose of the long tube and the short tube groups at 104 weeks after the instillation, although the incidences were not statistically significant. The genotoxicity of the SWCNTs was also evaluated *in vivo* comet assays using the lung cells of the rats at 26 weeks after the instillation. No significant change in % tail DNA was found in any group given SWCNTs. These findings suggested that most portion of the treated long-SWCNT tubes were deposited at the terminal bronchiole and considerable amount of the short-SWCNT reached at the alveolus resulting in the chronic inflammatory responses but possessed no potential for genotoxicity in the lung.

ABSTRACT FINAL ID: 3578 Poster Board: P270
TITLE: Genetic Determinants of Susceptibility to Silver Nanoparticle Induced Lung Inflammation

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KEYWORDS: Nanoparticles; Genetic Polymorphisms; Inflammation; Silver Nanoparticles

ABSTRACT BODY: Silver nanoparticles (AgNPs) are employed in a variety of consumer products and are reported to be non-toxic to humans. However, *in vivo* studies indicate that AgNPs can cause lung inflammation and toxicity in a size, coating and strain dependent manner. In this study, we exposed 25 inbred mouse strains to 0.25 mg/kg of 20 nm citrate-coated AgNPs or the equivalent volume of citrate buffer using oropharyngeal aspiration. We observed significant strain dependent increases in neutrophils in bronchoalveolar lavage fluid (BALF) in AgNP exposed mice compared to controls. We thus performed a genome wide association study to identify quantitative trait loci (QTLs) associated with susceptibility to AgNP induced lung inflammation. We tested 65,493 SNPs using the EMMA R-language package for association with levels of neutrophils in AgNP treated and control mice in 25 strains. We found 4 SNPs in QTLs on chromosomes 15 and 18 that were statistically significant. We performed qRT-PCR on candidate genes in these regions using lung tissue RNA from strains representing low, moderate, and high levels of neutrophilia. There were significant inverse correlations between fold change of *Ano6* mRNA and BALF neutrophils (r^2=0.45, $p<0.001$) in AgNP treated animals. *Nedd4l* was also inversely associated with BALF neutrophil levels (r^2=0.28, $p<0.01$). Moreover, combined reductions in *Nedd4l* and *Ano6* explains more of the variation in neutrophil levels than either gene alone. Both NEDD4L and ANO6 are thought to modulate the epithelial sodium channel (ENAC), which is important for epithelial lining fluid (ELF) homeostasis. Reductions in ANO6 and/or NEDD4L may thus lead to increased ENAC activity and subsequent alterations in ELF rheology, chemokine concentrations, and AgNP induced neutrophilia. Supported by NIEHS grants U19ES019545, U01ES020126 P30ES007033, P30ES000260, and T32ES007324, T32ES015459.

ABSTRACT FINAL ID: 3579 Poster Board: P271
TITLE: Gene Expression Differences in Peripheral Blood Mononuclear Cells (PBMCs) From Chronic Tobacco Consumers

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KEYWORDS: Biomarkers; Clinical Toxicology; Toxicogenomics; Moist Snuff; Smoking

ABSTRACT BODY: Cigarette smoking exerts diverse physiological effects on multiple organ systems. Several investigations have revealed chronic cigarette smokers exhibit marked changes in gene expression changes at sites of exposure (lung, buccal, and nasal) as well as in peripheral circulation and other organs that are not directly exposed to cigarette smoke. For example, genes involved in xenobiotic metabolism, oxidative stress, and immune responses are reported to be differentially regulated in smokers. The Swedish and US epidemiological data suggest that consumption of smokeless tobacco products (STPs), such as snus and moist snuff, is less harmful relative to cigarette smoking. However, an in depth understanding of molecular changes in the consumers of STPs is limited. Here, we present global gene expression changes in the PBMCs cells collected from smokers (SMK) and moist snuff consumers (MSC) in a biomarker discovery study. Generally healthy adult male study subjects were recruited into SMK, MSC, and Non-Tobacco Consumers (NTC) cohorts (40 subjects/ cohort). The subjects fasted overnight from food and tobacco and PBMCs were isolated. Global gene expression profiling was performed on Affymetrix U133 arrays, and differentially expressed genes (≥ 1.25 fold change and p≤0.05) among the cohorts were identified. While SMK exhibited 100 differentially expressed genes (15 up and 85 down) compared to NTC, there were 46 differences between SMK and MSC cohorts (31 up and 15 down). Notably, the MSC and NTC cohorts did not reveal any gene expression differences in this study. Twenty genes emerged as common differences between SMK vs NTC and SMK vs MSC comparisons. The top few genes include GRP15, LRN3, SASH1, GZM1 and CACNA2D2. The expression of these common genes and other differentially expressed genes was confirmed by RT-qPCR methods. Collectively these findings reveal that SMK exhibit distinct differences in PBMC gene expression relative to the NTC and MSC cohorts.

ABSTRACT FINAL ID: 3580 Poster Board: P272
TITLE: The Mechanism of Control of the Nrf2 Pathway after Repeated Xenobiotic Exposure

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KEYWORDS: Systems Biology; Oxidative Injury; Transcription Factors

ABSTRACT BODY: Exposure to various chemicals causes the cellular formation of reactive oxygen species (ROS), that consequently can lead to cytotoxicity. The ROS-mediated cellular injury is typically counteracted through activation of adaptive cellular stress responses. Oxidative stress leads to the activation of the Nuclear factor-erythroid-2-related factor 2 (Nrf2) pathway. In practice, individuals are typically repeatedly exposed to the same chemical, either by repeated dosing with medicines or in the workplace to other xenobiotics. Little is known about the dynamic regulation of the Nrf2 pathway in the context of such repeated exposures and whether the stress response will be additive in time or is truly adaptive in nature and will no longer progress. Here the effect of different repeated exposure scenarios on the Nrf2 pathway was investigated. To monitor the Nrf2 signaling pathway, two different stress response reporter cell lines were used: HepG2-Nrf2-GFP and HepG2-Srxn1-GFP, a direct downstream target of Nrf2. High throughput live confocal imaging was used to measure the time dynamics of these two components of the Nrf2 pathway after repeated exposure to different concentrations (12.5-200 μM) of diethyl maleate (DEM) and tert-butylhydroquinone (tBHQ). The results indicate that single exposure to DEM and tBHQ induce Nrf2 and Srxn1 over time in a concentration-dependent manner. A second exposure resulted in an even higher increase of the Nrf2 as well as the Srxn1 response. However, compared to the duration of the first exposure, the second response differed in dynamics with respect to the slope of the response and duration of the plateau value of the peak response. Moreover, differences were observed in the responses at the different concentrations between the first and second exposure, suggesting a truly adaptive response. The quantitative dynamics data are currently integrated in computational systems biology models for Nrf2 pathway activation.

ABSTRACT FINAL ID: 3581 Poster Board: P273
TITLE: Gene Expression Profile of Rat Kinome Varies Significantly Across Organs, Which May Influence the Organ-specific Toxicities of Tyrosine Kinase Inhibitors

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KEYWORDS: Kinase; Gene Expression/Regulation; Tyrosine Kinase Inhibitors

ABSTRACT BODY: Tyrosine kinase inhibitors (TKIs) are effective anticancer drugs and most of them are well-tolerated. However, several potentially serious adverse effects of TKIs have been described, including cardiotoxicity, hepatotoxicity and lung toxicity that may be due to inhibition of tyrosine kinases involved in normal cellular function. These toxicities were not well-predicted during preclinical studies; therefore, additional approaches are needed to understand and predict the mechanisms of TKI induced organ toxicities. We hypothesize that differences in transcriptional profiles of the Kinome may be responsible for differential susceptibility of specific tissues to the adverse effects of TKIs. Therefore, expression levels of kinase genes in F344 rats were evaluated. Eleven different organs (adrenal, brain, heart, kidney, liver, lung, muscle, spleen, testis, thymus and uterus) from untreated normal male (n=4) and female (n=4) rats of ages 2, 6, 21 and 104 weeks were collected and transcriptomics experiments were conducted using RNA-seq technology. Principal component analysis of gene expression data for 703 kinases revealed distinct clusters for each organ demonstrating unique patterns of expression across organs. Of 703 kinases, 694 showed significant ($p<0.05$, fold change>1.5) difference in the expression level between at least 2 organs at any of 4 ages in both male and female rats. The top 3 genes showing highest differential expression between multiple organs were Ak9, Kndc1 and Stk33 in males, and Ak9, Camk2a and Kndc1 in females. Skp1a and Ptk2 were among the top 10 highest expressed genes in 8 and 6 organs, respectively. These differentially expressed kinases may provide important insight on the molecular mechanisms that may be important in organ-specific susceptibility to the toxicity of TKIs and facilitate the development of safer drugs with greater tumor specificity and less off-target activity.

ABSTRACT FINAL ID: 3582 Poster Board: P274
TITLE: DEHP and Hexamoll(R) DINCH(R)—A Metabolomic Comparison

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KEYWORDS: Metabolomics; Metabonomics; Safety Evaluation

ABSTRACT BODY: Di(2-ethylhexyl)phthalate (DEHP), is used as plasticizer worldwide in a wide range of polyvinyl chloride (PVC) industrial and consumer products. Although still in use, DEHP is under scrutiny as it was shown to disrupt male sexual differentiation during development by reducing testosterone production in rodents e.g., in rats. This suggests DEHP is an endocrine disruptor. 1,2-Cyclohexanedicarboxylic acid diisononyl ester (brand name: Hexamoll® DINCH®) is a non-phthalate and non-aromatic, non-hazardous plasticizer for PVC and other polar polymers, which has been developed by BASF. In this study the effects of DEHP and DINCH on the rat plasma metabolome have been evaluated and compared using MetaMap®Tox database. The MetaMap®Tox database has been developed by BASF over the last ten years containing toxicity and metabolome profiles of more than 800 different compounds. To ensure maximum reliability, data was gained from plasma samples of highly standardized 4 week rat studies. Based on the common mode of action of certain reference substances, specific metabolic patterns have been generated for more than 120 different toxicological modes of action. With the extensive *in vivo* data available in the MetaMap®Tox database we were able to match the metabolic profiles of DEHP and DINCH with the respective patterns. DEHP had a strong influence on the liver and did match with patterns for peroxisome proliferation and phthalate induced toxicity to reproduction. This could not be confirmed for DINCH. The Pearson-based correlation of the metabolic profiles of DEHP with all profiles available in the MetaMap®Tox database showed a high number of matches with other phthalates and fibrates, whereas the metabolomic profile of DINCH did not. Furthermore, DEHP and DINCH are toxicologically different based on their metabolomic profile.

ABSTRACT FINAL ID: 3583 Poster Board: P275
TITLE: Inferring Toxicological Responses of HepG2 Cells from ToxCast High-Content Imaging Data

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KEYWORDS: Systems Biology; Computational Toxicology; Predictive Toxicology

ABSTRACT BODY: Understanding the dynamic perturbation of cell states by chemicals can aid in for predicting their adverse effects. High-content imaging was used to measure the state of HepG2 cells over three time points (1, 24, and 72 h) in response to 976 ToxCast chemicals for 10 different concentrations (0.39-200µM). Cell state was characterized by p53 activation, c-Jun activation, phospho-Histone H2A.x, phospho-Histone H3, alpha tubulin, mitochondrial membrane potential, mitochondrial mass, cell cycle arrest, nuclear size and cell number. Dynamic cell state perturbations due to each chemical concentration were utilized to infer coarse-grained dependencies between variables as Boolean networks (BNs). BNs were inferred from data in two steps. First, the data for each state variable were discretized into changed/active (> 1 standard deviation), and unchanged/inactive values. Second, the discretized data were used to learn Boolean relationships between variables. In our case, a BN is a wiring diagram between nodes that represent 10 previously described observable phenotypes. Functional relationships between nodes were represented as Boolean functions. We discovered that inferred BN show that HepG2 cell response is chemical and concentration specific. We observed presence of both single and cycle BN attractors. In addition, there are instances where Boolean functions were not found. We believe that this may be either because our time sample is too small or because our data lack additional cellular features. These results illustrate the utility of Boolean networks in characterizing regulatory networks within biological systems. This abstract does not necessarily reflect US EPA policy.

ABSTRACT FINAL ID: 3584 Poster Board: P276
TITLE: Kinetic Analysis of Gene Expression Responses to Tobacco Component Exposure for Risk Assessment

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KEYWORDS: Cardiovascular System; Gene Expression/Regulation; Toxicogenomics

ABSTRACT BODY: Smoking is a major risk factor for the development of cardiovascular diseases, chronic obstructive pulmonary diseases and lung cancers contributing significantly to morbidity and mortality worldwide. Among thousands of chemicals from cigarette smoke, we are interested in identifying the principal toxicants and how each affects vascular endothelium to predispose to vascular and cardiovascular disease. A new toxigenomics approach enables us to multiplex assays to explore the regulation of many genes by each toxicant treatment, and the high-throughput nature enables us to explore 19 toxicants at a range of concentrations, time points, and replicates. We applied an improved high-throughput gene expression detection method (RASL-seq) to discern the mechanisms of toxicity related to adverse events. RASL-seq method produced highly reproducible gene expression data with high-throughput and low cost to quantitatively assess the adverse effects of individual chemical components of tobacco smoke. In this study, expression of 347 stress genes was measured in HUVEC cells exposed to 19 tobacco components and detailed dose-response relationships on each gene was established using a quantitative analysis method derived from BMDexpress. The computational method identifies the BenchMark Dose (BMD) and Point Of Departure (POD), as well as the area under the curve (AUC) based on the change of gene expression over toxicant concentration for each gene and treatment. These measures quantify the dose-dependence of gene responses and enable a pathway analysis to clarify the mode of action of each toxicant. This strategy also enables pairs of chemicals to be analysed to identify synergistic interaction of tobacco components. The combination of well-validated cellular models, systematic and quantitative chemical-gene expression responses, and screening for dual-chemical synergies, will provide an improved quantitative basis for risk assessment.

ABSTRACT FINAL ID: 3585 Poster Board: P277
TITLE: Positive Allosteric Modulators of Acetylcholinesterase as Therapeutic for Organophosphate Poisoning Exhibit Low Cytotoxicity and High Potential Therapeutic Indices

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KEYWORDS: Organophosphates; Chemical and Biological Weapons

ABSTRACT BODY: One of the current therapeutic strategies for organophosphate (OP) poisoning includes pretreatment with the weak reversible acetylcholinesterase (AChE) inhibitor pyridostigmine bromide. As an AChE inhibitor, similar side effects exist as with OP poisoning. In an attempt to provide a therapeutic capable of mitigating AChE inhibition without such side effects, our high throughput screening efforts recently identified two compounds capable of increasing AChE's catalytic activity. Previously, our lab reported these two novel positive allosteric modulators (PAMs) of AChE increased AChE three-fold, but failed to upshift the apparent IC50 of a variety of OPs (paraoxon, diisopropyl fluorophosphates, and dicrotophos). These PAMs have been identified as Compounds II (4,4'-2-amino-5-hydroxycyclohexa-2,5-diene-1,4-diylidene)bis(azanylylidene))diphenol) and III (2,5-bis((4-hydroxyphenyl)amino)-4-((4-hydroxyphenyl)imino)cyclohexa-2,5-dien-1-one). Confocal laser scanning microscopy was used to evaluate overall PAM-induced toxicity in several lines. Cells were dosed at 0.6 and 6 µM of each, and transfected with mitochondria and nucleus fluorescent proteins. These images of compound-dosed MDCK (kidney), HepG2 (liver), PC-3 (adenocarcinoma), and SK-N-SH (neuroblastoma) demonstrate rounding of cells at the higher dose of each compound, evidence of toxicity. In addition, a 12-cell line panel, including liver, kidney, neuroblastoma, and carcinoma, was dosed with these two compounds to determine LD50 values. While both compounds appear least toxic in HepG2 (28.3µM±6.3 and 31.5±1.2), and most lethal in Hek-293 (6.43µM ±2.5 and 9.9±1.2), II appears slightly more toxic than III. Results suggest that at therapeutically relevant doses, these PAMs are minimally toxic *in vitro* and have the potential to treat and prevent OP intoxication independently or in combination with the current therapeutic standard of care.

ABSTRACT FINAL ID: 3586 Poster Board: P278
TITLE: The Cardio-Specific Effects of VX Exposure on Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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KEYWORDS: Organophosphates; Alternatives To Animal Testing; Cell Culture; VX

ABSTRACT BODY: Organophosphates are well known inhibitors of cholinesterases. Many of these are used for general pesticide and agricultural purposes, but several have also been developed for use as Chemical Warfare Agents (CWAs). These include compounds such as Sarin, Soman, and VX. Although the cholinergic nervous system effects have been well characterized for these agents. Many signs and symptoms unrelated to the nervous system are also observed. To better understand these secondary effects, efforts are ongoing to develop cell based models of exposure using human cell lines to better correlate toxicity. These models will add in the identification and characterization of secondary toxic effects which leading to better countermeasures and care for those exposed. To better understand cardio-specific symptoms, a human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) model has been developed and is being used to study the toxic effects of VX. The model evaluates hiPSC-CMs on both the impedance based xCELLigence System and High-Content Analyzer (HCA) platforms. Cell lines and media from several manufactures were tested to create a model that would meet the program goals for functionality and repeatability. The iCell Cardiomyocytes2 manufactured by CDI using the GE cell line recommended RPMI with the B27 supplement media gave the most repeatable results on the xCELLigence system while permitting the testing of compounds that are unstable in serum based media. Our models showed that VX has an immediate effect of increasing the beat rate will simultaneously decreasing the beat strength of the cells. This result was dose dependent with higher concentrations increasing and lower concentrations decreasing the toxic effect on both the beat rate and beat strength. These changes were not due to cell death. The HCA confirmed that cell death was not responsible for the effects, but that an immediate decrease in intracellular calcium levels may play a possible role. Calcium is well known to be responsible for cardiomyocyte signaling. With our models being able to both identify cardio function and measure calcium levels, we are next planning to test various drugs involved with calcium regulation in the heart to determine if any currently available compound can counteract the VX induced toxicity.

ABSTRACT FINAL ID: 3587 Poster Board: P279
TITLE: Acute Ammonia-Induced Respiratory Toxicity in Rats

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KEYWORDS: Respiratory Toxicology; Inhalation Toxicology; Lung; Pulmonary or Respiratory System; Ammonia

ABSTRACT BODY: Exposure to ammonia can be highly hazardous to health, as it is a severe irritant to the mucus membranes, eyes, skin, and gastrointestinal and respiratory tracts. The increasing production, transport, and utilization of ammonia for industrial, agricultural, and commercial uses enhance the likelihood of accidental release and toxic exposure scenarios. Extensive toxicological studies are required to determine the nature of acute respiratory toxicity associated with inhalation exposure to ammonia. The objective of this study is to evaluate and determine the mechanisms of toxicity for inhalation ammonia exposure. Male Sprague-Dawley rats (300-350 g) were exposed to air or 9,000, 20,000, 23,000, 26,000, 30,000, and 35,000 ppm of ammonia for 20 min in a custom head-out exposure system. Mortality, clinical observations, edema, body weight loss, and biochemical analysis of blood, bronchoalveolar lavage cells (BALC) and fluids (BALF) were used as measurements for inhalation toxicity 24 h post-exposure. Animals exposed to ammonia manifested dose-dependent increases in observed signs of intoxication, including increased chewing and licking, ocular irritation, salivation, lacrimation, oronasal secretion, and labored breathing. The LCt50 of ammonia within this head-out inhalation exposure model was determined by probit analysis to be 23,672 ppm for male rats. Edema measured by wet/dry weight ratio increased in all ammonia-exposed animals and was significant at 9,000 ppm. Weight loss at 24 h post-exposure, bronchoalveolar lavage (BAL) protein, cell death, and total cell counts significantly increased in animals exposed to 20,000 and 23,000 ppm of ammonia in comparison to controls. Differential cell counts of white blood cells, neutrophils, and platelets from blood and BAL significantly increased following exposure to 23,000 ppm of ammonia. This study further highlights the significant toxic effect that inhalation exposure to lethal concentrations of ammonia has on the respiratory system and will be used for the further development and evaluation of potential therapeutic countermeasures. The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the US Government.

ABSTRACT FINAL ID: 3588 Poster Board: P280
TITLE: Burn Due to Hydrofluoric Acid: *In Vitro* Experiment in a 3D Human Airway Reconstructed Epithelia

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KEYWORDS: Inhalation Toxicology; *In Vitro* and Alternatives; Burn; Hydrofluoric Acid

ABSTRACT BODY: Hydrofluoric acid (HF) is very corrosive and toxic for human exposure by skin and eye contact, ingestion and inhalation. Inhalation of hydrofluoric acid can be fatal (H330) following GHS/CLP regulation. Few experimental data are available to understand how hydrofluoric acid destroys lung tissues. We designed an experiment to explore the range of toxicological doses of HF and the effect on MucilAir™: MucilAir™ is a unique 3D Human Airway Epithelia reconstructed *in vitro*, with high standard of quality and reproducibility. Epithelia (MucilAir™) are reconstituted with human cells of bronchial origin (with donor consent). 100 µl of HF was applied on the epithelia during 20 min; the concentrations tested were 0.15, 0.75, 1.5, 7.5, 15, 75, 150 mM. The following endpoints were analyzed: morphology, tissue integrity monitoring (TEER), Lactate Dehydrogenase release quantification (LDH), Cilia Beating Frequency (CBF) and histology. Up to 1.5 mM of HF, no toxic effect has been detected by all the endpoints measured. Starting from 7.5 mM, the damage to epithelia were observed: the TEER dropped significantly, the pseudo-stratified structure was altered: only basal cell layer was present. Cilia beating frequency was reduced. However, based on TEER analysis, damage caused by HF below 7.5 mM could be repaired 7 days after exposure. And the CBF values were recovered despite some variations at earlier time-point, except when HF was used above 75 mM. HF at 75 mM and above caused severe damage to epithelia, which was not reversible. Histology clearly shows that the pseudo-laminate structure including the three cell types is well maintained for inspection and treatment HF 1.5 mM. 15 mM HF contact induced an abnormal structure with the presence of a single layer of basal cells. This is perfectly in line with the high value of TEER found to Day 7. From 15 to 150 mM strong and non-reversible toxicity was observed. MucilAir™ model allows characterizing the damages induced by HF and confirms that low concentration of hydrofluoric acid can induce irreversible damages. The model could be of interest to evaluate in further studies the benefit of early decontamination and treatments.

ABSTRACT FINAL ID: 3589 Poster Board: P281
TITLE: Development of an *In Vitro* Inhalation Toxicity Test for Improved Protection of Human Health

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KEYWORDS: Inhalation Toxicology; *In Vitro* and Alternatives; Toxicity; Acute

ABSTRACT BODY: Knowledge of acute inhalation toxicity and irritation potential is important for establishing safe handling, packaging, labeling, transport and emergency response procedures for chemicals. The US EPA High Production Volume Chemical Challenge, and the EU REACH programs have further increased the need for inhalation toxicity information. A UN treaty endorsed by the US, EU and others outlines a "Globally Harmonized System" (GHS) of Classification and Labeling of Chemicals. The GHS specifies 5 inhalation toxicity categories. The EPA has established a separate system that uses 4 toxicity categories. Acute inhalation toxicity tests currently accepted within the GHS and EPA systems involve *in vivo* 4 hr rat inhalation LC50 tests (OECD TG 403/436). In the current work, a newly developed *in vitro* toxicity test was evaluated in comparison to the established *in vivo* tests. The *in vitro* test exposes an organotypic human airway tissue model to test chemicals for 3 hrs, followed by measurement of tissue viability (IC75). 64 chemicals covering a broad range of toxicity classes, chemical structures and physical properties were evaluated. Results show that the *in vivo* and *in vitro* tests had 100% concordance for identifying highly toxic chemicals (GHS Cat 1-2 and EPA CAT I-II). However, the *in vivo* tests had only 29.0% (EPA system) or 61.4% (GHS system) sensitivity for identifying less toxic respiratory irritants. Numerous human respiratory irritants including acids, bases, aldehydes, amines and others were not classified as respiratory toxins/irritants by the *in vivo* tests. The *in vitro* airway model was very good (sensitivity of 81.1 - 82.4%) for distinguishing respiratory toxins and irritants (corresponding to GHS 1-3, EPA, I-III) from non-toxins, non-irritants (corresponding to GHS 4-5, EPA IV). Overall accuracy of the *in vitro* test was 81.2 - 84.1%. There were no false negative GHS Cat 1-2 or EPA Cat I-II predictions using the *in vitro* test. These data suggest that tests based on lethality in animals, while good for predicting highly toxic chemicals, produce a high percentage of false negative predictions for moderately/slightly toxic or irritating chemicals. The *in vitro* test using an organotypic human airway model was equal to current animal tests for predicting highly toxic inhaled chemicals, and better than animal tests for predicting moderately/slightly toxic respiratory irritants. The new *in vitro* testing approach should provide improved protection of human health compared to the current animal tests.

ABSTRACT FINAL ID: 3590 Poster Board: P282
TITLE: Welding Fume-Induced Generation of Reactive Oxygen Species and Activation of Inflammatory Signaling Pathways in RAW 264.7 Mouse Macrophages

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KEYWORDS: Particulates; Inhalation Toxicology; Inflammation; Metals; Welding Fume

ABSTRACT BODY: Welding fumes are a complex mixture of several toxic metals (e.g., Cr, Mn, Fe, Ni). Epidemiology indicates that welders have an increased risk for lung disease, including bronchitis, airway infections, and cancer. Animal toxicology studies show that specific welding fumes cause significant lung injury and inflammation, depending on the metal composition of the fume. The goal was to examine the potential mechanisms by which welding fumes with different metal compositions may generate reactive oxygen species (ROS) and activate inflammatory signaling pathways in an *in vitro* model. RAW 264.7 mouse macrophages were incubated with 0, 3.125, 6.25, 12.5, 25 and 50 µg/mL of either manual metal arc-stainless steel (MMA-SS) or gas metal arc-mild steel (GMA-MS) welding fumes for 24 hr. Cytotoxicity, ROS generation, and activation of different inflammatory markers were assessed. Metal composition and solubility of the fumes were different: MMA-SS (41% Fe, 29% Cr, 17% Mn) was highly water-soluble, whereas GMA-MS (85% Fe, 14% Mn) was water-insoluble. At 24 hr, MMA-SS significantly elevated ROS generation and dose-dependently increased cytotoxicity in the RAW 264.7 cells compared to GMA-MS and saline control. Welding fume-induced ROS generation led to production of the toxic lipid aldehyde, 4-hydroxynonenal (HNE). Both welding fumes induced the activation of the mitogen activated protein kinases (MAPK), such as extracellular signal-regulated kinases 1 and 2 (ERK1/2), leading to the increased expression of COX-2 in the RAW 264.7 cells. Also, welding fumes increased protein expression of Nrf2 and HO-1, activating the Nrf2-Keap-HO-1 pathway. In all cases, a significantly greater activation of the different inflammatory markers was observed for MMA-SS compared to the GMA-MS and saline control. These results suggest that the cytotoxicity of RAW 264.7 cells caused by MMA-SS is due to the presence of soluble and cytotoxic metals (Cr, Ni) that are absent in the GMA-MS fume.

ABSTRACT FINAL ID: 3591 Poster Board: P283
TITLE: Cilia of Human Mucilair Are Less Impacted by the Exposure to the Aerosol of a Candidate Modified Risk Tobacco Product Than to Whole Smoke From Conventional Cigarettes *In Vitro*

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S. Frentzel, D. Grandolfo, D. Kuehn, S. Majeed, M.C. Peitsch, J. Hoeng. *Philip Morris Products S.A., Neuchatel, Switzerland.*
KEYWORDS: Respiratory Toxicology; Cilia Beating Frequency

ABSTRACT BODY: Mucociliary clearance is an important defense mechanism that mediates removal of foreign particles and chemicals from the airways. Cilia beating thereby plays a key role that determines the rate of mucus clearance and thus constitutes a vital function of respiratory epithelia. Cigarette smoke has been reported to adversely impact cilia function *in vitro* and *in vivo*, by changing cilia beating frequency (CBF) or impairing ciliogenesis. To monitor CBF, semi-automated methods such as CiliaFA combine high-speed video recording with the ability to determine CBF. Using CiliaFA, we were able to confirm that the MucilAir CBF can be modulated *in vitro* by either 100 µM isoproterenol or a temperature shift to 4°C. Moreover, *in vitro* exposure of MucilAir with whole smoke from conventional cigarettes (3R4F) caused a decrease in the total surface area of the culture showing active cilia beating. Cilia on the epithelial cell surface that were detected to be still active after 3R4F exposure, showed variable beat frequencies, ranging from normal to decreased CBF. Compared to 3R4F cigarette smoke exposure, the effect of equivalent concentrations (based on nicotine) of a candidate modified risk tobacco product (THS2.2) aerosol was much less, judged from its effect on total surface area showing active cilia and from the determined cilia beating frequencies. Overall, this study clearly discriminated the effects of THS2.2 from the deleterious impact of 3R4F on cilia beating.

ABSTRACT FINAL ID: 3592 Poster Board: P284
TITLE: Respiratory Toxicity of Nitrogen Trichloride

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KEYWORDS: Lung; Pulmonary or Respiratory System; Respiratory Sensitization; Nitogen Trichloride

ABSTRACT BODY: Nitrogen Trichloride, (Trichloramine, NCl_3; CAS 10025-85-1) is formed in chlorinated swimming pools from nitrogen containing substances. Due to its limited solubility in water it enters the air of indoor swimming pools. Respiratory effects and asthma have been reported in exposed adults and children. The link to NCl_3 is controversial and further information is needed. Our studies comprised the following: 1. Lung function tests in volunteers exposed in an indoor swimming pool environment. 2. Respiratory symptoms in 1741 swimming pool workers who completed a mailed questionnaire. 3. Occurrence of asthma among 1866 children attending indoor swimming pools. The Regional Ethical Review Board in Umea granted permission for these human studies. 1) 37 volunteers without allergic sensitization were exposed to pool air with 0.23 mg/m3 of NCl_3 during 2 hours and for comparison in filtered air containing no NCl_3. Lung function was tested before and after in both cases. A statistically significant decrease in FEV1 was seen after exposure in pool air compared to exposure in NCl_3-free air ($p=0.01$). 2) A statistically significant relationship ($p<0.01$) was found between number of hours spent in pool environments and the percentage of workers reporting acute symptoms like eye- throat- and nose irritation, cough and dyspnea. 3) By parental questionnaires, attendance at indoor swimming pools and the prevalence of asthma in sensitized (determined by skin prick test) and non-sensitized children was determined. Swimming pool attendance was associated with a statistically significant increased Odds Ratio for asthma among sensitized children (OR 1.90 CI 1.09-3.32) after adjustment for sex, parental smoking, parental asthma and damp housing. No increased OR was found among non-sensitized children. Our studies support the idea that exposure to NCl_3 in indoor swimming pool environments increases the risk of respiratory symptoms and asthma particularly among those sensitized to other allergens.

ABSTRACT FINAL ID: 3593 Poster Board: P285
TITLE: Ozone-Induced Pulmonary Injury and Inflammation are Modulated by Adrenal-Derived Stress Hormones

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KEYWORDS: Respiratory Toxicology; Inhalation Toxicology; Lung; Pulmonary or Respiratory System; Ozone

ABSTRACT BODY: Ozone exposure promotes pulmonary injury and inflammation. Previously we have characterized systemic changes that occur immediately after acute ozone exposure and are mediated by neuro-hormonal stress response pathway. Both HPA axis and sympathetic tone alterations induce the release of epinephrine and corticosteroids to the blood after ozone inhalation. To understand the influence of these hormones in ozone-induced lung alterations, we eliminated their source by adrenalectomy. Male Wistar-Kyoto rats (12 week-old) underwent total bilateral adrenalectomy (ADREX), adrenal demedullation (DEMED) or sham surgery (SHAM). After 4 day of recovery, rats were exposed to air or ozone (1ppm), 4h/day for 1 or 2 days. Circulating medulla-derived adrenaline in DEMED and ADREX rats were significantly reduced relative to air-exposed SHAM. Cortex-derived corticosterone levels were decreased in DEMED rats and almost disappeared in ADREX rats. Ozone-induced lung protein/albumin leakage and neutrophilic inflammation were significantly reduced in DEMED and ADREX rats (ADREX>DMED). To characterize the influence of these hormones in ozone-induced inflammation, expression of different genes involved in inflammation were quantified in the lung by qPCR. Ozone-induced increases in Mip2 and Il6 in SHAM rats were diminished in DEMED and ADREX rats which coincided with neutrophilic inflammation. Ozone increased BALF eosinophil-like cells only in ADREX rats but not in SHAM rats. Th1/Th2 response measured by expression of specific cytokines such as Infg (Th1) and Il4 (Th2) were not significantly changed by adrenalectomy or ozone, although the expression of some secreted factors such as Il5, Il13 and Il12a tended to be higher in adrenalectomized rats regardless of exposure. However ozone exposure decreased Il13 expression in SHAM rats. Contrary to the accepted mechanism of acute ozone-induced lung cell injury, vascular leakage and inflammation which involves direct cellular damage and oxidative modification-mediated neutrophil recruitment, we show that ozone-induced lung injury and neutrophilic inflammation involving Mip2 and Il6 require circulating epinephrine and corticosterone. Additionally, acute ozone-induced neutrophilic inflammation in SHAM (normal) rats apparently may not involve acquired immune response or Th1/Th2 mechanisms. (Does not reflect EPA Policy).

ABSTRACT FINAL ID: 3594 Poster Board: P286
TITLE: Propylene Glycol and Glycerin in E-cigarette Liquids Modulate Murine Respiratory Irritation Responses and Human Sensory Irritant Receptor Function

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KEYWORDS: Inhalation Toxicology; Nervous System; Dose-Response; E-Cigarettes; Cinnamaldehyde

ABSTRACT BODY: Electronic cigarettes are unregulated and their toxicological and physiological effects remain poorly understood. E-cigarette liquids contain nicotine and flavorants dissolved in propylene glycol (PG) and/or vegetable glycerin (VG). Nicotine and some flavorants are respiratory irritants that activate sensory nerve endings in the airways, eliciting reflex responses including cough. However, a recent study observed attenuated cough reflexes in subjects inhaling E-cigarette vapor. Using barometric plethysmography we examined the effects of irritant-containing E-cigarette vapor on the respiratory irritation response in mice. Mice were exposed to commercial cinnamon-flavored E-cigarette vapor, with the E-liquid containing >7% cinnamaldehyde, a known sensory irritant, and 18mg/ml nicotine, with PG/VG 90:10 as vehicle using the V2 E-cigarette system. Mice displayed respiratory irritation, indicated by dose-dependent increases in time of breaking. Irritation developed relatively slowly with peak values lower than observed in mice exposed to cigarette smoke. Intriguingly, mice exposed to vehicle control (PG/VG 90:10) displayed irritant responses almost equal to responses to pure cinnamaldehyde vapors. However, cinnamaldehyde vapors produced more rapid irritation responses. Cinnamaldehyde elicits irritation by activating Transient Receptor Potential Ankyrin 1 (TRPA1), the irritant receptor ion channel expressed in sensory neurons. Using calcium microfluorimetry in HEK293t cells we examined responses of human and mouse TRPA1 to cinnamon E-liquids and vehicle. Some cinnamon E-liquids activated TRPA1 at dilutions of 1:100,000 , illustrating their high irritant content. Vegetable glycerin attenuated responses of TRPA1 to cinnamaldehyde, with the effect strongest on human TRPA1. These data demonstrate that irritant levels in E-cigarette vapors are sufficient to produce respiratory irritation. E-liquids contain large amounts of irritants specifically activating TRPA1 receptors. Vehicle components, PG and VG, have clear pharmacological effects *in vivo* and *in vitro*, attenuating irritant responses and, potentially, facilitating inhalation of higher irritant and nicotine levels in E-cigarette users.

ABSTRACT FINAL ID: 3595 Poster Board: P287
TITLE: Integrated Genomics and Proteomics Analysis of the Human Small Airway Epithelial Cells Exposed to Cigarette Smoke Condensate

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KEYWORDS: Systems Biology; Respiratory Toxicology; Cigarette Smoke Condensate

ABSTRACT BODY: Smoking affects nearly every organ of the body and some adverse health consequences of tobacco use may take decades to develop. Therefore, there is a need to identify mechanistically relevant and quantitatively valid biomarkers that predict long-term adverse health outcomes produced by exposure to tobacco smoke. The effects of cigarette smoke condensates (CSC) on mRNA and protein expression in human small airway epithelial cells (SAEC) using reference cigarette condensates under International Organization for Standardization (ISO) conditions at low (2.5 µg/ml) and high (5 µg/ml) concentrations for 7 and 30 days or without condensates as the control (0 µg/ml). mRNA and protein expression were analyzed using next-generation sequencing (NGS) and aptamer-based protein array, respectively. Pathway analyses for differentially expressed mRNAs and proteins were performed with MetaCore™ software. The results show that exposure time, CSC type and concentrations all affected mRNA and protein expression. Comparing genomics and proteomics analyses, we observed that for 16-28% of differentially expressed proteins, their corresponding mRNAs were also differentially expressed. Pathway analysis found that cell adhesion extracellular matrix remodeling was the most affected pathway at both the mRNA and protein level. Furthermore, one of the key molecules in the pathway, metalloproteinase inhibitor 1 (TIMP1), was consistently up-regulated at both the mRNA and protein level across all experimental conditions. TIMP1 is a natural inhibitor of the matrix metalloproteinases (MMPs) and promotes cell proliferation in a wide range of cell types, and it may also have an anti-apoptotic function. Taken together, CSC treatment may significantly affect cell adhesion extracellular matrix remodeling in SAEC, and TIMP1 and related MMPs may play an important role in this process.

ABSTRACT FINAL ID: 3596 Poster Board: P288
TITLE: Inhalation Toxicity in Rats Exposed to Respirable Particulate Matter From Southwest Asia in Combination with Model Burn Pit Emissions

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: B.A. Wong[1], N.M. Gargas[2], G.P. Lemmer[2], R.A. James[2], I.C. MacGregor[3]. *[1]Inhalation Toxicology, Henry M. Jackson Foundation, Wright-Patterson AFB, OH; [2]Camris, International, Wright-Patterson AFB, OH; [3]Battelle, Columbus, OH.*
KEYWORDS: Inhalation Toxicology; Particulates

ABSTRACT BODY: Adverse pulmonary effects have been reported in military personnel returning from deployment to the Middle East. Epidemiological studies to establish links between the reported effects and exposure to environmental particulate matter (PM) or emissions from burning solid waste (SW) in open air burn pits have been inconclusive. Male Sprague-Dawley rats were exposed to clean air, respirable PM, burn pit emissions, or a combination of respirable PM followed by burn pit emissions. PM collected from Camp Victory in Iraq was sieved and autoclaved prior to aerosolization using a Wright Dust Feeder. Rats were exposed to PM aerosol for 20 h/day, 5 d/wk over a 4 week span. The PM aerosol was respirable with a mass median aerodynamic diameter (MMAD) of 2.3 µm and average mass concentration of 4 mg/m3. Burn pit exposures were generated from combustion of SW in a model burn pit over a 6 h/day, over a 5 day span. The burn pit aerosol had an MMAD of 0.4 µm and a concentration of 0.8 mg/m3. CO2 and CO in the burn pit exposure averaged 666 ppm and 9.6 ppm, respectively. Necropsies were conducted 4, 32, and 90 days after the last exposure. Group average weight and weight gains were not statistically different between the exposure groups. Bronchial alveolar lavage fluid analyses showed decreased macrophage numbers and increased neutrophils in the animals exposed to PM. Resting tidal volume was lower in the sand exposed animals when compared with controls, but this difference diminished between the 4 and 32 day time points. Other biology endpoints showed little or no differences among groups compared with controls, statistically. In addition to the toxicology and biology data reported here, proteomic and metabonomic analyses of samples from this study will be used to identify potential biomarkers of exposure or response to these exposures.

ABSTRACT FINAL ID: 3597 Poster Board: P289
TITLE: Toxicants in E-Cigarette Aerosols—A Quantitative Survey and Comparison with Cigarette Smoke

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KEYWORDS: Dioxin; Halogenated Hydrocarbon; Metals

ABSTRACT BODY: Despite widespread use of e-cigarettes there have been few detailed chemical studies examining e-cigarette aerosol composition, with most studies to date focusing on specific compound groups. Here we report the most complete chemical comparison to date of emissions from an e-cigarette and a tobacco cigarette (~ 150 compounds), including FDA HPHC compounds, and species previously reported to be present in e-cigarette emissions. Vype e-Pen Blended Tobacco flavour, and the Kentucky Reference Cigarette 3R4F were examined. Vype e-Pen was puffed in two separate 100-puff blocks using a 55/3/30 puffing regime (volume(cm3)/ duration(s)/ interval(s)), and 3R4F smoke was collected (in separate rooms) using the Health Canada 55/2/30 regime (ventilation blocked). With anticipated low levels of some e-cigarettes constituents, air/method blank analysis was made at the concurrently with, and in the same way as e-cigarette measurements. Independent contract labs used ISO17025 accredited methods to quantify the following emissions: carbon/nitrogen oxides, carbonyls/dicarbonyls, alcohols/di-alcohols, phenols, o-heterocycles, chlorinated dioxins/furans; volatile, substituted and, polynuclear aromatic hydrocarbons; amides, azines, aromatic and aliphatic amines, nicotine & related compounds, nitrosamines, metals and radionuclides. 105 compounds were undetectable in Vype e-Pen emissions. 23 compounds were detected or quantified at comparable levels in Vype e-Pen emissions and air/method blank; hence it was concluded that e-Pen did not generate measurable levels of these 23. 15 compounds were quantified at higher levels in Vype E-pen emissions than the blank, but at substantially lower per-puff levels than 3R4F. Similar or higher per-puff emissions of four compounds (propylene glycol, glycerol, menthol and chromium) were measured from Vype E-pen in comparison to 3R4F. Nearly 100 of the compounds investigated were measurable in 3R4F emissions. This study shows substantial chemical differences between emissions from e-cigarettes and tobacco cigarettes. Most cigarette toxicants examined could not be detected in the e-cigarette aerosol. Measuring air/method blanks is an essential step for identifying experimental artefacts amongst trace-level e-cigarette aerosol constituents.

ABSTRACT FINAL ID: 3598 Poster Board: P290
TITLE: Airway Macrophage Uptake of Particulates is Differentially Affected by Inhalation of Fine and Ultrafine Carbonaceous Particulates Combined with Vehicular Gases

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KEYWORDS: Particulates; Cardiopulmonary; Volatile Organic Compounds

ABSTRACT BODY: Near-roadway exposures have been associated with increased risk of cardiopulmonary health effects. It remains unclear if this effect is related solely to increased concentrations of particulate matter (PM), or to interactions with gaseous species formed in the combustion process. The present study tested this by exposing mice to vehicle engine (diesel and gasoline combined)-derived PM, separated as fine (FP) and ultrafine (UFP) sizes, in order to determine if the vehicle engine PM could induce markers of pulmonary and vascular inflammation in WT and Apolipoprotein E knockout mice. Mice were exposed to 300 μg/m3 of either UFP or FP for 6h/d for 1-d or 30-d by whole-body inhalation. Two additional groups were included, which received the FP or UFP combined with gaseous copollutants derived from fresh gasoline and diesel emissions, to examine the toxicological contribution of gas-particle interactions. Body weights were consistent for all groups except for mice exposed to UFP+gases, where both ApoE-/- and WT mice exhibited significant reductions in growth relative to filtered air controls. After 30 days of exposure, no pulmonary inflammation or injury was noted in any exposure group, in terms of bronchoalveolar lavage cellularity, total protein, or lactate dehydrogenase levels. However, macrophages in mice exposed to UFP+gases exhibited substantially more PM inclusions compared to UFP or FP alone. Macrophages from FP+gas-exposed mice exhibited virtually no PM inclusions. Thus, the size of the PM may effect either the interaction with gaseous components from vehicular engine emissions, or the biological responses to gas+PM mixtures. These results may provide clues as to the origins of systemic inflammatory signals from inhalation exposures to fresh emissions.

ABSTRACT FINAL ID: 3599 Poster Board: P291
TITLE: Establishment and Characterization of a Novel 3D Human *In Vitro* Small Airway Model

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S. Huang, B. Boda, J. Vernaz, L. Wiszniewski, S. Constant. Epithelix. *Plan-les-Ouates, Switzerland.*
KEYWORDS: Lung; Pulmonary or Respiratory System; Respiratory Toxicology; *In Vitro* and Alternatives

ABSTRACT BODY: The small airways are non cartilaginous airways with a diameter < 2mm, which are extremely venerable to external insults such as tobacco smoke, mineral dust, air-pollutants, allergens, drugs, bacterial and viral infections. They play an important role in many lung diseases including chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), sarcoidosis and obliterative bronchiolitis (OB). However, the small airways constitute one of the least understood areas of the lungs due to the inaccessibility *in vivo*. Therefore, an *in vitro* human small airway model would be tremendously valuable for toxicity testing of chemical substances and for studying various respiratory diseases. We report here the establishment and characterization of a novel *in vitro* human small airway model (SmallAir). The primary epithelial cells were isolated from the distal lungs by enzymatic digestion. After amplification, the cells were seeded on the microporous membrane of Transwell inserts. Once confluent, the cultures were switched into air-liquid interface. After 3 weeks of culture, the epithelium became fully differentiated, with morphology of columnar epithelium, and a thickness of 10-15 µm. The epithelium is electrically tight (TEER ≈ 400 ohm.cm2). Most significantly, CC-10, a specific marker of Clara cells, was highly expressed in SmallAir. CC-10 was detected by both immuno-histochemisty and Western Blot. As expected, SmallAir contained few Muc5-Ac positive cells (goblet cells). In contrast, CC-10 was not detected in MucilAir™, an *in vitro* model of the human bronchial epithelium. Instead, Muc-5Ac was highly expressed in MucilAir™. SmallAir contain also basal cells and ciliated cells, showing cilia beating (8 Hz) and mucociliary clearance (15 um/s). SmallAir represents a unique and powerful tool for studying the physiology and function of small airways and it should provide new insights into this major area of lung diseases.

ABSTRACT FINAL ID: 3600 Poster Board: P292
TITLE: Semi-Volatile Organic Components of Ultrafine Particles Promote Atherosclerosis in ApoE -/- Mice

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KEYWORDS: Respiratory Toxicology; Cardiopulmonary; Particulates

ABSTRACT BODY: Coronary artery disease (CAD) is the most prevalent cause of disease-related death in the US and emissions related particulate matter (PM) exposures are thought to be associated with thousands of excess deaths per year. Ultrafine particles (UF) contain a large proportion of redox-active organic compounds that can exist in either the volatile or particle phase and are considered semi-volatile organic compounds (SVOCs). After inhalation, these compounds may be responsible for initiating a cascade of oxidative stress and inflammation in the body that can promote atherosclerotic plaque development that can lead to CAD. ApoE -/- mice, which are prone to developing atherosclerosis, were exposed to unmodified UF CAPs (CAPs) or CAPs with the SVOC components removed by a thermodenuder (deCAPs) for 8 weeks for 5 hours/day, 4 days/week at the UC Irvine campus. A control group was exposed to purified, filtered air. The particle number concentrations of each atmosphere were monitored real-time and the CAPs concentration was adjusted to match the deCAPs concentration which is lowered by denuding. The aortic arch and brachiocephalic arteries of each mouse were removed and sectioned to analyze arterial morphology and composition. Serum was analyzed for levels of inflammatory cytokines. A higher percentage of mice exposed to CAPs formed arterial plaques compared to deCAPs and air exposed mice. Furthermore there was greater vascular wall thickening and fibrosis in the arteries of mice exposed to CAPs compared to deCAPs. Cytokines IL-6 and IL-1b, both of which have been implicated in the inflammatory processes that promote atherosclerotic plaque development, were increased in the serum of mice exposed to CAPs relative to deCAPs, indicating that the SVOCs are promoting accelerated atherosclerosis through a pro-inflammatory mechanism. The attenuation of atherosclerostic plaques with deCAPs exposure indicate that SVOCs in CAPs exposures are possibly important contributors to the toxic cardiovascular effects and these components deserve consideration in discussions of regulation of PM emissions and public health.

ABSTRACT FINAL ID: 3601 Poster Board: P293
TITLE: Exposures to Old Technology Diesel Emissions to Evaluate Biological Response in Non-Human Primates

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J. Brower, H. Irshad, M. Doyle-Eisele, Y. Tesfaigzi, J. McDonald. *Lovelace Respiratory Research Institute, Albuquerque, NM.*
KEYWORDS: Exposure, Environmental; Inhalation Toxicology; Lung; Pulmonary or Respiratory System; Diesel

ABSTRACT BODY: The adverse health effects of exposure to standard diesel vehicle emissions (DE) are a major concern among urban populations. Studies investigating the biological effects of diesel exposure date back more than 30 years, but combustion technology is constantly improving. We intended to establish a non-human primate model of acute DE exposure in order to test future hypotheses related to exhaust fractionation and comparisons with alternative fuel technologies. This study evaluated the biological responses of female cynomolgus macaques after acute (4 hour) whole body exposure to filtered air (FA) or whole DE. Flows and dilutions in the exposure system were targeted for 300 µg/m3 particulate in the DE atmosphere. The exposure atmospheres were characterized for mass concentration, particle size distribution, carbon monoxide, carbon dioxide, nitrous oxides, and hydrocarbons. At baseline and at 4 hours post the end of exposure, blood was collected for hematology and serum collection, bronchoalveolar lavage (BAL) was performed, and bronchial brushings and biopsy were conducted at two sites within the bronchial tree for various endpoint analyses. At 1 hour post exposure an additional hematology sample was collected. BAL and serum samples were analyzed for various cytokines. Exposure to DE induced inflammation in terms of hematology measurements with neutrophils increasing as early as 1 hour post the acute exposure, and lymphocytes increasing at the 4 hour time point. Because the FA exposure also induced increases in both total white blood cells and in lymphocytes, the biological significance of this finding is unclear. When analyzing BAL cell counts and differentials, both FA and DE induced increases in neutrophils and macrophages relative to baseline samples, but DE also induced significant increases in lymphocytes. Analysis of cytokine levels in BAL and serum showed non-significant decreases in IL-1b and CC-16 and non-significant increases in IL-6 after DE exposures, in both BAL and serum. Analysis of gene expression of bronchial brushing samples showed markedly greater, though non-significant, expression of CC-16, Muc5ac, and SPDEF following DE exposure and small non-significant increases following FA exposure. It is possible that the changes observed in these samples represent the earliest changes to the inhaled atmospheres. Perhaps later time points would manifest greater observed changes.

ABSTRACT FINAL ID: 3602 Poster Board: P294
TITLE: Phytochemical Nrf2 Agonist Sulforaphane Prevents Oxidative Lung Injury in Mice

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: H.-Y.Cho[1], L. Miller-DeGraff[1], P. Talalay[2], M. Yamamoto[3], S.R. Kleeberger[1]. *[1]Inflammation, Immunity, and Disease Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, NC; [2]School of Medicine, Johns Hopkins University, Baltimore, MD; [3]Graduate School of Medicine, Tohoku University, Sendai, Japan.*
KEYWORDS: Antioxidants; Inhalation Toxicology; Oxidative Injury; Nrf2

ABSTRACT BODY: Nrf2 is essential to airway protection against oxidative insults via antioxidant response element (ARE)-mediated induction of cytoprotective genes. To test the hypothesis that enhanced Nrf2-ARE pathway protects lungs from subsequent oxidative insult, wild-type (*Nrf2*$^{+/+}$) and *Nrf2*-deficient (*Nrf2*$^{-/-}$) mice were orally given with a phytochemical Nrf2 agonist, sulforaphane (SFN), or PBS before (-5, -3, and -1 d) air or hyperoxia (>95% O2) exposure. Alternatively, mice were fed with standardized broccoli sprout extract (SBE, containing SFN precursors)-mixed diet or regular diet (AIN) for 14 d before hyperoxia or air exposure. At the end of exposure, mice were killed and lung injury was evaluated by bronchoalveolar lavage and histopathologic analysis. Lungs from the study with oral SFN were processed for cDNA microarray analysis. In *Nrf2*$^{+/+}$mice, hyperoxia-induced lung neutrophilia, epithelial cell injury, and histopathologic injury were significantly decreased by SFN or SBE. Lung injury phenotypes in *Nrf2*$^{-/-}$ mice were not significantly changed by SFN or SBE while mild-to medium histopathologic protection was observed in SBE-given *Nrf2*$^{-/-}$ mice, indicating Nrf2-independent action of SFN. Hyperoxia-induced body weight loss was significantly attenuated by SBE in *Nrf2*$^{+/+}$mice, but not in *Nrf2*$^{-/-}$. Transcriptome analysis determined that SFN significantly stimulated lung genes involved in mitochondrial energy metabolism as indicated by enhanced oxidative phosphorylation complex assembly subunits. During hyperoxia exposure, genes altered by SFN in an Nrf2-dependent manner associated not only with antioxidant defense and mitochondrial function but also cellular assembly and lipid/amino acid/nucleic acid metabolism associated with NF-κB signaling pathway, indicating their role in pulmonary protection. Results suggest a potential role for dietary SFN in normal lung energy metabolism and its preventive intervention for oxidative lung disorders mainly through Nrf2-dependent manner.

ABSTRACT FINAL ID: 3603 Poster Board: P295
TITLE: Involvement of Nrf2 Signaling Pathway in Usnic Acid-induced Toxicity in Human Hepatic Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S. Chen, Z. Zhang, Z. Ren, D. Yu, L. Couch, W. Tolleson, B. Ning, N. Mei, L. Guo. *US FDA–National Center for Toxicological Research, Jefferson, AR.*
KEYWORDS: Oxidative Injury; Cytotoxicity; Mechanisms; Nrf2 Pathway; Usnic Acid

ABSTRACT BODY: Usnic acid extracts and pure usnic acid, as dietary supplements, have been marketed as weight loss agents. Severe hepatotoxicities including acute liver failure have been reported to be associated with the use of usnic acid-containing products. Our previous mechanistic studies revealed that autophagy, calcium homeostasis disturbance, and ER stress involve in usnic acid-induced toxicity. In this study, we investigated the role of oxidative stress and Nrf2 signaling pathway in usnic acid-induced toxicity in HepG2 cells. We found that 24 h treatment of usnic acid caused S phase cell cycle arrest and DNA damage in a concentration-dependent manner. Treatment of usnic acid triggered oxidative stress as demonstrated by increased reactive oxygen species (ROS) generation and glutathione depletion. Short-term treatment (6 h) of usnic acid significantly increased mRNA and protein expression of Nrf2 (nuclear factor erythroid 2-related factor 2), provoked Nrf2 translocation to the nucleus, up-regulated antioxidant response element (ARE)-luciferase reporter activity, and increased the expression levels of Nrf2 targets including glutathione reductase, glutathione S-transferase, and NAD(P)H quinone oxidoreductase (NQO1). Furthermore, knockdown of Nrf2 with shRNA aggravated usnic acid-induced DNA damage and cytotoxicity. Taken together, our results suggest that usnic acid causes cell cycle dysregulation, DNA damage, and oxidative stress; and that Nrf2 signaling pathway plays an important role in usnic acid-induced cytotoxicity.

ABSTRACT FINAL ID: 3604 Poster Board: P296
TITLE: γ Radiation Induced Activation of Nrf2-Regulated Antioxidant Enzymes: Protective Effect of a Multiple Antioxidant

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: C. Hai. *Fourth Military Medical University, Xi'an, China.*
KEYWORDS: Oxidative Injury; Exposure, Environmental; Mechanisms

ABSTRACT BODY: Oxidative stress has been believed to play a crucial role in the development of radiation-induced damage. Our previous results have showed that the traditional Chinese medicine ANTIOXIN (Guoyao Zhunzi Z20040084, developed by our department) is a multiple antioxidant that can effectively inhibit oxidative stress. The present study was designed to evaluate the radio-protective effect of ANTIOXIN in γ-radiation-induced toxicity. Firstly, several *in vitro* models were used to evaluate the antioxidative effect of ANTIOXIN. Compared with each constituent, ANTIOXIN exhibited more comprehensive and stronger inhibitory effect in the indicated *in vitro* models. In rats, radiation induced significant hepatic and systematical dysfunction, as evidenced by the changes of liver pathology and serum and hepatic biochemical parameters. In addition, radiation resulted in notable increase of various antioxidant enzymes and proteins. Nuclear factor erythroid 2 p45-related factor 2 (Nrf2) silence could inhibit the increase of these enzymes induced by radiation, indicating that Nrf2-regulated various antioxidant enzymes may consist of an “antioxidant enzyme chain” to solid the defense in response to oxidative stress. Radiation-induced activation of Nrf2 may be due to increased reactive oxygen species (ROS), which was mainly generated by NADPH oxidases. The administration of ANTIOXIN could prevent radiation-induced oxidative stress, since those changes of oxidative stress markers induced by radiation were inhibited by ANTIOXIN. In conclusion, ANTIOXIN may protect against radiation-induced oxidative injury, through either scavenging ROS directly or decreasing ROS generation induced by radiation via the inhibition of NADPH oxidases expression. These results provide new insight into the studies of antioxidants and oxidative stress-associated diseases.

ABSTRACT FINAL ID: 3605 Poster Board: P297
TITLE: TCBQ-Induced DNA Double-Strand Breaks Repair Involves Rad51-Regulation through the p53 Signaling Pathways in MDA-MB-231 Cells

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KEYWORDS: Environmental Toxicology; Dna Repair; P53; Rad51

ABSTRACT BODY: Tetrachlorobenzoquinone (TCBQ) is a reactive metabolite of environmental pollutant pentachlorophenol(PCP). Although the genotoxic effect of PCP has been comprehensively investigated, there is little known about TCBQ's genotoxic effects. Our previous study found that TCBQ can generate a large amount of reactive oxygen (ROS) through redox active oxyfen process, that can lead to DNA doublestrand breaks (DSBs) and apoptosis in mammalian cells. DSBs can cause genomic instability, mutations, and neoplastic transformations, and improper repair of DSBs may lead to the development of cancer. Rad51 is a key protein in the homologous recombination (HR) pathway of DSBs repair. The effect of TCBQ on Rad51 expression and its putative role in the TCBQ-induced DSBs in MDA-MB-231 cells are still not clear enough. In the current study, TCBQ was tested for its genotoxicity using MDA-MB-231 cells as experimental model. The aim of the study is to elucidate the role of Rad51 in TCBQ-induced DSBs in MDA-MB-231 cells. First, cell viability was measured and were used for further investigation. The cytotoxic effect of TCBQ to MDA-MB-231 cells were represented in **Fig 1**. TCBQ gradually decreases the viability of MAD-MB-231 cells in a dose- and time-dependent fashion. Then we demonstrated TCBQ-induced Rad51 gene expression. MDA-MB-231 cells were treated with TCBQ (25 μM) for 0 (vehicle control), 3, 6, 12 and 24 h. Cells were treated with TCBQ for indicated times and then total protein in cell lysate were extracted for immunoblotting, **Fig 2-3**. Total cell lysate protein were detected over time course showed that Rad51 protein increased and reached a peak at 1 h, then fell dramatically with further exposure to 25 μM TCBQ. DNA damage induced cell cycle delay or block, the protein of cell cycle arrest can also be induced to participate in DNA damage repair and apoptosis process, such as p53 protein. We results indicated that TCBQ induced p53 and p-p53 expressions significantly by immunoblotting, **Fig 4**. Knockdown of p53 with siRNA effectively reversed the downregulation of Rad51 and decreased the TCBQ-induced DSBs. TCBQ activates p53 signal pathway and regulates the expression of Rad51 in MDA-MB-231 cells, **Fig 5**. Thus, the results suggest that TCBQ induced Rad51 downregulation at high doses and long time in MDA-MB-231 cells. And p53 signaling pathways are involved in the process. In conclusion, the interaction of p53 and RAD51 can maintain the stability of cell genome.

ABSTRACT FINAL ID: 3606 Poster Board: P298
TITLE: Polychlorinated Biphenyl Quinone Induces Autophagic Flux Through Different Autophagic Structures

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: Q. Shi, Y. Song. *Southwest University, Chongqing, China*.
KEYWORDS: Oxidative Injury; Polychlorinated Biphenyls; Signal Transduction; Autophagic Flux

ABSTRACT BODY: Autophagy is an active degradative process to remove or turnover bulk cytoplasmic constituents through the endosomal and lysosomal fusion system resulting in the formation of autophogosomes in eukaryotic cells, which plays a critical role in removing long-lived proteins and damaged organelles. Under stress situations, autophagy plays a key pro-survival role in cells by providing the nutrients to maintain the metabolism. However, extensive activation of autophagy can also lead to cell death3. Beclin-1, the autophagy-related protein, is essential for the initiation of autophagy. Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants, which have been banned for their production and most of their applications in industrialized countries from 1970s. However, due to their persistent physicochemical properties, PCBs become widely distributed geographically, and accumulate in plants and animals through food chain, which pose severe threat to human health nowadays. The diverse toxicities of PCBs have been extensively documented, including endocrine disruption, neurotoxicity and immunotoxicity. Our previous studies demonstrated that PCB quinones exposure induce reactive oxygen species (ROS)-driven DNA damage, oxidative damage, cytotoxicity, genotoxicity and apoptosis in HepG2 cells. However, the effect of PCB29-pQ on autophagy remains unknown. The aim of the present study was, therefore, to investigate the role of PCB29-pQ on autophagy regulation and the mechanisms underlying this process. In our research,PCB29-pQ (**Fig 1**) treatment significantly induced the formation of red fluorescent AVOs compared to control in HepG2 cells (**Fig 2**). In addition, intracellular MDC was quantified by a fluorometric analysis showed that the most influorescence intensity was 5 μM of PCB29-pQ compared with the control cells (**Fig 3**). PCB29-pQ influences the level of transcriptional of LC3 (**Fig 4**). Furthermore, the results suggesting that CQ challenge resulted in increased LC3B-II net flux expression in the cells treated with PCB29-pQ, interestingly, the most effective concentration for western blotting was 5 μM of PCB29-pQ (**Fig 5**). These findings indicated that PCB29-pQ induced AVs increasement was caused initially by an increase in AV biogenesis, but the molecular mechanism was need to further study.

ABSTRACT FINAL ID: 3607 Poster Board: P299
TITLE: Glutamate Cysteine Ligase Overexpression Attenuates the Toxicity of Chemical-induced Oxidative Stress, and Modulates the Adaptive Expression of Nrf2-target mRNAs

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KEYWORDS: Glutathione; Computational Toxicology; Green Chemistry

ABSTRACT BODY: An important principle of Green Chemistry is to mitigate toxicity based on predictions made from structure/activity models. We examined the toxicity of various oxidative stress-inducing chemicals (tert-butyl hydroquinone (tBHQ), hydroquinone (HQ), R-carvone, tert-butyl hydroperoxide (tBHP), cumene hydroperoxide (CHP), bis-phenol A (BPA), perfluorooctanoic acid (PFOA) and Dinoseb). Cells with higher levels of glutathione (GSH), should be protected against such chemicals and show less activation of the Keap1/Nrf2 antioxidant response pathway. Glutamate cysteine ligase (GCL) is rate-limiting for GSH synthesis. CR cells are genetically modified mouse Hepa-1 liver cells with higher GCL catalytic (GCLC) and modifier (GCLM) subunit expression, and GSH than plasmid-vector controls (HV cells). CR and HV cells were exposed to these chemicals and assessed for viability, GSH levels and mRNAs for Nrf2 target genes Gclc, Gclm, Nqo1 and Hmox1. CR cells had increased resistance toward most of these chemicals (with HQ > CHP = tBHQ = tBHP = BPA > PFOA). Adaptive increases in GSH and Gclc, Gclm, Nqo1 and Hmox1 mRNAs were greater in HV cells, suggesting that GCL overexpression attenuated Nrf2 activation. Chemical reactivity was assessed with orbital analysis and via transition state modeling using density functional theory calculations. This modeling supported the observed trends in CR cell resistance toward HQ, tBHQ, CHP and tBHP. These analyses highlight the promise of quantitative structure-activity modeling for predicting toxicity, and the need to further explore biotransformation and toxicokinetic parameters in order to refine such models. Supported by NSF CHE-1339637 and NIEHS P30ES070033.

ABSTRACT FINAL ID: 3608 Poster Board: P300
TITLE: Characterizing Redox Potentials in Adipose: Implications for Endocrine Disrupting Chemicals and Obesity

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KEYWORDS: Oxidative Injury; Endocrine Disruptors; Redox Biology

ABSTRACT BODY: Obesity has been linked to exposure to endocrine disrupting chemicals (EDCs), such as bisphenol A (BPA). Adipose responds to hormones and is an important component of the endocrine system, regulating energy storage and utilization. Recent studies suggest that redox imbalances in adipose may be contributing to obesity pathology; however, redox potentials have not been previously characterized in adipose. To 1) investigate methods for estimating redox potentials in adipose and 2) characterize redox potentials in different types of adipose, high performance liquid chromatography (HPLC) was performed on subcutaneous (SF), visceral (VF), and gonadal (GF) fat in adult male and female lean a/a and obese Avy/a mice of the agouti strain (93% C57BL/6) (N=20). A set of replicate tissues from the same animals were analyzed separately and used to confirm findings. One-way ANOVA demonstrated that redox potentials for oxidized and reduced glutathione (GSSG:GSH) in VF (-203.7 mV) were significantly more reducing than in SF (-177.1 mV, $p<0.01$) and GF (-167.6 mV, $p<0.01$). Similarly, cystine and cysteine (CYSS:CYS) redox potentials in VF (-153.6 mV) were significantly more reducing compared to SF (-113.8 mV, $p<0.01$) and GF (-113.7, $p<0.01$), although in a set of biological replicates, VF was only significantly different from GF ($p<0.01$). In females, adipose was more reducing (GSSG:GSH) than in males ($p<0.01$). When stratified by depot, females had significantly more reducing GSSG:GSH redox potentials in SF ($p=0.03$), but not in GF ($p=0.66$) or VF ($p=0.55$). GSSG:GSH redox potentials became more oxidizing with increasing body weight ($p=0.01$), but this relationship was not significant in a set of biological replicates. Redox potentials did not differ by genotype or age. These results indicate that HPLC is a reliable method for determining adipose redox potentials, and have implications for evaluating obesity outcomes in EDCs studies. Investigations are currently underway to examine whether exposure to BPA alters redox potentials in VF.

ABSTRACT FINAL ID: 3609 Poster Board: P301
TITLE: Histones are Targets for Modification by Glucose-Derived Methylglyoxal

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KEYWORDS: Kidney; Oxidative Injury; Epigenetics

ABSTRACT BODY: Diabetic nephropathy (DN) is the major cause of morbidity and mortality in diabetic patients. The sustained hyperglycemia associated with diabetes results in substantial oxidative stress and inflammation, leading to the generation of the reactive α-oxoaldehyde, methylglyoxal (MGO). MGO has been shown to play an integral role in DN pathogenesis and its effects are often seen in DN patients, despite glycemic control, via a phenomenon called metabolic memory. Metabolic memory is hypothesized to result from alterations in histone posttranslational modifications. Here, we describe histones as targets for modification by MGO in a cell culture model of hyperglycemia. Utilizing novel LC-MS/MS methodologies developed in our laboratory, histones were purified from cultured HEK293 cells treated with low (5mM) or high (25mM) glucose. Following exhaustive proteolytic digestion, absolute concentrations of histone posttranslational modifications were quantified by LC-MS/MS. These analyses revealed both Lys and Arg-derived MGO adducts, with the Arg hydroimidazalone (MG-H) adducts being the most prevalent. To validate that these adducts were indeed derived from cellular MGO, glyoxalase 1 was knocked down, resulting in substantial increases in both cellular MGO and MG-H adducts. Sites of MGO modification were identified via high-resolution mass spectrometry, which revealed Lys and Arg adducts on all four-core histones. We hypothesize that MGO adduction of histones offers a novel link between inflammation, hyperglycemia, and DN pathogenesis. The effects of these modifications on cellular homeostasis are the subject of current investigation.

ABSTRACT FINAL ID: 3610 Poster Board: P302
TITLE: *In Vitro* Oxidative Stress Responses of Cigarette Smoke Extracts in Human Lung Epithelial Cells

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KEYWORDS: Toxicity; Acute; Oxidative Injury; Inhalation Toxicology; Tar Delivery; Cigarette Smoke Extract

ABSTRACT BODY: Cigarette smoke contains more than 5,000 chemicals, as well as reactive oxygen species (ROS) such as free radicals. These may induce oxidative stress responses and induce damage to DNA, protein and lipids, which in turn can trigger and promote the development of many smoking related diseases. The objective of this study was to analyze the oxidative stress induced by cigarette smoke aqueous extracts (AqE) with different ISO tar yields using *in vitro* assays. Three commercial brands of cigarettes of different ISO tar yields (4 mg, 7 mg and 11 mg) and 3R4F reference cigarette (9.4 mg) were tested. AqE was produced by bubbling smoke (ISO smoking regime) through 20ml cell culture medium. Nicotine levels (UPLC-MS) and optical density (OD320nm, a proxy measurement of tar), were measured as a quality control for each AqE batch. Human lung epithelial cells (H292) were exposed to a range of AqE concentrations, along with appropriate controls. Four *in vitro* assays, including the DCF assay, glutathione (GSH:GSSG), ARE (antioxidant response element) reporter assay and cell apoptosis assay were used for the detection of cellular ROS, antioxidant imbalance, activation of Nrf2 antioxidant pathway and cell apoptosis, respectively. Nicotine levels in the AqE stock solutions ranged from 11.9 µg/mL (11 mg tar product) to 3.1 µg/mL (4 mg tar product). OD320nm readings were ranged from 0.76 from the highest tar delivery product and 0.35 in the lowest delivery product. AqE from all four cigarettes gave clear dose-response relationships in these *in vitro* assays. In general, the degree of response correlated with tar yields of the cigarettes. However, oxidative stress induced by AqE from the 9.4 mg tar yield cigarette (3R4F) was lower than that of the 7 mg tar yield cigarette. This may be in part due to different tobacco blends used in these cigarettes. This study shows that these assays are sufficiently sensitive to discriminate different cigarette types, and that the ranking of the *in vitro* responses corresponds largely with their tar deliveries. This panel of assays will be useful for assessment of tobacco products and alternative nicotine delivery devices.

ABSTRACT FINAL ID: 3611 Poster Board: P303
TITLE: A Live-Cell Kinetic Assay for Glutathione Oxidation in HaCat Keratinocytes Using the roGFP2 Biosensor

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KEYWORDS: Alternatives to Animal Testing; Glutathione; Cutaneous or Skin Toxicity

ABSTRACT BODY: Cellular oxidative stress—an increase in reactive oxygen species that can damage macromolecules—is a key event in multiple adverse human health outcomes. Glutathione mitigates oxidative stress by chemically reducing reactive oxygen species and oxidized macromolecules. Oxidation of the glutathione pool is thus an indicator of acute oxidative stress. To facilitate high throughput screening of compounds for their potential to induce oxidative stress, an assay was developed utilizing a human keratinocyte HaCat cell line stably expressing Grx1-roGFP2—a genetically-encoded, ratiometric, fluorescent biosensor for glutathione redox status. Fluorescence emission from the biosensor in living cells was monitored kinetically with a microplate reader. Assay optimization using the reactive oxygen species hydrogen peroxide as a reference compound yielded an assay dynamic range of 4.58 fold-change, intra-assay CV of 4.98%, and a Z' factor of 0.80. Concentration-dependent responses to hydrogen peroxide, the glutathione oxidizing agent diamide, and the redox cycling K vitamer menadione were observed. Furthermore, recovery to a reduced state was observed for all three compounds with time to complete recovery inversely related to dose. Cytotoxicity measurements were multiplexed with the redox readout by adding the cell-impermeant nucleic acid dye propidium iodide and reading its fluorescence at wavelengths orthogonal to the roGFP2 signal. At the highest concentrations tested (1.0 and 0.5 mM, respectively), hydrogen peroxide and diamide did not cause cytoxicity within three hours of addition, but moderate cytotoxicity was observed for menadione. The HaCat-roGFP2 assay is a scalable, live-cell kinetic assay for monitoring dynamic glutathione redox status for bioactivity profiling of direct oxidative stress induced by xenobiotic exposures.

ABSTRACT FINAL ID: 3612 Poster Board: P304
TITLE: Increased Susceptibility of Vitamin D Receptor Null Mice to Hyperoxia: Role of Nrf2

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KEYWORDS: Transcription Factors; Lung; Pulmonary or Respiratory System; Oxidative Injury

ABSTRACT BODY: Bronchopulmonary dysplasia (BPD) remains one of the most common complications of premature birth with multifactorial etiology. Oxygen exposure has been shown to be one of the major risk factors. Recent studies have shown that vitamin D has multiple anti-inflammatory and anti-proliferative effects, besides its role in calcium homeostasis, and exerts its properties through vitamin D receptor (VDR). Nuclear factor, erythroid 2 related factor 2 (Nrf2) is a transcription factor which activates cellular rescue pathways against oxidative stress, apoptosis, carcinogenesis. Vitamin D has been shown to activate the Nrf2-Keap antioxidant pathway. In this investigation, we tested the hypothesis that VDR-null mice will be more susceptible to hyperoxia, compared to wild type (WT) mice, via mechanisms entailing attenuated Nrf2 activation and augmentation of inflammatory pathways. Six to ten week old male WT or VDR-null mice were exposed to hyperoxia (95% oxygen) or room air for 72 h. Lung injury was evaluated by H&E staining and neutrophil infiltration. Nrf-2, NADPH quinone reductase (NQO1), and NF-kB protein and mRNA expression were determined by western blot and RT-PCR. IL-6 expression was evaluated using RT-PCR. VDR-null mice had worsening pulmonary edema and injury after hyperoxia exposure compared to WT animals. Hyperoxia exposure led to significant induction of Nrf2 and NQO1 mRNA and protein expression in lungs, and this induction was attenuated in VDR-null mice. Hyperoxia induced IL-6 mRNA expression in WT mice while having opposite effect in VDR-null mice. In addition, hyperoxia down regulated NF-kB mRNA expression in WT, but not in VDR mice. Interestingly, VDR-null mice showed higher levels of Nrf2 and NQO1 mRNA and protein expression when maintained in room air conditions. In conclusion, our results support the hypothesis that decreased Nrf2 activation and NQO1 induction by hyperoxia in the VDR-null mice may have contributed to increased inflammation and worsening lung injury in these animals after oxygen exposure. Further studies are needed to understand the molecular mechanisms involved in the hyperoxic lung injury in VDR null mice compared with wild type, which could lead to new therapies to prevent and/or treat BPD.

ABSTRACT FINAL ID: 3613 Poster Board: P305
TITLE: Analytical Method Validation and Development for Some Persistent Organic Pollutants in Water and Sediments by Gas Chromatography Mass Spectrometry

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KEYWORDS: Persistent Organic Chemicals; Polychlorinated Biphenyls; Methods/Mechanism; PBDE

ABSTRACT BODY: Many of the persistent organic pollutants (POPs) with endocrine disrupting properties are monitored due to their high priority related to their adverse health effects. Analyses of chemical pollutants in environmental samples are laborious, expensive, and not ecofriendly which is difficult to perform due to limited resources. Moreover, these analyses were frequently developed targeting one class of pollutants, consequently cannot meet the need of monitoring a rapidly increasing number of pollutant chemicals in the environment. The objective of this present study was to examine the feasibility of using a single sample pretreatment procedure and multiple GC-MS runs for the analyses of multiple groups of semi volatile organics; PCBs (28, 52, 101, 118, 138, 153, 180), PBDEs (17, 47, 66, 100, 153, 183) and OCPs(α-HCH, HCB, γ-HCH, Heptachlor, p,p-DDD, p,p-DDE, p,p-DDT) in sediment and water samples at the same time. Extraction for water involved SPE using C18 and for sediment using self-made column with florisil, PSA and magnesium with ultrasonication step by acetone. The optimized procedure was validated and applied tap, river and lake water, and river and lake sediments. For both matrices and all analytes, high linearity (0.995-0.999), recovery (88-106%) with all RSD values below 20 and LOQ levels below the tested limits were achieved. This reliable and effective procedure for monitoring selected multiple POP levels in water and sediment; does not require a complicated apparatus or intensive manual labor, and minimizes the consumption of organic solvent. This simple and cost-effective method, based on rapid and safe procedures, can be effectively used for routine analysis of selected PCBs, PBDEs and OCPs.

ABSTRACT FINAL ID: 3614 Poster Board: P306
TITLE: Strategies to Address Reach Annex X Requirements for Developmental Toxicity Testing in a Second Mammalian Species

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KEYWORDS: Reproductive and Developmental Toxicology; Safety Evaluation; Alternatives to Animal Testing; REACH

ABSTRACT BODY: Annex X of the European REACH regulation specifies the physicochemical, toxicological and ecotoxicological information required for substances manufactured or imported into the EU in quantities >1000 tonnes pa. Before new tests are performed, all available *in vitro* and *in vivo* data, historical human data, data from (Q)SARs and from structurally-related substances must first be assessed. To assess potential effects on reproduction, REACH requires information on fertility and developmental toxicity, as specified in Column 1 of Annex X, including: prenatal developmental toxicity (OECD 414) studies in two species and an OECD 443 (extended one generation reproductive toxicity). The core objectives of the REACH requirements are: (1) to generate adequate information to identify reproductive hazards and decide whether classification and labelling as a reproductive toxicant is warranted; and (2) to have sufficient information for human health risk assessment. This poster describes testing strategies to address both of the main objectives of the REACH requirements for developmental toxicity testing, while optimising the use of alternative testing approaches and reducing the number of animals required for higher tier studies. Two scenarios are considered: (1) no existing reproductive or developmental toxicity data available for a substance or (2) existing rodent reproductive or developmental data (OECD 422 and 414) available for a substance. A stepwise approach is followed in each scenario, in which the available data generated at each step are assessed for significant reprotoxic effects and hazard classification. Each scenario considers use of OECD 414 limit tests (1000 mg/kg) before moving to full OECD 414 studies. Where hazard classification is triggered no further testing is proposed. Rat or rabbit embryo culture studies are also included in each scenario. The results are evaluated in conjunction with QSAR data for developmental toxicity, foetal development data from the OECD 443 study, and the results of genotoxicity studies, to determine if additional developmental toxicity data in a 2nd species are needed or whether sufficient information is available to support the development of a waiver request for the developmental toxicity study in a second species.

ABSTRACT FINAL ID: 3615 Poster Board: P307
TITLE: Derivation of a No Significant Risk Level for Aloe Vera Extract

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KEYWORDS: Regulatory/Policy; Natural Products; Carcinogenesis; Proposition 65; Aloe Vera

ABSTRACT BODY: Aloe vera is widely-used in products such as shaving cream, aftershave lotion, depilatories, deodorant, wound healing cream, and burn/sunburn care products. Aloe vera (non-decolorized whole leaf extract) was recently added to the State of California's list of chemicals known to the State to cause cancer or reproductive toxicity (Proposition 65), due to its identification by IARC as a Group 2B possible human carcinogen. In a National Toxicology Program study, non-decolorized Aloe leaf extract produced a significant, dose-dependent increase in the incidence of large intestine adenomas and carcinomas when administered to rats but not mice in drinking water for 2 years. The NTP rat tumor data served as the basis for non-decolorized Aloe leaf extract's classification by IARC and its listing on Proposition 65. Non-decolorized Aloe leaves contain anthraquinones and their C- and O-glycosides. Its mode of carcinogenic action involves bioactivation of anthranoid glycosides by intestinal microorganisms. Liberated anthraquinone aglycones are oxidized to form genotoxic species such as aloe-emodin. Oxidized aglycones are absorbed in the small intestine and glucuronidated in the liver, followed by biliary excretion and microflora-mediated liberation of free anthraquinone in the large intestine. The tumorigenic effect in rats but not mice has been attributed to species-specific differences in the intestinal microflora spectrum and intestinal transit time. Products containing Proposition 65 carcinogens are subject to warning requirements unless exposure is below California's "safe harbor" No Significant Risk Level. As no NSRL for non-decolorized Aloe leaf extract has been established, the goal of this work was to derive a NSRL. Following California's procedure for NSRL development, we conducted benchmark dose modeling on combined benign and malignant tumor incidence from NTP's 2-year rat study. BMD modeling identified the oral dose of non-decolorized Aloe leaf extract associated with a 10% increase in tumor incidence and yielded animal cancer slope factors to which conventional interspecies scaling factors were applied. We used human equivalent CSFs to calculate the dose associated with a 1 in 100,000 increased cancer risk. The resulting NSRL was 500 microg/day. This value may serve as the basis through which manufacturers can assess consumer exposures to non-decolorized Aloe leaf extract and, ultimately, demonstrate compliance with Proposition 65 requirements.

ABSTRACT FINAL ID: 3616 Poster Board: P308
TITLE: A Predictive Simulation of Subacute PCB-Induced Oxidative Stress in the Human Liver Using a Combination of Techniques

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KEYWORDS: Physiologically-Based Pharmacokinetics; Predictive Toxicology; Biological Modeling; PCB

ABSTRACT BODY: Toxicology testing in the twenty-first century (TT21C) heavily encourages risk assessment methods that deviate from the classically extensive *in vivo* models. Alternative testing methods, if effective, would allow toxicologists to greatly reduce and ideally replace full animal use in toxicity assays and would immensely accelerate the risk assessment process. The strategy proposed in this study is based on coupling different modeling techniques to determine the magnitude of oxidative stress in human livers subacutely exposed to a combination of environmental contaminants. Both coplanar and non-coplanar polychlorinated biphenyls (PCBs) are known for inducing such a response and were focused on in this particular modeling application. This strategy required an initial physiologically based toxicokinetic (PBTK) model to estimate the dosimetry of the PCB mixture within the human body; thus the amount distributed to the liver over time. This study coupled several modeling techniques to assess the relevant PBTK parameters and subsequently simulate dynamic responses, with literature on relevant hepatotoxicity studies and current *in silico* methods. The predictive simulation showed that a PCB-treated liver emulated purely *in vivo* experimental changes in biomarkers associated with oxidative damage in a subacute dose-dependent manner, specifically malondialdehyde (MDA) and glutathione (GSH) alterations. In further studies, computational models of this nature can be coupled with *in vitro* assays to determine more sophisticated response mechanisms and toxicological endpoints, ultimately decreasing or eliminating the demand for extensive *in vivo* experiments.

ABSTRACT FINAL ID: 3617 Poster Board: P309
TITLE: Comparison of Hexabromocyclododecane (hbcd) Biomonitoring Data and High Throughput Screening Dose-Responses Using Pharmacokinetic Modeling

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KEYWORDS: Computational Toxicology; Risk Assessment; Biological Modeling; HBCD

ABSTRACT BODY: The past few years, advances in toxicity testing methods such as systems biology and high-throughput screening have brought new ways to assess the potential harm from chemicals. Margin of exposures between endpoints and exposure levels can be used to bridge the data generated from these new toxicity methods and effectively screen biological activities into a common measure. The purpose of this work evaluates the margin of exposure between extrapolated *in vitro*/*in vivo* endpoints with Canadian biomonitoring data for flame retardants. A physiologically-based pharmacokinetic (PBPK) model for HBCD was developed to estimate exposure from biomonitoring levels in the population. The PBPK model suggests that HBCD is highly bound to lipoproteins which govern the distribution of the chemical into adipose tissue stores. By reverse dosimetry, the model was then applied to estimate daily population intake of HBCD based on blood concentrations measured in the Canadian population. The toxicokinetic descriptions were also used to compare Biomonitoring Equivalents (BE) of *in vivo* toxicity endpoints and extrapolated human equivalent dose metrics from *in vitro* endpoints from the ToxCast^{TM} database. From the reverse dosimetry, daily estimated intakes corresponding 25, 50, 95 and 99th percentiles of blood concentrations were 5.40, 6.72, 11.55 and 13.9 ng/kg respectively. These exposure values are far below the human extrapolated equivalent daily intake calculated from *in vivo* BE values and *in vitro* ToxCast^{TM} assays measures which were between 952 and 121000 ng/kg. Margin of exposure between the *in vitro* and *in vivo* endpoints ranged between 80 and 2340 fold. The magnitude of the ratio provides an indication of the level of concern for the chemical that is important for the risk assessment prioritization of chemicals. Case studies such as this work will be used to assist in developing predictive tools and informing the utility of non-traditional toxicity data for the next generation of risk assessment.

ABSTRACT FINAL ID: 3618 Poster Board: P310
TITLE: Magnetically 3D Bioprinted Hepatocyte Spheroids for *In Vitro* Metabolic Studies

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KEYWORDS: Cytochrome P450; Alternatives To Animal Testing; Cell Culture; Spheroids

ABSTRACT BODY: Three-dimensional (3D) liver models can be of great utility for hepatoxicity screening in preclinical drug development, as they represent native tissue environments, but existing platforms suffer from various technical limitations and a lack of scalability for high-throughput screening. Here, we demonstrate magnetic 3D bioprinting (M3B) as an alternative platform for creating hepatocyte spheroids. In M3B, cells are magnetized by binding a biocompatible nanoparticle assembly (NanoShuttleTM) to their membrane, then aggregated into spheroids using a mild magnetic field. The magnetization of cells and spheroids overcomes technical limitations of other platforms. Magnetization can be done within hours of thaw or harvest with lower cell numbers needed and without adherence, thus making use of nonadherent hepatocyte populations often lost due to low plating efficiencies. The magnetized spheroids can also be held with magnetic forces, improving sample retention during media exchange. We assembled primary human hepatocyte spheroids with M3B in cell-repellent 384-well plates and compared them to monolayer cultures from the same donor. After 48 hrs, cultures were exposed to the test compounds: rifampicin, verapamil, omeprazole, ticlopidine, and α-napthoflavone. After 5 days of culture, we used luminescent assays to assess overall viability and activity of major CYP450 enzymes (3A4, 2B6, 1A2). An undosed population of spheroids was then stained for presence of cytoskeletal and liver-specific markers, albumin, MRP-2, HNF4α, and aPKC. M3B spheroids showed improved CYP3A4, CYP1A2, and CYP2B6 basal activity and fold-induction over concurrent monolayer culture. Immunofluorescence staining confirms presence of liver-specific markers. The localization of MRP-2, F-actin, and aPKC suggest the presence of functional bile canaliculi, even after a short period in culture. Overall, magnetic 3D bioprinting can form highly relevant hepatic microtissues for studies in drug metabolism and interaction.

ABSTRACT FINAL ID: 3619 Poster Board: P311
TITLE: Quantitative Bias Analysis for Epidemiological Associations of Perfluroalkyl Substance Serum Concentrations and Early Onset of Menopause

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KEYWORDS: Physiologically-Based Pharmacokinetics; Biological Modeling; Epidemiology

ABSTRACT BODY: A cross-sectional epidemiologic study reported an association between increased serum concentrations of perfluoroalkyl substances (PFAS) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and early menopause (Taylor et al 2014). This association may be explained by the fact that women who underwent menopause no longer excrete PFAS through menstruation. The objective of this study was to use physiologically-based pharmacokinetic (PBPK) modeling to assess how much of the epidemiologic association between PFAS and altered timing of menopause might be explained by reverse causality. We developed a population life-stage PBPK model of PFOS and PFOA characterized by realistic distributions of physiological parameters including age at menopause. We then conducted a Monte Carlo simulation of a cross-sectional National Health and Nutrition Examination Survey (NHANES) population to match the epidemiological population reported by Taylor et al. The population characteristics, including age, body mass index, age at menopause and serum PFAS concentrations were similar between the simulated and reported data. We compared the simulated hazard ratio estimates to the reported associations. In the simulated data, the hazard ratios for PFOS serum concentration tertile 2 and 3 were 1.36 (95% Confidence Interval, 1.31-1.41) and 1.91 (1.85-1.98) while the reported associations were 1.23 (1.04-1.44) and 1.16 (0.91-1.48). Simulated hazard ratios for PFOA serum concentration in tertile 2 and 3 were 1.24 (1.20-1.29) and 1.31 (1.27-1.36) while the reported associations were 1.22 (0.92-1.62) and 1.36 (1.05-1.75). Under our current model structure, pharmacokinetics can fully explain the reported associations between PFAS serum concentrations and early onset of menopause. In the future, we will simulate another epidemiologic study of this association (Knox et al 2011).

ABSTRACT FINAL ID: 3620 Poster Board: P312
TITLE: Characterization of the Interplay Between Intra-Thyroidal Transporter and Metabolic Enzyme on Thiocyanate (SCN^-) Kinetic: Development and Use of a SCN^- PBPK Model

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KEYWORDS: Physiologically-Based Pharmacokinetics; Biological Modeling; Endocrine; Thyroid; Thiocyanate

ABSTRACT BODY: Exposure to a mixture of contaminants, such as SCN^- and perchlorate (ClO_4-), can disturb thyroid homeostasis. The mode of action of SCN^- is poorly quantified, contrary to that of ClO_4-, probably due to its greater complexity. SCN^- has been shown to inhibit both the thyroidal uptake of iodide (I-), via the sodium-iodide symporter (NIS), and thyroid hormone (TH) synthesis, via the thyroid peroxidase (TPO) within the thyroid. TPO also metabolizes SCN^- to its major metabolite, sulfate. A quantification of the net effect of the NIS- and TPO- mediated interactions is needed to evaluate the dose-response of SCN^- on circulating TH levels. The characterization of the intra-thyroidal exposure to SCN- is therefore crucial. In an extension of our exploratory efforts, we have completed the development of a PBPK model for SCN^- by including a description of its kinetics by NIS and TPO and evaluating the uncertainties of the parameter estimates. The PBPK model of SCN^- has 3 compartments: serum, thyroid (modelled as diffusion limited) and rest of body, and was linked to a sub-model for sulfate. The model was calibrated using 4 different published kinetic studies in rats. Six kinetic parameters were estimated simultaneously in a Bayesian framework, and the uncertainties of the estimates and model predictions were quantified. The predicted serum and thyroid concentrations of SCN^- were in good agreement with the observations. The mean of the *in vivo* metabolic clearance by TPO was estimated for the first time at $7.5 \pm 1.8x10\text{-}^5 L/h/kg^{0.75}$. The model also captured the dose-dependent nonlinearities of SCN^- kinetics after an acute exposure in rats. Model behavior was evaluated by a Morris screening test; intra-thyroidal tissue concentrations in SCN^- were more sensitive to thyroid partition coefficient and NIS maximum rate of uptake. Extrapolation of the model to humans underlined inter-species discrepancies, specifically that of renal elimination kinetic of SCN^-. Overall, our work allowed for quantifying the intra-thyroid concentrations of SCN^- necessary for characterizing its disproportional dose-response on TH synthesis.

ABSTRACT FINAL ID: 3621 Poster Board: P313
TITLE: Extension and Use of the Biologically Based Dose-Response (BBDR) Pregnancy Model for the Thyroid Axis to Estimate Iodine Nutrition and Thyroid Function Status of Pregnant Women in the United States

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KEYWORDS: Risk Assessment; Biological Modeling; Biomonitoring; BBDR, Thyroid, Pregnancy; Iodine

ABSTRACT BODY: A deterministic BBDR model for the thyroid axis was developed as a quantitative risk assessment tool to evaluate the effects of varying iodine intake and perchlorate exposure on thyroid hormone (TH) levels in near-term pregnant woman and fetus. A previously developed model was calibrated for normal thyroid and marginal iodine deficiency conditions, with a lower bound daily dietary iodine intake rate of 75μg/day, to evaluate moderate thyroid axis disturbances. In the current study, we extended the functional range of the model to address severe iodine deficiency conditions, including accommodating the effects of homeostatic mechanisms. Available information on interrelationships between serum TH levels and varying iodine status were acquired from the literature. Several model calibration strategies were tested and the appropriate ones were selected to capture empirically the net effects of adaptive and compensatory mechanisms for iodine intake rates below 75 μg/day. Confidence intervals for the fitted statistical parameters of the nonlinear regression model indicated that the predicted trends in TH levels of the newly extended model were comparable to observed trends from published data. A probabilistic evaluation of the extended full-range model was used to interpret the available biomonitoring data on urinary iodine levels in late gestation pregnant women in the United States. The model estimated an average iodine intake rate of 225 μg/day. Median values of the corresponding trimester-specific distributions predicted for the maternal total and free TH levels were 144.9 nmol/L and 8.3 pmol/L, respectively. The ratio of the 97.5th to 2.5th percentile of the model-predicted distribution in the maternal free thyroxine levels was 2.2. Overall, the current efforts provide an improved tool for evaluating impacts on maternal serum TH levels for a wider range of severity in thyroid axis perturbations and can be adapted to assess relevant risk of exposure to thyroid active chemicals, such as perchlorate.

ABSTRACT FINAL ID: 3622 Poster Board: P314
TITLE: Aggregate Exposure Models to Estimate Safety of Fragrance Ingredients in Cosmetic Products

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: V. Rocha, B. Buza, C. Zacarias, H. Arcuri. *Safety, Natura Innovation, Cajamar, Brazil.*
KEYWORDS: Safety Evaluation; Chemical Allergy; Exposure Assessment; Cosmetic Toxicology

ABSTRACT BODY: Risk assessment of each ingredient is an important step during cosmetics products development to ensure the safety use for consumers and meets all regulatory requirements. Some cosmetics could have more than 40 different ingredients besides the fragrances, which are mixtures of dozens of ingredients, some with allergenic potential. Use of several personal care products are part of the daily routine. Actual models to evaluate product safety consider a scenario that evaluates the hazard of all ingredients presents on certain formula, the target public and the mode of use, however do not consider that some ingredients could be also present in multiple products types presenting an aggregate exposure. This requires a more real life estimation of consumer exposure, including for example the understanding of the real frequency, amount of use, areas of exposure. To develop models and predictions algorithms that take into account the aggregate exposure of 26 labeling fragrance ingredients presents on different cosmetic products used by Brazilian women, to perform a more realistic risk assessment approach. 602 women aged 25-55 years residing in São Paulo, Recife and Porto Alegre answered a questioner made by Provoker Institute regarding their habits of using face cosmetic products. The aggregate exposure model was building based on following formula: Agg Exp = (Frequency of product use daily x Amount per use x Concentration of fragrance ingredient per product x Retention factor x Penetration factor)/Surface area or Body weight. Considering the habits and the exposure scenario for face cosmetic products on Brazil women, together with the safety levels for a fragrance allergenic ingredient established by the Research Institute for Fragrance Materials on Quantitative Risk Assessment Model (QRA) our preliminary data have shown that this approach can guide a better safety design of products. This model allowed the calculation and estimation of the exposure for the same allergenic ingredient in a more realistic scenario of consumer habits considering the presence of this material on an entire line of products, and so the safety assessment of the combination, not a single product as used to be done. Aggregate exposure models have the advantage to combine different variables to simulate a more realistic exposure scenario adding more safety for consumers and less concern for regulators.

ABSTRACT FINAL ID: 3623 Poster Board: P315
TITLE: Contributions of Hydronic Heaters to Neighborhood 3-Hour Average PM2.5 Levels

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KEYWORDS: Cardiopulmonary; Particulates; Risk Assessment; Fine Particles; PM2.5

ABSTRACT BODY: Considerable epidemiologic evidence supports the hypothesis that adverse cardiac effects are associated with elevated fine particulate (PM2.5) exposures of subdaily duration. In response, the US EPA developed a new (Reff) NowCast method that considers approximate 3- to 12-hr time-weighted average PM2.5 concentrations (TWAs), depending upon air quality variability. Thus 3-hr TWAs are of specific interest from a public health perspective. In an earlier study of outdoor air near six hydronic heaters (HHs), we reported that substantial 5- and 10-minute PM2.5 TWA excursions were experienced by HH neighbors, and that the odds of an excursion were increased when an HH neighbor was downwind (at 3 sites), and during calm conditions (at 4 sites). In this follow-up analysis, autoregressive models were specified to evaluate contributions to neighborhood-scale PM2.5 air pollution from 5 of 6 HHs previously investigated, considering sequential (not rolling) 3-hr TWAs, and after controlling for 3-hr TWAs at paired reference ("background") monitors. The average percent increases in 3-hr TWA at nearfield monitors were 8 to 17% for each hour spent downwind, and 5 to 13% for each hour under calm conditions. If application of the US EPA Air Quality Index (AQI) were extended to HH settings, where PM2.5 TWA can increase rapidly, then HH PM2.5 contributions would be expected to sometimes change the NowCast Air Quality Index category from "good" to "moderate."

ABSTRACT FINAL ID: 3624 Poster Board: P316
TITLE: Formaldehyde and Acetaldehyde Generated by Electronic Cigarettes: Meta-Analysis of Aerosol Concentrations and Comparison to Tobacco Cigarette Smoke

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KEYWORDS: Volatile Organic Compounds; Exposure Assessment; E-Cigarette; Formaldehyde and Acetaldehyde

ABSTRACT BODY: As the popularity of electronic nicotine delivery systems (ENDS) has increased, so has the concern regarding exposure and potential health effects associated with aerosol constituents of ENDS. Formaldehyde (FA) and acetaldehyde (AA) can be generated by the oxidation of propylene glycol or vegetable glycerin, which are the primary constituents of ENDS liquids. The aim of this meta-analysis was to evaluate existing analytical measurements of FA and AA in aerosols produced by various ENDS devices and the potential influence of device and user features on FA and AA aerosol concentrations. In addition, FA and AA concentrations from ENDS aerosols were compared to that of tobacco cigarette smoke. Daily FA and AA exposures were calculated from aerosol analytical data reported in 14 different studies and from topography data (169 puffs, 98.5 mL/puff) collected from 24 different studies. The average weighted aerosol concentration of FA was 22.71 µg/L (median 1.05 µg/L, range ND to 760 µg/L, N=97) and AA was 11.66 µg/L (median 0.58 µg/L, range ND to 511.44 µg/L, N=95). A significant number of samples were below the analytical limit of detection: 22.7% for FA and 24.2% for AA. Additionally, the majority of the estimated daily concentrations of FA and AA from the pooled data were below estimated daily levels from cigarettes at 89% and 98%, respectively. Only 13 of 192 total samples were measured above the average concentration of FA or AA in cigarettes (22-74 µg/cigarette and 372- 1240.3 µg/cigarette, respectively). Further analysis of these 13 samples suggested unrealistic device use and sampling conditions, such as long puff durations (e.g., 8 second puffs), as well as high voltage and coil temperatures that can lead to an undesirable 'dry puff' scenario. While the majority of studies did not indicate device power settings, such information along with other device features (e.g., temperature, voltage, topography parameters) will be critical to report as ENDS chemical emissions patterns are compared across future exposure studies, product types and reasonable use scenarios for human risk assessment purposes. A set of standard experimental design reporting parameters are recommended for future studies.

ABSTRACT FINAL ID: 3625 Poster Board: P317
TITLE: Exposure Monitoring of Graphene Manufacturing Workplaces

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KEYWORDS: Nanotechnology; Exposure, Environmental; Exposure Assessment; Workplace; Graphene

ABSTRACT BODY: This study monitored the possible exposure to graphene release at two graphene research facilities; one facility manufactures graphene using graphite exfoliation and CVD (chemical vapor deposition), while the other facility manufactures graphene using CVD to make a layer of graphene on a copper plate, which is then transferred to a PET sheet. To estimate the potential exposure of researchers and workers to graphenes and evaluate the status of the protective control measures, personal and area monitoring were conducted at both facilities. To estimate the nanomaterial release process, a variety of direct reading instruments were used, including a dust monitor, condensation particle counter, nanoparticle surface area monitor, scanning mobility particle sizer, and aethalometer. The graphenes and other structures released from the work process were identified using a transmission electron microscope (TEM). Furthermore, the elemental carbon (EC) concentration was determined from the respirable dust sampling. The gravimetric concentrations of the total suspended particulate at workplace A and B were very low, and the EC concentrations were mostly below the detection limit, indicating very low exposure to graphene or any other particles. The real-time monitoring, especially the aethalometer, showed a good response to the released black carbon, providing a signature of the graphene released during the opening of the CVD at workplace A. The TEM observation of the samples obtained from workplace A and B showed graphene-like structures and aggregated/agglomerated carbon structures. Therefore, when taken together, the present results indicate very minimal graphene or particle exposure at facilities manufacturing graphenes with good manufacturing practices.

ABSTRACT FINAL ID: 3626 Poster Board: P318
TITLE: Determination of E-Caprolactam Target Concentrations in Nasal Fluid After Controlled Exposures of Human Volunteers

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KEYWORDS: Inhalation Toxicology; Cutaneous Or Skin Toxicity; Volatile Organic Compounds; Caprolactam

ABSTRACT BODY: ε-caprolactam (ECL) is an important industrial chemical that is used worldwide primarily in the production of plastic fibres such as nylon 6. It has relatively low human toxicity; of importance is local irritation that occurs after exposure to higher concentrations of ε-caprolactam as aerosols or vapors. An exposure study was performed to examine, if ECL in low concentrations cause local irritation in the upper respiratory tracts. 52 healthy adults (26 men, 26 women) were exposed in a 30 m^3 chamber for six hours daily on four consecutive days to 0, 0.05, 0.5 and 5 mg/m^3 ECL in a cross-over design. Nasal lavage was sampled from each volunteer before and after daily exposure. ECL concentrations in the nasal fluids (NALF) were determined by a HPLC-UV procedure (LOQ 20 µg/L). In pre-exposure samples and in samples after zero exposure ECL could not be detected definitely. Positive peaks in the analysis of pre-exposure samples are explained by the relatively low speficicity of the applied analytical technique. The mean ECL concentrations were 41 (± 15) µg/L after 0.05 $mg/^3$, 150 (± 102) µg/L after 0.5 mg/m^3 and 492 (±358) µg/L resp. after 5.0 mg/m^3. The NALF levels of ECL after exposure to 0.5 and 5 mg/m^3 differ statistically significant from the levels before exposure as well as after zero or 0.05 mg/m^3 exposure. It is concluded that individual exposures to ECL vapors in concentrations above 0.05 mg/m^3 over 6 hours can be objectified by analysis of NALF samples. Acknowledgement: For financial support, we would like to thank the *European Caprolactam and Fiber Producer*, the *US Carpet and Rug Institute* and the *Verein zur Förderung der Arbeitsmedizin an der Universität Heidelberg.*

ABSTRACT FINAL ID: 3627 Poster Board: P319
TITLE: Synthesis and Identification of Hydroxylated Metabolites of Vinclozolin

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KEYWORDS: Endocrine Disruptors; Biomonitoring; Biomarkers; Vinclozolin; Fungicide

ABSTRACT BODY: Vinclozolin (V) is a fungicide used in agricultural settings. V is biotransformed to at least 9 metabolites, denominated from M1 to M9. V and its metabolites M1 and M2 are classified as endocrine disruptors by competitively inhibiting the androgen receptor. Anti-androgenic effects cannot be completely explained by organ and serum levels of V and M1 and M2 metabolites. *In vivo* and *in vitro* studies indicate that M5 metabolite is the most abundant metabolite found in serum, organs, and urine and may be used as an exposure biomarker. There is no available information about toxicological and anti-androgenic properties of M4-M9 metabolites. The aim of this study was to synthesize and to identify to V metabolites. Metabolites were synthesized from V by the Sharpless reaction with some modifications. V was incubated in water: tert-butanol (1:1) containing OsO4, hydroquinidine-1,4-ftalazinedil diether, potassium ferrocyanide, methyl sulfonamide and potassium carbonate for 24 h at room temperature. The reaction products were analyzed and identified by HPLC/DAD/MSD through tret, UV spectrum and mass spectrometry using purified standards obtained from serum of rats administered repeated doses oral of V and extracts of enzyme assays using V or M1 as substrates and rat liver microsomes. Five metabolites were identified as products from V by Sharpless reaction. These corresponded to M1, M2, M5, M6, and M7. M1 and M5 represented the main products (37.4 and 33.5%, respectively), followed by M7 (12.1%), and M2 and M6 (4.3%). These results indicate that Sharpless reaction represent an excellent method to produce enough amounts of M5, M6 and M7 metabolites to carry out toxicological studies. In addition, they could help to explain the anti-androgenic effects on male reproductive system associated to this fungicide.

ABSTRACT FINAL ID: 3628 Poster Board: P320
TITLE: Prenatal Heavy Metal Exposures and Neurodevelopmental Outcomes in a Thai Agricultural Birth Cohort

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KEYWORDS: Developmental Toxicity; Prenatal; Exposure Assessment; Metals; Heavy Metals

ABSTRACT BODY: Current research indicates low-level prenatal exposure to heavy metals can have increasing neurotoxic effects, especially when exposure occurs during critical windows of development. The present study aimed to assess the relationship between *in utero* heavy metal exposure from maternal pesticide application and neurologic integrity at birth. Female farmworkers (N=59) were recruited in their first or early second trimester of pregnancy between March 2011 and February 2012 at the local antenatal clinic in Northern Thailand. These farmworkers are participating in a pilot birth cohort called the **S**tudy of **A**sian **W**omen **A**nd their off**S**pring's **D**evelopment and **E**nvironmental **E**xposures (SAWASDEE). Participants were administered a questionnaire at the time of enrollment, at 28 weeks, and 36 weeks of gestation. Biological samples were collected at each prenatal visit and at parturition. A urine sample was collected at each visit and blood samples were collected at the same time points that the questionnaire was administered. Maternal, infant, and cord blood and urine were collected at birth. Neurobehavioral function was measured using the **B**razelton **N**eonatal **B**ehavioral **A**ssessment **S**cale (BNBAS), which utilizes seven clusters (Habituation, Orientation, Motor, Range of State, Regulation of State, Autonomic Stability, and Abnormal Reflex). Trimester-resolved concentrations of chromium, arsenic, cadmium, mercury, and lead were measured in blood to assess exposure to the fetuses of participants. Significant associations were seen between arsenic levels and diminished Orientation and clusters. For the Abnormal Reflexes cluster of BNBAS, there is a significant association between increased heavy metal levels and increased abnormal reflexes for each metal analyzed. The greatest significances were seen in arsenic in enrollment samples and arsenic in cord blood samples. These results are suggestive of a negative association between prenatal heavy metal exposure and neurobehavioral functioning at birth. This study is one of the first to examine the impact of trimester-specific exposure to heavy metals on neurodevelopment using several measures of exposure biomarkers in a highly exposed agricultural population.

ABSTRACT FINAL ID: 3629 Poster Board: P321
TITLE: Estimation of Exposure Amounts of 2,2',4,4'-Tetrabromodiphenyl Ether (BDE-47) in the South Korean General Population With a Physiologically Based Pharmacokinetic Model and Conventional Exposure Model

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KEYWORDS: Exposure Assessment; Physiologically-Based Pharmacokinetics; Persistent Organic Chemicals; BDE47

ABSTRACT BODY: Several studies reported major congeners of PBDEs were found in general population and there have been trails to estimate the exposure amounts; however, we still have limited information on exposure amounts and internal dose in humans.The objectives of this study were estimate intake level of BDE-47 for the general population using PBPK model and exposure model using conventional risk assessment, respectively. We adopted a PBPK model of BDE-47 developed with rats to apply it to humans with appropriate parameters to general population. Then, empirical relationship between exposure and BDE-47 in blood was constructed. For conventional risk assessment, the distribution of body weight, inhalation rate and so forth were carefully applied to algorithm for calculating exposure amounts. According to the ECR from PBPK model, the 50th percentile of average daily intake amounts were 0.5 ~ 1.2 for female (n = 200) and 0.5 ~ 1.9 ng/kg/day for male (n = 194), respectively. Estimated intake amounts from the exposure algorithm were 0.02 ~ 0.08 for female and 0.02 ~ 0.12 ng/kg/day for male, respectively. The hazard quotient (HQ) for BDE-47 (100 ng/kg/day) were below unity in all methodologies. Although the exposure levels of BDE-47 seemed lower than RfD, estimates of the internal dose were different by models. Those from PBPK were several times higher than from exposure model, which indicate exposure model might need another source of exposure. Further researches are required for reduce the knowledge gaps.

ABSTRACT FINAL ID: 3630 Poster Board: P322
TITLE: Comparison of Urinary PAHs Among Firefighters and Asphalt Pavers

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KEYWORDS: Polycyclic Aromatic Hydrocarbons; Exposure Assessment; Risk Assessment

ABSTRACT BODY: Firefighters and asphalt pavers are exposed to polycyclic aromatic hydrocarbons (PAHs) during various work activities. The purpose of this study was to evaluate urinary PAH levels and compare these bio-monitoring levels among firefighters, asphalt pavers, and non-occupationally exposed individuals. The National Institute of Standards and Technology (NIST) urinary PAH levels were used for non-occupationally exposed controls. When compared to the NIST standard for smokers and non-smokers, firefighters demonstrated statistically significant differences in urinary concentrations for the following metabolites: 2-OH-fluorene, 3-OH-fluorene and 1-OH-pyrene, which were lower in firefighters than the NIST mean for smokers. 1-OH-phenanthrene, 2-OH-phenanthrene and 3-OH-phenanthrene were higher among world trade center exposed firefighters than the NIST mean for smokers. When firefighters were compared to the NIST non-smoker standard, firefighters demonstrated elevated levels in all tested PAH biomarkers due to a mixture of smokers and non-smokers in the firefighter cohort. Asphalt workers had statistically significant higher urinary concentration elevations in 2-OH-fluorene, 1-OH-phenanthrene and 3-OH-phenanthrene as compared to the NIST smoker mean. When asphalt pavers were compared to the NIST non-smoker mean, asphalt pavers had statistically significant increases in all tested PAH biomarkers, with the exception of 2-OH-phenanthrene. While firefighters did not demonstrate a substantial change in urinary PAH metabolite levels compared to control populations of smokers and non-smokers, asphalt pavers experienced concentrations that were in some cases increased by orders of magnitude compared to NIST controls. Future research may be needed to evaluate any potential health risk posted to occupational exposed asphalt pavers.

ABSTRACT FINAL ID: 3631 Poster Board: P323
TITLE: Personal Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) During Cookstove Use in Rwandan Households

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KEYWORDS: Exposure Assessment; Polycyclic Aromatic Hydrocarbons; Epidemiology

ABSTRACT BODY: The World Health Organization has estimated that exposure to toxins and smoke emitted from cookstoves leads to 4.3 million premature deaths annually. Nearly three billion people, mostly in developing countries, rely on solid-fuel-burning cookstoves to prepare food and heat their homes. The relationship between personal exposure to indoor air pollutants such as carbon monoxide and fine particulate matter and adverse health effects is well established. Little attention has been given to polycyclic aromatic hydrocarbons (PAHs); one of the primary sources of PAH exposure is indoor cookstove use. The goal of our study is to quantify and characterize the exposure to PAHs produced by cookstove smoke in peri-urban Rwandan households. Comprehensive exposure assessments were carried out in 180 households during July-August 2015. In each household, the primary cook's exposure to PAHs was measured using a PUF/XAD2 sampler (SKC, Eighty Four, PA) over a 24-h period. We also recorded kitchen ventilation and size as well as fuel type, quantity, and moisture content. Of the households surveyed, 74% cooked primarily inside during the seven days prior to sample collection. The majority used charcoal stoves as their primary cooking method; 70% used portable charcoal stoves, 18% used fixed charcoal stoves, and the remaining 12% used a clay stove, three-stone fire, or other stove. Charcoal was used as the primary fuel source in 94% of the households. Preliminary GC-MS analysis has shown that the most prominent PAHs emitted from the charcoal-burning cookstoves were chrysene and indeno(1,2,3-cd)pyrene (IP). Other PAHs detected included naphthalene, benzo(ghi)perylene (BghiP), fluorene, and pyrene. According to relevant literature, the experimental ratio of 0.66 for IP:IP^{+}BghiP concentrations indicates that the observed PAHs originate from the burning of biomass, rather than from liquid fossil fuels. The results of this research will aid in both understanding the personal exposure from cookstove emissions and the development of new, safer methods for food preparation and home heating. This has the potential to improve the health outcomes of millions of people globally.

ABSTRACT FINAL ID: 3632 Poster Board: P324
TITLE: Clues to Adaptation of Humans to Air Pollution—A Review of Findings From Czech Biomonitoring Studies

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KEYWORDS: Polycyclic Aromatic Hydrocarbons; Biomarkers; Epigenetics

ABSTRACT BODY: Air pollution causes diseases, allergies or premature deaths. During the last decade Czech biomonitoring research focused on the investigation of genetic damage in populations living in locations with various levels and sources of air pollution: In Prague (P) (moderate levels of pollutants due to traffic), Southern Bohemia (SB) (moderate levels of pollutants due to local heating), and Moravian-Silesian Region (MS) (a highly polluted region due to steel industry). E.g., in 2011, average concentration of benzo[a]pyrene (B[a]P) in the air was 0.9 ng/m^3, 1.3 ng/m^3, 10.1 ng/m^3, in P, SB and MS respectively. As some results of biomarker analyzes were unexpected, we revised cytogenetics and -omics findings with the aim to suggest mechanisms of the effect of air pollution on the integrity of DNA and function of genes. We found that: (i) DNA damage increased with elevated concentration of B[a]P in subjects living in P; (ii) DNA damage in subjects from MS had different trend and in general did not increase with exposure to B[a]P; (iii) Subjects that lived in P, temporarily moved to MS and were exposed to very high concentrations of B[a]P exhibited significant increase of DNA damage; (iv) Subjects from studied locations differed by gene expression and DNA methylation pattern; (v) Increased exposure during prenatal development significantly impacted function of genes and DNA damage later in life. We suggest, that prenatal and early life exposure is critical for sensitivity to negative factors later in life and that it specifically programs the organism for survival in the conditions in which the prenatal development was completed. Subjects living in MS seem to be adapted to the increased level of air pollution, probably due to changes in DNA methylation pattern during the prenatal development and its impact on gene expression levels. Supported by Ministry of Education Youth and Sports CR (#LO1508) and EU (EU FP7/ENV-2012-308524-2/CITI-SENSE).

ABSTRACT FINAL ID: 3633 Poster Board: P325
TITLE: Particulate Matter vs. Bioaerosol-Comparison of Toxicological Effects and Chemical Characteristics

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: W. Dott, S. Michael. *Institute of Hygiene and Environmental Medicine, RWTH Aachen University, Aachen, Germany.*
KEYWORDS: Metals; Environmental Toxicology; Cytokines; Chemical Characterization

ABSTRACT BODY: Clean air is a basic requirement for human health and well-being. In the last years, numerous studies investigated the association between air pollution level and various health effects. In this context particularly ambient airborne particulate matter (PM) and bioaerosol concentrations are considered as important environmental cofactors for respiratory and sensitization symptoms. However the pathophysiological mechanisms and possible triggers behind these effects are still unclear. The objective of this study was to compare the toxicological effects of different source-related particles in regard to their chemical composition. In this context we investigate airborne PM from an urban traffic and a rural site (Aachen, Germany) as well as biogenic particles from a poultry farm. A detailed description of the PM sampling sites and procedures as well as the prevalent air quality was previously published (Michael et al., 2013). Bioaerosol samples were collected from the air filter systems within the barn (mechanical retention) and include a parallel particle size distribution determination (PM10, PM2.5, PM1, UFP). The chemical characterization comprised the detection of total carbon, metals, inorganic ions and endotoxins. For the *in vitro* toxicity testing, a human alveolar basal epithelial cell line (A549) was exposed to water-soluble PM10 extracts from both PM and bioaerosol samples at concentrations of 1-100 µg/ml for 0-96 h. For the toxicological characterization the following biological responses were analysed: cytotoxicity, inflammatory (IL-8 and 6) and oxidative stress (total GSH, SOD, CAT) response.The chemical analysis of these particles indicated the presence of 21 elements, water soluble ions and strong differences in the endotoxin level (bioaerosol > PM). The toxicological characterization of the analysed samples shows a particle specific source-, concentration- and time-dependent effect of the measured endpoints. The direct comparison between PM and bioaerosol samples demonstrates clear variation in particle induced effect spectrum and intensity. Therefore, the present study confirms the hypothesis that PM but also bioaerosols are an important environmental risk factor for human health. Michael, S., Montag, M., Dott, W. (2013): Pro-inflammatory effects and oxidative stress in lung macrophages and epithelial cells induced by ambient particulate matter, Environmental Pollution, 183, 19-29

ABSTRACT FINAL ID: 3634 Poster Board: P326
TITLE: Evaluation of Pulmonary Function Among Workers Engaged in the Manufacture of Hydraulic Fracking Ceramic Proppant

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KEYWORDS: Respiratory Toxicology; Ceramic Proppant, Unconventional Gas, Spirometry

ABSTRACT BODY: Workers involved in hydraulic fracking processes are exposed to various types of chemicals and dusts in their workplaces, such as proppants, which hold open the fissures created in the fracking process. Recently, ceramic proppants have been developed that may be less hazardous to workers than traditional proppants. Pulmonary function testing of workers producing ceramic proppant was used to assess the potential inhalation hazards of ceramic proppant. Male workers (n = 100) from a producer of ceramic proppant were evaluated with pulmonary function test data collected and evaluated using The American Thoracic Society (ATS) acceptability criteria. A comparison group was selected from the Third National Health and Nutrition Examination Survey (NHANES III) spirometry laboratory subset. No pulmonary function deficits were found in the worker group in comparison to the NHANES III population. Multiple linear regression analysis showed that the mean FEV1(Forced Expired Volume in One Second) and FVC(Forced Vital Capacity) values in workers were 0.11 and 0.08 liters respectively, and were greater as compared to the NHANES III population. Unexpectedly, an FEV1/FVC ratio of less than 0.8, when compared to the NHANES III group, produced an odds ratio of 0.44 in worker group, indicating less risk of preclinical pulmonary dysfunction. Overall, exposure to ceramic proppant was not found to produce an adverse impact on pulmonary function in workers engaged in the manufacture of ceramic proppant.

ABSTRACT FINAL ID: 3635 Poster Board: P327
TITLE: Influence of Breathing Pattern on Derivation of Human Equivalent Concentrations of Inhaled Diacetyl for Deep Lung Effects in Rodents

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KEYWORDS: Inhalation Toxicology; Lung; Pulmonary Or Respiratory System; Dose-Response; Diacetyl

ABSTRACT BODY: Multiple analyses have been performed with the aim of quantifying the potential exposure-response relationship between exposure to diacetyl and the onset of respiratory disease. Recently, a refined BMC analysis was performed of subchronic inhalation data from Morgan et al. (2008) and NTP (2008), wherein rodents were exposed to airborne diacetyl concentrations up to 100 ppm for 90 days. For each observed bronchiolar and alveolar endpoint, a range of human equivalent concentrations (HECs) were calculated via a sensitivity analysis that incorporated varying input parameters, such as the BMC model employed, a “scrubbing” factor, and different breathing patterns. The aim of this study was to evaluate the influence of breathing patterns on the derivation of HECs. Using two published CFD-PBPK models, diacetyl concentrations associated with deep lung effects were modeled under two occupational breathing scenarios: 1) mixed breathing with 2.5h of nose-breathing and 5.5h of mouth breathing, and 2) nose-breathing-only for 8h. The calculated HEC range for minimal bronchiolar epithelial hyperplasia in rats was 1.35-29.0 ppm and 8.04-33.3 ppm for mixed breathing and nose-breathing-only, respectively. In mice, the range of HECs for minimal peribronchiolar lymphocytic inflammation was 4.26-124 ppm and 25.3-143 ppm for mixed-breathing and nose-breathing-only, respectively. These HECs were influenced differentially, depending on the input parameters used. Specifically, the HEC values obtained using the data from one CFD-PBPK model were virtually insensitive to changes in breathing pattern assumptions, while the HEC values obtained using an alternative and updated model for nose-breathing-only were roughly four- to five-fold higher than those obtained for mixed breathing. Occupational breathing pattern assumptions appear to influence the resulting HEC ranges and, therefore, could significantly augment the estimated risk of respiratory disease development. Future studies should accurately characterize the components that determine breathing patterns, such as minute volume and respiratory rate, when evaluating the risk of respiratory diseases following diacetyl exposure.

ABSTRACT FINAL ID: 3636 Poster Board: P328
TITLE: TTC for Non-Cancer Endpoints: Is the TTC Concept Applicable to Compounds with Pharmaceutical Properties?

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KEYWORDS: Non-Genotoxic; Pharmaceuticals; ; Threshold, Systemic Toxicity, TTC; Active Pharmaceutical Ingredients

ABSTRACT BODY: The TTC concept is currently not applied to active pharmaceutical ingredients (APIs) as those are tested in animal and human clinical studies; hence the risk assessment is based on compound specific data. The situation differs for impurities in pharmaceuticals. Impurities are often not amenable to experimental testing due to limited available amounts. Therefore, it would be favorable to use a threshold approach below which a risk for human health is negligible. The TTC concept is already in use to assess the risk of genotoxic impurities, as described by the ICH M7 guideline. But to this date there is no approach to derive thresholds for non-genotoxic impurities. For non-genotoxic organic chemicals, the current TTC values can be applied: Cramer class 1 (30 µg/kg/d); Class 2 (9 µg/kg/d); Class 3 (1.5 µg/kg/d). These thresholds are e.g. in use to assess food ingredients or impurities in pesticides. Our first approach is to construct a master TTC dataset based on existing non-cancer TTC databases (DBs) including in addition API specific data provided by eTOX. eTOX is an European IMI project where 13 pharmaceutical companies share proprietary toxicity data and ontologies in a toxicity DB and prediction models to support decision making process. The TTC dataset was grouped by pharmacological targets and analyzed for cumulative distributions. As an example, 360 APIs associated with over 120 pharmacological targets were analyzed. Six APIs were classified as Cramer Class I, including Aspirin and Tretinoin; one (Trimoprostil) as Class II. All other APIs were grouped to Class III. Targets include Prostaglandin, chemokine, metabotropic glutamate, 5-HT receptors, and tyrosine kinases. The median NO(A)EL values of these targets were 18.4, 30, 5.7, 4.7, 1.42 mg/kg/day, respectively. Our goal is to understand whether a new API enriched TTC dataset can contribute to thresholds for endpoints beyond genotoxicity in the field of pharmaceuticals e.g. for non-genotoxic impurities or Occupational Safety of APIs.

ABSTRACT FINAL ID: 3637 Poster Board: P329
TITLE: Considerations in Selecting a Principal Study for Deriving a Chronic Inhalation MRL for 1-Bromopropane (1-BP)

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KEYWORDS: Risk Assessment; Regulatory/Policy; Toxicity; Chronic; 1-Bromopropane

ABSTRACT BODY: The best available principal studies for deriving an ATSDR chronic-duration inhalation MRL for 1-BP are (1) a human cross-sectional occupational exposure study identifying a point of departure (POD) of 0.46 ppm based on diminished vibration sense in workers exposed to 1-BP and (2) a 2-year bioassay identifying a POD of 0.78 ppm for respiratory tract histological lesions in mice. Advantages of selecting the human study are (1) use of human data and (2) identification of a neurological critical effect, as neurological effects appear to be the most sensitive effect for workers repeatedly exposed to 1-BP and in animals exposed to 1-BP for acute and intermediate durations. However, the human study has a number of limitations, some of which were identified by the investigators or pointed out by others in the published literature, including (1) the cross-sectional study design which limits interferences that can be made, (2) potential selection bias for the control group, (3) potential underestimation of 1-BP exposure levels, (4) lack of biomonitoring data for controls, and (5) the relative insensitivity of the method used to measure vibration sense. Although the chronic animal study is more robust, selecting mouse respiratory lesions as the critical effect is of concern because (1) neurological effects in chronically exposed animals have not been adequately studied to characterize the relative sensitivity of neurological effects versus respiratory effects, and (2) available animal data indicate species differences in susceptibility to respiratory lesions. Potential MRL values based on the candidate human and animal PODs, after application of uncertainty factors, are nearly equivalent. After consideration and deliberation of the data, the human study appears to be the best available basis for the MRL despite acknowledged limitations, principally because it presents human evidence of neurological impairment as the most sensitive effect of inhaled 1-BP. ATSDR's final assessment should be available for public comment by March of 2016.

ABSTRACT FINAL ID: 3638 Poster Board: P330
TITLE: Derivation of the Biomonitoring Equivalent for Inorganic Tin

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KEYWORDS: Biomonitoring; Risk Assessment; Metals; Tin

ABSTRACT BODY: A Biomonitoring equivalent (BE), as defined by Hays, Aylward and co-workers, reflects the concentration or range of concentrations of a chemical or its metabolites in a biological medium (blood, urine, or other medium) that is consistent with an existing health-based exposure guidance value such as a reference dose (RfD) or a Tolerable Daily Intake (TDI). BEs have been developed for a large number of chemicals and have been used to interpret exposure data from biomonitoring programs in the US and Canada. The objective of the present study was to derive a urinary BE for inorganic tin (BE-urine) for the TDI established by Netherlands National Institute for Public Health and the Environment (RIVM). The TDI of 0.2 mg/kg bw per day is based on a NOAEL of 20 mg/kg bw per day from a 2-year feeding study in rats where a small increase in tin accumulation in bone and a decrease in feed efficiency were observed at 40 mg/kg bw per day. A mass balance approach was used to derive a urinary BE associated with the chronic exposure guidance value. An assumption of steady-state exposure to inorganic tin is made in the BE derivation. Accordingly, the amount of tin excreted in urine each day was set equal to the amount ingested (i.e. exposure guidance value x body weight) and multiplied by a factor representing the urinary excretion fraction (UEF). While the UEF for basal diet was 25%,a UEF of 0.24% was associated with the supplemented dose group based on the results from a peer-reviewed human volunteer study. Absorption decreases with increasing oral intake and the urinary excretion fraction is lower with increasing oral intake. A BE-urine of 25 μg/g creatinine was derived for inorganic tin. This BE represents the concentration of inorganic tin in urine that is consistent with the TDI of 0.2 mg/kg bw/day established by RIVM (2009). Overall, the robustness of pharmacokinetic data forming the basis of the BE-urine development is medium, and the availability of internal dose and kinetic data in the animal species forming the basis of the assessment could improve the overall confidence in the present assessment.

ABSTRACT FINAL ID: 3639 Poster Board: P331
TITLE: Derivation of Permissible Daily Exposure Limits for 15 Generic Small Molecule Therapeutics: Practical Considerations for the Implementation of EMA Guidance

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KEYWORDS: Regulatory/Policy; Pharmaceuticals; Risk Assessment; Tenoxicam

ABSTRACT BODY: The 2014 European Medicines Agency (EMA) "*Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal product in shared facilities*"(EMA/CHMP/CVMP/SWP/169430/2012) is intended to address potential cross-contamination concerns by setting Permitted Daily Exposure (PDE) limits for pharmaceutical substances. These thresholds are intended to protect the patient from adverse effects of unintended lifetime exposure to pharmaceutical substances. PDE values were derived for a range of therapeutic classes using publicly available information. PDE values were 80, 500, 1500 and 2500 µg/day were derived for the antibacterial drugs vancomycin, ofloxacin, cefuroxime sodium, and clarithromycin, respectively. For the non-steroidal anti-inflammatory drugs thiocolchicoside, tenoxicam, and etodolac, PDE values were 1, 400, and 1000 µg/day, respectively. The gastric H_2 blocker famotidine had a PDE of 24 µg/day, while the proton pump inhibitors pantoprazole and lansoprazole had PDE values of 250 and 400 µg/day, respectively. For the calcium channel blockers amlodipine besylate and diltiazem, PDE values were 70 and 75 µg/day, respectively. Additionally, a PDE of 60 µg/day was derived for antipsychotic drug aripiprazole, 1500 µg/day for the antifungal fluconazole, and 4100 µg/day for the antiviral acyclovir. In this sample set of 15 generic small molecule therapeutics, PDE values range from 1-4100 µg/day and in all but one instance, the PDE has a margin of safety of less than $1/1000^{th}$ of the lowest therapeutic dose. These PDE values were documented using an adaptation of the PDE Determination Strategy as outlined in the Annex of the Guideline. Specifically, the hazard identification in terms of "YES", "NO" or "UNKNOWN" checkboxes could potentially be misinterpreted. The derivation and documentation of the PDE for tenoxicam is shown in detail as a case study. Successful implementation of any regulatory guidance must consider the practical implications including any potential for misinterpretation of information when complex scientific and medical information is presented in a simplistic, binary manner.

ABSTRACT FINAL ID: 3640 Poster Board: P332
TITLE: Margin of Safety Derivation for Pyridoxine Hydrochloride (Vitamin B6)

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KEYWORDS: Risk Assessment; Margin Of Safety

ABSTRACT BODY: Pyridoxine hydrochloride is the hydrochloride salt form of pyridoxine, or Vitamin B6. Vitamin B6 is a water-soluble vitamin required by the body for the synthesis of the neurotransmitters serotonin and norepinephrine, as well as for myelin formation, and functions as a coenzyme in the metabolism of amino acids. Supplementation with Vitamin B6 is used to treat various conditions, such as carpal tunnel syndrome and premenstrual syndrome. High dose oral consumption of Vitamin B6 has been reported to induce sensory neuropathy. Pyridoxine-induced sensory neuropathy is characterized by numbness, burning, shooting or tingling pain, clumsiness, and ataxia. An adult male reported reversible neuropathy in his right leg following short-term (30 days) supplementation of a commercially marketed supplement containing 30 mg of Vitamin B6 as pyridoxine hydrochloride, which is 1,500% of the daily value. In the United States, the median intake of Vitamin B6 from food is 2 mg/day for men. Therefore, the individual's Estimated Daily Intake (EDI) of Vitamin B6 is 32 mg/day. In 1998, the United States Institute of Medicine (IOM) established a Tolerable Upper Intake Level (UL) of 100 mg/day for pyridoxine based on sensory neuropathy in humans above 200 mg/day along with an uncertainty factor (UF) of 2 based on limited data on doses less than 500 mg/day. As part of a comprehensive human health risk assessment, ToxServices calculated a margin of safety (MOS) for pyridoxine (as pyridoxine hydrochloride) by dividing the UL by the individual's EDI (i.e., UL / EDI = MOS; 100 mg/day / 32 mg/day = 3.125), resulting in an MOS of 3. The MOS represents the margin between the level of Vitamin B6 unlikely to cause adverse effects in adults and the intake level of Vitamin B6 from both the diet and the supplement. Based on the MOS of 3, it is unlikely that the neuropathy was caused from exposure to pyridoxine present in the supplement.

ABSTRACT FINAL ID: 3641 Poster Board: P333
TITLE: Implications of EPA's Proposed Dermal Slope Factor on Risks Posed by Dermal Contact with Grilled Meats

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KEYWORDS: Polycyclic Aromatic Hydrocarbons; Cutaneous Or Skin Toxicity; Percutaneous Absorption

ABSTRACT BODY: USEPA's revised Toxicological Review of Benzo(a)pyrene (BaP) (US EPA 2014) proposed a Dermal Slope Factor (DSF) of 0.006 (µg/day)-1. With this DSF, cancer risk estimates for PAH exposures through multiple exposures would be dominated by risks posed by dermal rather than ingestion exposures. The DSF was checked for biological plausibility by estimating the skin cancer risks posed by dermal contact with PAHs from char-broiled meats. It is well known that cooked meats contain PAHs, but no studies were found in the literature on the levels of PAHs on the surfaces of the cooked meats which would be contacted by the hands, lips, and oral cavity. Chicken breasts, hamburgers, and salmon burgers were char-broiled at the company café, and the surfaces of the meats (representing < 2.5% of the mass from each piece of cooked-meat) were removed and analyzed for an extended list of PAHs. Meats were cooked using a charcoal-gas grill until considered 'well-done' by the café's griller. Average benzo(a)pyrene toxic equivalent concentrations (BaP-TE) were calculated using EPA's proposed Relative Potency Factors (2010). BaP-TE concentrations were 874 (± 171), 720 (± 51), and 149 (± 14) µg/kg of surface material respectively for the three meats. The average lifetime risk for skin cancer among the US white population is 20%. For black Americans, it is 70 times lower. Only a small fraction of these cancers appear on the hands, and finger cancer is exceedingly rare. Using standard EPA risk assessment methods and low end assumptions for the frequency and duration of skin contact with cooked meats, the DSF predicts that PAHs on the surfaces of cooked meats explain a large fraction of the total hand cancer burden in the US white population and more than 100% of hand cancer in the black population. For instance, when it is assumed that black Americans touch char-broiled hamburgers with three fingers only three times a year, PAHs predict over 100% of the observed skin cancer on the hands. Given that people are dermally exposed to PAHs from many other sources, these values are minimum over estimates of the role of PAHs if the DSF were true. Dermatologists overwhelmingly agree that exposure to sunlight is the primary cause of human skin cancer, so this validation exercise demonstrates that EPA's DSF is not an accurate predictor of human cancer risk.

ABSTRACT FINAL ID: 3642 Poster Board: P334
TITLE: Risk Assessment of Honey Bees Potentially Exposed to Acrylonitrile

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: A. Pawlisz[1], D. Smith[2], B. Bullock[3]. *[1]GHD, Dallas, TX,[2]GHD, Exton, PA,[3]CSX, Jacksonville, FL.*
KEYWORDS: Exposure Assessment; Ecotoxicology; Risk Assessment; Honey Bee; Acrylonitrile

ABSTRACT BODY: A railroad car with acrylonitrile (ACN) sustained failure and subsequent fire. ACN became airborne, along with cyanide CN, a combustion byproduct. Bee keepers have raised concerns regarding impacts on bees and honey; the State suspended the sale of honey. Based on discussions with stakeholders, two main exposure pathways of concern were identified: (1) ACN/CN could somehow contaminate the nectar; and (2) honey made from uncontaminated nectar could become contaminated with ACN/CN by bees cooling the hive using water from impacted creek. To address these concerns a risk assessment was performed. The assessment contained a conceptual exposure model; air concentration data; plume modeling; physicochemical properties, toxicology, fate, and transport of ACN/CN; honey bee ecology, behavior, and feeding strategies; weather conditions; and exposure duration. The conceptual exposure model revealed that a large portion of the potential exposure was during rain and at night, when bees are largely inactive. The release occurred during the early summer "nectar dearth," a period when flowers, nectar, and nectar gathering are limited compared to spring and fall. Plume modeling suggested that the layer of impacted air was thin and well above the ground level; and the prevailing wind directed the plume, after dispersion, over areas not intersected by any apiaries. The duration of potential exposure was short and ACN/CN were not detected in air beyond the derailment area. Given the high volatility of ACN/CN, there would have been loss of any residues at multiple points in process between nectar gathering and honey formation in the hive. Physicochemical properties implied that ACN/CN in the plume would not have been efficiently scavenged by rain or air particles for sufficient deposition on flowers or soil. Root uptake and translocation to flower and nectar was estimated as highly unlikely. The evaporative cooling pathway was deemed incomplete due to cool/rainy conditions limiting bee activity, as well as the availability of clean water. Based on the multiple lines of evidence, it was concluded that bees and honey were unaffected by the acrylonitrile release.

ABSTRACT FINAL ID: 3643 Poster Board: P335
TITLE: 1,4-Dioxane Reanalysis in Support of a Regenerative Hyperplasia Mode of Action (MOA)

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: P. Nance, M. Dourson. *Department of Environmental Health, TERA Center, University of Cincinnati, Cincinnati, OH.*
KEYWORDS: Carcinogenesis; Toxicity; Chronic; Dose-Response; Mode of Action; 1,4-Dioxane

ABSTRACT BODY: The State of Kentucky petitioned the Alliance for Risk Assessment (ARA) to obtain additional information from Japanese studies to inform 1,4-dioxane's cancer mode of action (MOA) based on a recent reanalysis. Additional information and translations of the Japanese studies are also supportive of a regenerative hyperplasia MOA but with one exception, specifically, the reported findings from the histopathology and clinical chemistry of the mouse liver in the Japanese studies are contradictory. The reanalysis of data leads to the conclusion that these rodent tumors are evoked by a regenerative hyperplasia mode of action (MOA) that stimulates existing background mutations. Regenerative hyperplasia in this context is due to an overwhelming toxicity in the rodent liver as evidenced by an increase in blood levels of enzymes indicative of liver cell damage and associated histopathology due to 1,4-dioxane exposure that occurs in a dose and time related manner throughout the lifespan. This contradiction may be due in part to the investigators changing criteria for liver histopathology scoring during the course of reporting their results. A limited amount of additional information from the Japanese studies, including potentially rereading some of the mouse liver histopathology slides, may be helpful. The intent of this ARA project is to obtain this limited, additional information from the Japanese studies, or other information as appropriate, in order to resolve the hypothesized MOA for 1,4-dioxane's liver tumor formation (and potentially other tumors).

ABSTRACT FINAL ID: 3644 Poster Board: P336
TITLE: Implications for Risk Characterization of Non-Monotonic Shaped Dose Response Curves

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KEYWORDS: Dose-Response; Reproductive System; Endocrine Disruptors; Hormesis

ABSTRACT BODY: The existing risk assessment and management model is a framework that supports e assessment of numerous types of chemicals, including essential elements. It likewise is applicable to several kinds of phenomena such as hormesis and other low dose non-monotonic behaviors; for example the masking of less severe effects by effects of greater severity at higher doses. The nature of risk assessment, however, often precludes precise estimate of risk and only gives assurances of accuracy in the subthreshold range of population dose by way of a judicious use of conservative safety/uncertainty factors and other assumptions. Application of a mode of action framework provides complementary approaches that may be both more precise and accurate for example, characterizing both the risks and the benefits of exposures to essential elements, but such a framework must still address the differing severity of adverse effects. Contemporary modeling methods, such as categorical regression can be of use in several cases, for example: 1) when essentiality of certain elements provides evidence that two thresholds likely exist in an individual for adverse effects; 2) when hormetic effects suggest adaptation and compensation at low dose; or 3) when toxicity masking occurs routinely at higher doses leading to non-monotonic curves for less severe effects. Non-monotonic effects that fall outside of these areas will need to be carefully examined before relevance can be determined.

ABSTRACT FINAL ID: 3645 Poster Board: P337
TITLE: Quantitative High-Throughput Gene Expression Analysis Using a Modified RASL-Seq Platform Enables Treatment-Response Kinetic Analysis for Risk Assessment

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KEYWORDS: Risk Assessment; Genetic Toxicology; Dose-Response; Tobacco

ABSTRACT BODY: Toxicogenomics represents the merging of toxicology, genetics, molecular biology and bioinformatics to describe the response of organisms to chemical exposure. It opens a new paradigm in drug development and risk assessment. With the advent of next-generation sequencing (NGS) technologies, genome-wide RNA sequencing (RNA-seq) and targeted sequencing methods have recently been exploited for comprehensive transcriptome analysis. Transcriptome analysis not only reveals mechanistic information of observed toxicity but also captures the early stage of adverse events which may not show in the endpoint cell-based assays. Despite the genome-wide information provided by RNA-seq method, biases introduced during library preparation and inter-experiment variability limit precision, and the expense limits the number of doses, replicates, and compounds analyzed. We demonstrate an improved gene expression platform applied to risk assessment, called RNA-mediated oligonucleotide Annealing, Selection, and Ligation with Next-Gen sequencing (RASL-seq), to generate a highly reproducible, automated, high-throughput gene expression detection platform. RASL-seq platform bypasses the cDNA synthesis step that contributes the most variation between assays, to obtain a direct measurement of relative abundance of target transcripts. The improved method currently multiplexes 1041 assays for 347 genes per sample, and gathers information for 384 samples in one sequencing reaction; using RNA input as low as 0.5 ng total RNA. Reproducibility is remarkably high; the inter-experiment R-square ~0.9 and intra-experiment R-square >0.95. A quantitative analysis method as an extension to BMDexpress has been developed to elucidate the dose-response relationships for each gene upon treatment and identify the BenchMark Dose (BMD) and Point of Departure (POD). The detailed dose-response relationships, in combination with pathway analysis, allow researchers to identify the biological events that drive adverse events and to classify uncharacterized toxicants by comparison to a reference database. An example reveals detailed concentration-response relationships on gene expression for 18 tobacco chemicals was used to demonstrate this toxigenomics application for risk assessment.

ABSTRACT FINAL ID: 3646 Poster Board: P338
TITLE: Quantitative Analysis of Genotoxicity Dose Responses by Five Tobacco-Related Compounds in the Human TK6 Cell *In Vitro* Micronucleus Assay

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KEYWORDS: Dose-Response; Genetic Toxicology; Risk Assessment

ABSTRACT BODY: Genotoxicity assessments are currently used mainly for human health hazard identification. Recent work has attempted to use quantitative analysis of genetic toxicity data as a tool for human health risk characterization. In this study, we conducted *in vitro* micronucleus tests in human TK6 cells with five genotoxic carcinogens that are found in cigarette smoke, namely 4-aminobiphenyl (4-ABP), benzo[a]pyrene (BaP), cadmium chloride (CdCl2), 2-amino-3,4-dimethyl-3H-imidazo[4,5-f] quinoline (MeIQ), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). TK6 cells were treated with 4-ABP, BaP, MeIQ, and NNK with metabolic activation and with CdCl2 with and without metabolic activation. Data from 20 closely spaced concentrations were used for generating dose responses. The six dose responses were then analyzed using the DRSMOOTH/PROAST and Benchmark Dose (BMD) software packages to determine points of departure (PoDs). BMD10, BMD50, BMD100, and BMD200 were calculated based on 10%, 50%, 100%, or 200% increases in the background frequency, respectively. The upper (U) and lower (L) 95% confidence limits (BMDU and BMDL) were established for each of the BMDs and used to rank the chemicals from most to least potent based on micronucleus formation. The order of genotoxic potency for the three most potent responses was similar using the No Observed Genotoxicity Effect Level and PROAST BMDL values. The six dose responses could also be divided into 3-4 groups based on BMDL and BMDU for BMD10, BMD50, and BMD100, and into two different groups for BMD200. The results suggest that the quantitative analysis might be useful in assessing risk associated with the genotoxicity of different chemicals found in tobacco products or tobacco smoke.

ABSTRACT FINAL ID: 3647 Poster Board: P339
TITLE: Use of High Throughput Screening Data in IARC Monograph Evaluations

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: I. Rusyn[1], W. Chiu[1], K. Guyton[2], M. Martin[3], D. Reif[4]. *[1]Texas A & M University, College Station, TX;[2]International Agency for Research on Cancer, Lyon, France;[3]US Environmental Protection Agency, Durham, NC;[4]North Carolina State University, Raleigh, NC.*
KEYWORDS: Regulatory/Policy; Alternatives to Animal Testing; Predictive Toxicology

ABSTRACT BODY: Evaluation of carcinogenic mechanisms serves a critical role in IARC monograph evaluations, and can lead to "upgrade" or "downgrade" of the carcinogenicity conclusions based on human and animal evidence alone. Three recent IARC monograph Working Groups (110, 112, and 113) pioneered analysis of high throughput *in vitro* screening data from the U.S. Environmental Protection Agency's ToxCast program in evaluations of carcinogenic mechanisms. For monograph 110, ToxCast assay data across multiple nuclear receptors were used to test the hypothesis that PFOA acts exclusively through the PPAR family of receptors, with activity profiles compared to several prototypical nuclear receptor-activating compounds. For monographs 112 and 113, ToxCast assays were systematically evaluated and used as an additional data stream in the overall evaluation of the mechanistic evidence. Specifically, ToxCast assays were mapped to 10 "key characteristics of carcinogens" recently identified by an IARC expert group, and chemicals' bioactivity profiles were evaluated both in absolute terms (number of relevant assays positive for bioactivity) and relative terms (ranking with respect to other compounds evaluated by IARC, using the ToxPi methodology). PFOA activates multiple nuclear receptors in addition to the PPAR family in the ToxCast assays. ToxCast assays offered substantial coverage for 5 of the 10 "key characteristics," with the greatest coverage for modulation of receptor-mediated effects. The patterns of bioactivity observed in ToxCast assays provided additional support to Working Group evaluations of the mechanistic evidence. High throughput *in vitro* screening data such as those from ToxCast provide a useful resource for evaluating both specific mechanistic hypotheses as well as the overall strength of the mechanistic evidence. However, the current ToxCast assay set does not sufficiently cover many of the 10 key characteristics of carcinogens that form the framework for current IARC mechanistic evaluations, highlighting the need to identify or develop additional assays for high throughput screening in order to more comprehensively cover mechanisms relevant to carcinogenicity.

ABSTRACT FINAL ID: 3648 Poster Board: P340
TITLE: Integrating QSARs in Rapid Screening of New Cosmetic Ingredients

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S.-T. Kim[1], S. Sieber[1], C. Choi[2], T. Schatz[2]. *[1]Ashland Inc., Dublin, OH; [2]Ashland Inc., Bridgewater, NJ.*
KEYWORDS: Alternatives to Animal Testing; QSAR; Safety Evaluation; Safer Alternative Assessment; Safety of Cosmetic Ingredients

ABSTRACT BODY: In order to compete in the current economic climate, personal care product companies are required to produce large numbers of new, innovative and effective products whilst significantly reducing development time and costs without compromising the consumer safety. With the advent of combinational chemistry and high-throughput screening, the numbers of new candidate structures coming out of the discovery cycle has increased significantly. This creates a demand for faster screening of the toxicological properties of these candidates. Unfortunately, advancing international regulations, along with increasing effort to ban animal testing, often inhibit a scientist's ability to appropriately and timely determine the hazard and risk of any given chemical. We have used a wide range of computer-based (quantitative) Structure-Activity Relationship (qSAR) models in predicting physicochemical properties, toxicological endpoints and other biological effects, as well as fate in the environment and biological organisms. The applicability of any given software tools are carefully evaluated and documented in integrating qSARs in comparative safer alternative assessments of cosmetic ingredients with their main strengths and limitations. While using qSARs in the rapid comparative safer alternatives for cosmetic ingredients, we also find that relying on structural alerts alone would lead to over-classification of chemical hazards, particularly for endpoints such as sensitization and reproductive and developmental toxicity, suggesting the importance of using professional judgement to validate structural alert-based predictive toxicity.

ABSTRACT FINAL ID: 3649 Poster Board: P341
TITLE: A Systematic Decision-Tree Framework Approach for Assessing Consumer Safety of Polymers Used in Cosmetics and Personal Care Products

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S.-T. Kim[1], C. Choi[2], T. Schatz[2]. *[1]Ashland Inc., Dublin, OH; [2]Ashland Inc., Bridgewater, NJ.*
KEYWORDS: Safety Evaluation; QSAR; Risk Assessment; Cosmetic Polymer Risk Assessment; Polymer Safety Assessment

ABSTRACT BODY: Polymers represent a large class of ingredients in cosmetics and personal care products. A diverse range of polymers are applied in cosmetic and personal care products as film formers, fixatives, rheology modifiers, thickeners, emulsifiers, conditioners, stabilizers, antimicrobials, and other skin beneficial agents. The use of polymers in these products is highly developed and innovative advances in polymer sciences are deriving the creation of sophisticated products. In this presentation, we are discussing a systematic decision-tree framework approach that we have used, in order to: (1) Provide appropriate safety support for intended product application; (2) identify potential safety and/or external-relation issues, and (3) mitigate risk regarding the commercialization of new innovative product. This systematic decision-tree framework involves: (1) Collect physico-chemical characteristics of polymer (composition, MW, charge, extractable/impurity profile, etc.), (2) collect/review all available toxicology/safety data (polymer classification/categorization, toxicological data, qSAR, read-across, data reliability, etc.), (3) determine whether the toxicological/safety data are adequate to derive a screening level toxicological risk assessment [based on (a) polymer/analog class, (b) residual monomers and/or crosslinkers and/or side-chain modifiers, (c) other impurity profile], (4) complete toxicity assessment and exposure assessment (use Toxicology Review Form to identify the appropriate NOAEL, LOAEL, and Toxicity Rating to serve as the basis for the Toxicity Assessment), (5) complete the risk characterization for the polymer, and (6) conduct periodic data reviews. By using the systematic decision-tree framework approach in assessing consumer safety of polymers used in cosmetics and personal care products, we were able to quickly screen polymers and to efficiently and effectively prioritize resources to the assessment of new polymers.

ABSTRACT FINAL ID: 3650 Poster Board: P342
TITLE: The Role of Feature Selection and Statistical Weighting in Predicting *In Vivo* Toxicity Using *In Vitro* Assay and QSAR Data

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KEYWORDS: Computational Toxicology

ABSTRACT BODY: Our study assesses the value of both *in vitro* assay and quantitative structure activity relationship (QSAR) data in predicting *in vivo* toxicity using numerous statistical models. The models were built on datasets of (i) 586 chemicals for which both *in vitro* and *in vivo* data are currently available in EPA's Toxcast and ToxRefDB databases, and (ii) 769 chemicals for which both QSAR data and *in vivo* data exist. Similar to a previous study (based on just 309 chemicals, Thomas et al. 2012), after converting the continuous values from each dataset to binary values, the majority of more than 1,000 *in vivo* endpoints are poorly predicted. Even for the endpoints that were well predicted (about 40 with an F1 score of >0.75), class imbalances in *in vivo* endpoint data or cytotoxicity across *in vitro* assays may be skewing results. We investigated whether use of best practices for data preprocessing and model fitting in real-world contexts would improve model predictions. This included options for dealing with missing data, including omitting observations, aggregating variables, and imputing values. We also examined the impacts of feature selection (from both a statistical and biological perspective) on performance and efficiency, and we weighted outcome data to reduce endpoint imbalances to account for potential chemical selection bias and assessed revised performance. For example, initial weighting strategies decrease the number of models with an F1 score >0.75 drastically (to 6), but these models are more able to predict nontoxic chemicals in certain contexts. The results of these analyses can be used to inform screening or other decisions, especially in the context of future data enhancements, such as more biologically relevant *in vitro* assays, additional *in vivo* endpoint data, and extension of chemical space. Disclaimer: This abstract does not necessarily reflect US EPA policy.

ABSTRACT FINAL ID: 3651 Poster Board: P343
TITLE: Assessment of Potential Tremolite Exposures from Historical Vermiculite-Containing Consumer Products

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KEYWORDS: Exposure Assessment; Inhalation Toxicology; Asbestos

ABSTRACT BODY: For nearly 100 years, vermiculite has been commercially mined in the United States for use in consumer products. Some vermiculite deposits are known to contain asbestiform and/or non-asbestiform tremolite. While it has been shown that processing and exfoliation remove impurities from vermiculite ore, it has been reported that vermiculite-containing consumer products may still contain trace levels of tremolite asbestos. The objective of this analysis was to estimate the total potential cumulative exposure to tremolite experienced by consumers associated with the use of four vermiculite-containing products, and to determine whether this exposure would result in an increased risk of mesothelioma. A literature search was conducted to locate studies that described the composition of and/or airborne asbestos exposure associated with the use of the following vermiculite-containing products: packaging material, fire-rated wallboard, fertilizer, and loose attic insulation. Using conservative assumptions regarding typical consumer use patterns, the total potential cumulative tremolite exposure was estimated for each of these products. These cumulative exposure estimates were then compared to published no-observed adverse effect levels (NOAELs) and lowest-observed adverse effect levels (LOAELs) for mesothelioma that were determined based on the epidemiological literature evaluating tremolite asbestos-exposed cohorts. Under typical use scenarios, reported maximum air concentrations (f/cc) were 0.48 for packaging material, 0.08 for fire-rated wallboard, 0.094 for fertilizer, and 14.4 for loose attic insulation. Maximum cumulative exposures (f/cc-yr) to tremolite were estimated to be 0.0095 for packaging material, 1.3E-06 for fire-rated wallboard, 3.7E-05 for fertilizer, and 0.0002 for attic insulation. These exposures are well below published mesothelioma NOAELs for fibrous tremolite (0.05-2.6 f/cc-yr) and therefore consumers using these products are not expected to be at an increased risk of mesothelioma.

ABSTRACT FINAL ID: 3652 Poster Board: P344
TITLE: Categorization of UVCBs Using Chemical-Biological Read Across

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KEYWORDS: Alternatives To Animal Testing; Cell Culture; Dose-Response

ABSTRACT BODY: Chemicals of **U**nknown or **V**ariable composition, **C**omplex reaction products, and **B**iological materials (UVCBs) present a major challenge for registrations under the REACH and US High Production Volume regulatory programs. In addition to frequent variations in their chemical composition, many gaps in available toxicity data preclude confident groupings of these substances for read across applications. Here, we present a comprehensive experimental and computational approach to categorize UVCBs according to global similarities in (1) their chemical composition using Ion Mobility Mass Spectrometry (IMMS) and (2) their bioactivities using a suite of *in vitro* models. For chemical read across, we analysed 20 petroleum substances from four distinct product groups by IMMS to determine substance-specific quantitative parameters including m/z distribution, drift time, carbon numbers, and double bond equivalents. For biological read across, we exposed induced pluripotent stem cell-derived cardiomyocytes and hepatocytes to a DMSO-soluble extract series of 26 petroleum substances comprising six product groups for up to 72 hours. Dose-response profiles for live cell cardiophysiology assessments (calcium-flux), high-content cell imaging (cytotoxicity and mitochondrial integrity), and targeted transcriptomics (TempO-seq) revealed product group-specific similarities. Data integration in ToxPi software and subsequent correlation analysis revealed group-specific clustering and a high degree of correlation between biological and chemical data sets. Altogether, we demonstrate how novel analytical chemistry and *in vitro* screening approaches can be effectively utilized to categorize UVCBs thereby indicating their potential applicability in regulatory submissions.

ABSTRACT FINAL ID: 3653 Poster Board: P345
TITLE: Measurement of Cyp2b1 Protein Induction in Laser Dissected FFPE Liver Samples by Nano-LC Mass Spectrometry

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KEYWORDS: Cytochrome P450; Pesticides; Non-Genotoxic

ABSTRACT BODY: Cytochrome p450 (CYP) gene induction is an early associative key event in the mode of action (MOA) of non-genotoxic carcinogens that act via activation of nuclear hormone receptors such as the constitutive androstane receptor (CAR). Cyp protein measurement in formalin fixed paraffin embedded (FFPE) samples has utility for retrospective read-across approaches for risk assessment. However, this task is challenging in FFPE tissue due to difficulties in applying traditional methods such as Western blotting to these samples. To address this issue we have developed a method for the direct measurement of Cyp2b1 protein induction in laser dissected FFPE samples using mass spectrometry (LC MS/MS). We measured Cyp2b1 protein changes (relative to control) in laser dissected FFPE liver sections derived from Wistar rats treated with a known CAR activator - a pyrazole carboximide succinate dehydrogenase inhibitor (SDHI1). Proteins were extracted from the liver sections using the Qiagen Q proteome kit and trypsin digested prior to performing LC MS/MS using an Orbitrap mass spectrometer. The protein yield was in the range 0.7-1.4 ug/mm2 tissue in the control and SDHI1 treated liver sections. Fold change values were calculated from intensity levels of unambiguously identified peptides that mapped uniquely to Cyp2b1. Statistical analysis was performed using a students t test. SDHI1 caused a 60 fold induction of Cyp 2b1 ($P<0.05$). We also detected significant changes to Cyp3a1 and several other proteins many of which are regulated by CAR/PXR. There was no induction of Cyp1a1 or Cyp4a1. The Cyp protein induction reflected the biochemical data (PROD, EROD activity). The results suggest that the use of proteomics has utility for unambiguous assessment of Cyp protein induction, and quite possibly other proteins, aiding MOA investigations with the potential for integrated pathways analysis.

ABSTRACT FINAL ID: 3654 Poster Board: P346
TITLE: Analysis of Chemical Properties of Drinking Water and Soil Qualities in Ihiala, Southeast Nigeria

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KEYWORDS: Food Safety/Nutrition; Metals; Safety Pharmacology

ABSTRACT BODY: Surface water is the commonest source of drinking water in Ihiala town, and there are no good waste disposal systems making water prone to contamination. Many unreported cases of water-borne diseases and sudden deaths abound in and around Ihiala and indeed Nigeria. This study analyzed the chemical properties of drinking water sources and soil samples in Ihiala, South-East Nigeria, with a view to assessing the safety of the drinking water sources. Twenty water samples (streams 4, rivers 2, springs 3, taps 5 and sachet 6) and 10 soil samples collected from within the river, stream and spring water sources were analyzed. Chemical parameters analyzed included inorganic constituents (pH, alkalinity, total hardness, total dissolved solids, cyanide, free chlorine and nitrate), and metals (aluminum, cadmium, copper, chromium, lead, and nickel). The methods and/or instrument used for the analysis were: metals – atomic absorption spectrophotometer, pH – pH meter, alkalinity – acidimetric titration method, nitrate – sodium salicylate method, total hardness (TH) – EDTA titration method, total dissolved solids (TDSs) – measurement of specific conductance, free chlorine – iodometric method, and cyanide – spectrophotometric method. Results showed that only lead was significantly increased ($p = 0.0481$) in the river compared to the sachet water samples. Compared to the Standard Organization of Nigeria (SON) Guideline for drinking water in Nigeria, the sachet water samples showed a significant increase in the levels of lead, nickel, cadmium, chromium and aluminium, and significant decrease in the pH and nitrate levels. The levels of copper, TH and TDSs in the sachet water samples were within the guideline values of SON. All the chemical parameters in the springs, streams, boreholes and rivers were statistically equal to the SON's guideline for drinking water ($p > 0.05$). There were no significant differences in the levels of alkalinity, TH, aluminium, copper, chromium, cadmium and lead between the soil and water samples, but there was significant increase in pH, nitrate and TDS levels of soil compared to water. From this study, only water samples from springs and boreholes in Ihiala met the guideline for chemical properties of the Nigerian Standard for Drinking Water Quality of SON.

ABSTRACT FINAL ID: 3655 Poster Board: P347
TITLE: The Determination of Nine N-Nitrosamines at Nanogram Per Gram Levels in Tobacco and Tobacco Product

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KEYWORDS: Safety Evaluation; Volatile N-nitrosamines, GC-MS/MS, D-SPE, Tobacco, Tobacco Products

ABSTRACT BODY: A method for determination of nine volatile N-nitrosamines (VNAs) in tobacco and tobacco products has been developed. Targets are as following: N-Nitrosodimethylamine (NDMA), N-Nitrosopyrrolidine (NPYR), N-Nitrosopiperidine (NPIP), N-Nitrosomorpholine (NMOR), N-Nitrosoethylmethylamine (NEMA), N-Nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosobuylmethylmine (NBMA), N-nitrosodibutylamine (NDBA). The method uses dispersive solid phase extraction (D-SPE) followed by gas chromatography coupled to tandem mass spectrometry. The type of adsorbents in D-SPE was optimized. 1 g of primary secondary amine and 0.5 g of carton were used for purification. The method was validated for linearity, precision, accuracy and matrix effect. The method allowed recoveries between 84% and 118% for nine targets with <16% relative standard deviations at three spiking levels of 1, 2 and 5 ng/g. The limits of quantification and limits of detection ranged within 0.03-0.15 and 0.10-0.49 ng/g at a signal-to-noise ratio of 10 and 3, respectively. Application of the method for samples (burley tobacco, flue-cured tobacco, snuff, chewing tobacco and reference cigarettes) showed the presence of six VNAs (NDMA, NDEA, NMOR, NPYR, NPIP and NDBA) ranging within not detected (ND)-30.7 ng/g. NMEA, NMBA and NDPA were not detected in any sample.

ABSTRACT FINAL ID: 3656 Poster Board: P348
TITLE: Electronic Cigarette Puff Topography: Analysis of Monitoring Method Among Naïve and Experienced Users

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KEYWORDS: Inhalation Toxicology; Exposure Assessment; Electronic Cigarettes

ABSTRACT BODY: Puffing topography for users of electronic nicotine delivery systems (ENDS) is necessary to accurately characterize aerosol exposures and potential health risks from ENDS. A variety of qualitative and quantitative methods have been utilized to assess topography among ENDS users. This analysis involved a pooled assessment of existing data involving different topography parameters (puff volume, duration, and frequency) and monitoring methods of users of different ENDS types. Topography information was pooled from 24 different studies utilizing the following monitoring methods: 1) self-reported survey/questionnaire, 2) video surveillance, and 3) ENDS monitoring device. Additionally, puffing parameters from 40 aerosol exposure studies (not involving human subjects), as well as data utilized in past health risk assessments of ENDs were compared with those reported in topography studies. Analysis of topography data showed that puff parameters, including fluid consumption, puff count, duration, volume, flow rate, and interval, varied across the three monitoring methods. Topography parameters appeared to vary based on user experience, device type (first, second, third generation), and nicotine concentration. Additionally, three primary populations of ENDS users were represented across studies: experienced, naïve, and dual (smoked traditional tobacco cigarettes and used ENDS). Analysis of the topography studies indicates that 1) self-reported surveys of puff topography generally resulted in higher estimates of puff frequency than quantitative methods using real-time monitoring devices; 2) device type influenced topography among naïve and experienced users, and; 3) with the exception of first generation devices, experienced users showed a puff volume, duration, and frequency higher than naïve users. Furthermore, puff volume, duration, and interval parameters utilized in experimental aerosol exposure studies differed from that reported in topography studies involving human subjects. Considering the variances in topography across device types and user experience, these findings indicate that proper exposure estimations should take into account a range of puffing parameters to accurately assess the potential health risks from ENDS aerosol exposures. This analysis should provide useful information for researchers planning aerosol exposure studies, identifying data gaps, and improving the design of future topography studies.

ABSTRACT FINAL ID: 3657 Poster Board: P349
TITLE: Determination of a Peptide in Rabbit Plasma: Sensitivity Enhancement Using an Api6500 Qtraptm

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M. Encabo[1], L. Folguera[1], X. Esparza[1], I. Lenthéric[1], M. Abellán[1], S. Martín[2]. *[1]Envigo CRS SA, Santa Perpetua de La Moguda, Spain; [2]Kubus, Madrid, Spain.*
KEYWORDS: Pharmaceuticals; Toxicokinetics; Exposure Assessment; Peptide

ABSTRACT BODY: The present communication describes the use of an ULC-MS/MS (API6500 QTRAPTM) system in the bioanalysis of a small peptide to achieve a challenging LLOQ for a preclinical study in Rabbit involving intra-vaginal administration. Comparative results between two MS/MS detectors (API4000TM vs. API6500 QTRAPTM) are provided. The ULC-MS/MS method used to determine this peptide involved protein precipitation followed by solid-phase extraction (SPE) to extract and purify the peptide from rabbit plasma. Chromatography involving a short gradient elution was performed on an Acquity UPLC® CSH™ C18 column with a mass transition: m/z 584.5 > m/z 249.1. This transition was followed for quantitative purposes after positive electrospray and collision induced dissociation. Initially the Lower Limit of Quantification (LLOQ) achieved for the studied peptide was 500 pg/mL on the API6500 QTRAPTM, but, in order to cover *in vivo* studies by vaginal administration, additional development efforts were made and a new LLOQ of 50 pg/mL was reached. Sample bioanalysis was achieved with a 3.5 min run time. The method was linear from 50 to 10,000 pg/mL, with specific reagents employed to avoid carry-over in the system. The accuracy and precision of the assay was within 15%. A similar peptide was used as internal standard. The developed method was considered suitable for routine sample analysis and the development effort was reduced due to the use of a state of the art ULC-MS/MS (API6500 QTRAPTM) system.

ABSTRACT FINAL ID: 3658 Poster Board: P350
TITLE: Toxicity and Fob Measurement of Ulught after Single IV Administration with 2-Week Recovery Period in Rats

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: K. Kabirov[1], J. Sorger[2], M. Lindeblad[1], A. Zakharov[1], J. Fischer[1], A. Lyubimov[1]. *[1]University of Illinois at Chicago, Chicago, IL; [2]Intuitive Surgical Inc., Sunnyvale, CA.*
KEYWORDS: Pharmaceuticals; Toxicity; Acute; Pharmacokinetics; Ulight

ABSTRACT BODY: The toxicity, toxicokinetics and the functional status of the CNS were evaluated in Crl:CD (SD) rats after a single IV infusion of Ulight (a new imaging agent) at 0, 2, 20 and 200 mg/kg followed by up to a 2-week observation period. No mortalities were seen in all dosing groups. Clinical signs observed in rats were related to Ulight administration and its excretion with urine, feces and through skin (resembling the color of the test article). In the 200 mg/kg dose group, diarrhea and soft stool were seen during the FOB tests. All animals were within normal limits at the time of ophthalmic examinations on Day 2 and Day 15. There were no significant differences in body weights, food consumption, or hematology and coagulation parameters assessed. On Day 2, there was a statistically significant increase in mean total bilirubin concentrations in the 200 mg/kg dose group when compared to the control group. In males on Day 8, there were statistically significant increases in mean TBILI and albumin (ALB) levels seen only at 200 mg/kg dose group when compared to the control. FOB measurements performed on Day 1 showed statistically significant decreases in mean number of squares entered at 200 mg/kg in males and arousal response at 200 mg/kg in females when compared to the control (slight CNS depression). On Day 2, there was a statistically significant increase in testes/epididymis weight in the 200 mg/kg dose group when compared to the control (not confirmed by histopathology). Peak plasma levels occurred at the 5 min time-point after Ulight administration. Ulight was rapidly eliminated in each sex-dose group (half-life (t1/2-λ) ranged from 1.04 to 1.23 hrs). Sex and Ulight dose were found to independently influence Ulight pharmacokinetics and systemic exposure. In summary, slight toxicity was seen at the Ulight dose level of 200 mg/kg. The NOAEL was established at the Ulight dose level of 20 mg/kg. This study was sponsored by Intuitive Surgical.

ABSTRACT FINAL ID: 3659 Poster Board: P351
TITLE: Investigation of Mechanisms of Skin Toxicity Observed in Cynomolgus Monkeys

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KEYWORDS: Cutaneous or Skin Toxicity; Bioinformatics; Safety Evaluation; Non-Human Primate

ABSTRACT BODY: The purpose of this presentation is to describe a case of drug-induced cutaneous toxicity observed in cynomolgus monkeys and to share our attempt to elucidate mechanisms of toxicity. The test-article (Compound-X) was a small molecule with a ubiquitously distributed target, especially in rapidly dividing cells, and which modulated cell cycle regulation. After seven consecutive days of oral dosing, animals developed dose-dependent multifocal skin lesions. The lesions were distributed mainly in thin-skinned areas of the ventral body including cheek, chest, abdomen and inner limbs. Microscopically, the lesions were confirmed as epidermal vesicle formation and ulceration. Immunohistochemical (IHC) staining for epidermal and leukocyte markers revealed that the levels within the epidermis where separation occurred were not consistent and predominant inflammatory cell infiltrate were comprised of neutrophils and macrophages without involvement of lymphocytes. These IHC staining results indicated that the conditions observed in our case did not fit to any reported cases of immune-mediated blistering skin lesions. As a next step, we investigated the mechanisms of toxicity specific to Compound-X using Mechanism of Action (MoA) Central, a novel target/mechanism of action search engine developed at Novartis. An MoA Central searches using Compound-X identified structural analogs with cell line proliferation profiles similar to PI3K inhibitors. This suggested that direct or indirect modulation of PI3K/AKT/mTOR pathway modulation could play a role in our skin toxicity case. IHC staining for pAKT showed there was decreased pAKT expression level in the epidermis of the skin lesion, which also supported the MoA Central search result. To the best of our knowledge, similar cutaneous toxicity has not been reported previously, although there are many reports of other types of cutaneous toxicities. The mechanisms of cutaneous toxicity in our case could not be determined due to limitations in investigative possibilities. However, after running an MoA Central search, we are now investigating possible on- or off-target effects by Compound-X in association with PI3K pathway modulation. Understanding the mechanisms of the toxicity is very important when assessing human relevancy during drug development. Our investigative approach can be utilized when unusual skin toxicities are observed in the future.

ABSTRACT FINAL ID: 3660 Poster Board: P352
TITLE: A Comparison of Micro- and Macro- Blood Sample Collection for Bioanalysis of a Large Molecule and the Impact on Hematology Parameters

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KEYWORDS: Toxicokinetics; Exposure Assessment

ABSTRACT BODY: A comparative assessment of toxicokinetic (TK) results obtained through use of standard macrosamples (0.4 or 0.5 mL) versus those from microsamples (32 or 80 µL) was conducted. Presented here is data from two separate studies, both involving IV and SC administration of a large molecule (biologic) to rats once weekly, for either 2 doses or 13 doses. For the 2-dose study, TK microsamples were collected from all animals at all time points while reference macrosamples were collected for a limited number of predetermined time points. Microsamples were collected via tail vein and macrosamples by jugular venipuncture. For the 13-week dose study, TK and immunogenicity (IG) blood microsamples were collected over a full timecourse from main study animals, the same animals from which hematology parameters were evaluated. Macrosamples were collected for a limited number of predetermined time points. Both micro- and macrosamples were collected via jugular venipuncture. A single microsample was used for assessment of both TK and IG. Validated MSD assays (adapted for analysis of low volume serum microsamples) were used for assessment of TK and IG. Bioanalytical results obtained from micro and macro TK samples were considered comparable in both studies. IG results from the 13-week study correlated well with the TK parameters in individual animals. Consequently the microsampling technique is considered to be adequate also for assessment of IG. Hematology samples collected at usual volumes were within historical range for the species, strain and age of the animals, and were therefore considered unaffected by the repeat sampling. In conclusion, bioanalytical results obtained from micro and macrosamples were comparable at concurrent time points for the large molecule evaluated, the use of microsampling allowed a full TK profile evaluation from main study population with no impact on hematology parameters.

ABSTRACT FINAL ID: 3661 Poster Board: P353
TITLE: Evaluation of the Renal and Systemic Effects of Single and Repeated Doses of ISIS 141923 Administered by Subcutaneous Injection to Nephrectomized CD-1 Mice

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: R.A. Fey[1], J.R. Kolb[1], J.A. Engelhardt[2], S.P. Henry[3]. *[1]Preclinical Development, Toxicology, Ionis Pharmaceuticals, Inc., Carlsbad, CA; [2]Preclinical Development, Pathology, Ionis Pharmaceuticals, Inc., Carlsbad, CA; [3]Preclinical Development, Ionis Pharmaceuticals, Inc., Carlsbad, CA.*
KEYWORDS: Pharmaceuticals; Antisense Oligonucleotides; 2'-O-Methoxyethyl Phosphorothioate

ABSTRACT BODY: To assess whether reduced glomerular filtration rate (GFR) effects the toxicity profile of 2'-O-methoxyethyl phosphorothioate (2'-MOE) antisense oligonucleotides (ASO), ISIS 141923 was administered subcutaneously to intact, hemi- and 5/6-nephrectomized CD-1 mice as either single doses (on Day 1) or as repeated doses administered over 6 weeks (4 loading doses on Week 1 followed by once-weekly doses, thereafter). Because a fraction of any individual ASO dose is rapidly cleared from plasma via glomerular filtration and tubular reabsorption in kidney with subsequent elimination in urine, the study was intended to evaluate whether reduced GFR alters the pattern of systemic tissue accumulation of ASO or toxicity. Vehicle or ISIS 141923 was administered to 20 intact, hemi- and 5/6-nephrectomized female mice at doses of 5, 25 and 75 mg/kg/wk. Animals were sacrificed on Days 3 and 45 (10/group), 48 hr following first or final dosing. At necropsy, blood, urine and tissues specimens were taken for clinical pathology, toxicokinetic and histopathologic evaluation. Plasma and tissue toxicokinetics demonstrated dose-dependent exposure following and single and multiple doses with higher concentrations observed following multiple doses. Plasma, liver, kidney and spleen concentrations were generally higher in the nephrectomized mice relative to intact mice following both single and multiples doses, with the increases being more prominent in 5/6 nephrectomized mice. In liver, increased exposure was not associated with any microscopic indications of hepatotoxicity. In kidney, increases in BUN, creatinine and urine protein excretion were observed in the nephrectomized mice, consistent with the experimental model, but not associated with any oligonucleotide-related increases in indications of renal pathology. Thus, presumed decreases in GFR were associated with increases in ASO accumulation of <6-fold in plasma and <2-fold in tissue in the nephrectomized mice relative to intact mice, but did not produce any increases in hepato- or renal toxicity.

ABSTRACT FINAL ID: 3662 Poster Board: P354
TITLE: Human Primary Proximal Tubule Cell Monolayers as a Predictive Model of Nephrotoxicity

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: G.W. Chung[1], S.F. Billington[1], J.K. Zolnerciks[2], C.D.A. Brown[1]. *[1]Medical School, Newcastle University, Newcastle Upon Tyne, United Kingdom; [2]SOLVO Biotechnology, Seattle, WA.*
KEYWORDS: *In Vitro* and Alternatives; Kidney; Predictive Toxicology

ABSTRACT BODY: Accurate prediction of human drug toxicity is a major challenge in drug development. It is estimated that around 50% of pre-clinical *in vivo* screening of new potential drug molecules in rodents fail to predict subsequent human toxicity. Regulatory authorities have called for the development of alternative *in vitro* cell based screening assays to improve predictiviness during pre-clinical screening. Here we demonstrate that the aProximate™ *in vitro* human proximal tubule cell (hPTC) model can be used to model nephrotoxicity using KIM-1, Clusterin and NGAL as biomarkers of toxicity. hPTCs were grown to confluence on Transwell filter supports for 3 days, then exposed to either gentamycin (200 µg/ml), cisplatin (10 µM), cyclosporin A (10 µM) or methotrexate (10 µM) for up to 120 hours. Samples of the apical media were analysed for the presence of KIM1, Clusterin or NGAL using an ELISA approach. Monolayer integrity was assessed by measuring transepithelial electrical resistance (TEER). All data is expressed as mean ± SEM from 4 replicates. Exposure of hPTC cells to gentamycin resulted in a significant increase ($P < 0.001$) in the levels of clusterin (25.2 ± 1.2 v 266.3 ± 12.5 pg/ml), KIM1 (4.1 ± 1.9 v 43.8 ± 1.2 pg/ml) and NGAL (21.4 ± 0.4 v 72.9 ± 4.3 pg/ml) at 24 hours versus 120 hours exposure time. Similar results were found for cisplatin, with significant ($P < 0.001$) increases in clusterin (7.5 ± 0.9 v 287.5 ± 6.1 pg/ml), KIM1 (3.3 ± 0.6 v 18.2 ± 3.6 pg/ml) and NGAL (26.3 ± 1.2 v 73.7 ± 5.3 pg/ml) production. Likewise, exposure to cyclosporin A resulted in a significant ($P < 0.001$) increase in clusterin (9.8 ± 0.8 v 349.6. ± 10.1 pg/ml), KIM1 (2.9 ± 0.2 v 9.8 ± 0.4 pg/ml), and NGAL (14.8 ± 2.4 v 61.4 ± 2.1 pg/ml). Exposure of cells to methotrexate (10 µM) also resulted in significant increases in clusterin (11.2 ± 1. 2 v 222.7 ± 8.6 pg/ml). KIM1 (2.9 ± 0.6 v 9.5 ± 1.1 pg/ml) and NGAL (6.6 ± 0.9 v 17.7 ± 1.4 pg/ml) production. For cisplatin, cyclosporin A and methotrexate exposure, the increased biomarker levels at 120 hours exposure were accompanied by significant decreases (>60%) in TEER suggesting changes in monolayer integrity were also present. No changes in TEER values were found with exposure of the cells to gentamycin. These data suggest that aProximate™ hPTC cell monolayers express key biomarkers of toxicity and may be an excellent predictive *in vitro* model to study nephrotoxicity during drug development.

ABSTRACT FINAL ID: 3663 Poster Board: P355
TITLE: *In Vivo* Phototoxicity (photoirritation) Assay in a Pigmented Model

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: T. van Huygevoort, J. Latour, P. van Sas, K. Scase, H. Emmen. *GIT, WIL Research Europe, 's-Hertogenbosch, Netherlands.*
KEYWORDS: Pharmaceuticals; Photosafety

ABSTRACT BODY: Drugs and compounds which absorb UV-VIS light in the range of 290-700 nm and show partitioning in the skin after oral administration need to be tested for phototoxicity (ICH guideline S10 "Guidance on photosafety evaluation of pharmaceuticals"). ICH S10 states that "although non-pigmented animals are generally more sensitive to light for detecting phototoxicity, a pigmented model should be considered if a compound has a high affinity to melanin". A 3 day systemic phototoxicity model in rat is developed for skin and ocular phototoxicity since no nonclinical *in vivo* assay has been formally validated yet. Criteria will be defined for the selection of a pigmented model following the quantification of the melanin binding capacity *in vitro*. The Wistar Albino strain was used as non-pigmented model, the Brown Norway (BN) rat as fully pigmented model and the Long Evans (LE) rat as a strain comprising both highly pigmented dark skin areas and less pigmented light skin areas. Groups of 3 females were dosed orally with 100 mg/kg Sparfloxacin (SPX) for 3 consecutive days, followed by a single irradiation on Day 3 using the Oriel Solar Simulator providing artificial sunlight matching the total spectrum when the sun is at a zenith angle of 48.2°. The UVB part was attenuated in order to maximize the UVA dose which did not exceed the Minimal Erythema Dose. SPX was used as phototoxic reference substance showing high melanin affinity in the *in vitro* assay. The albino rat showed higher sensitivity to sunlight exposure which limited the maximum UVA dose. Differences in skin responses between the BN and albino rat were seen for SPX. The LE rat showed positive skin responses in the light skin areas and no responses in the dark skin areas. Ophthalmological and histopathological examination of the eyes of the LE rat did not reveal abnormalities. Both negative control groups (dosed with SPX and not exposed to sunlight or dosed with vehicle and exposed to sunlight) showed no abnormalities of the skin and eyes. The results of the eye investigations of the albino and BN rat will be presented on the poster. Overall the results suggest that with the LE rat it is not possible to induce a higher response in dark skin with a high melanin binder. Both, the fully pigmented BN and albino strain are suitable models for the assessment of phototoxicity, but additional investigations are needed to quantify the response and to correlate the *in vitro* melanin binding capacity with the *in vivo* skin accumulation of melanin binding compounds.

ABSTRACT FINAL ID: 3664 Poster Board: P356
TITLE: A Combined 16-26 Week Chronic Toxicology Study in Mice with a Surrogate Mab to Support the Development of a First-in-Class Molecule: Study Design, Operational and Technical Challenges

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KEYWORDS: Pharmaceuticals; Non-Mammalian Species; Toxicity; Chronic

ABSTRACT BODY: NI-0101 is a humanized monoclonal antibody that potently blocks Toll-like receptor 4 (TLR4) dimerization, suppressing downstream inflammatory signals. As the epitope of NI-0101 on human TLR4 resides in a hypervariable region that shows diversity between species, this antibody does not cross-react with any of the standard laboratory species. In accordance with alternative approaches for species-specific molecules outlined in ICH S6, an anti-mouse TLR4 surrogate mAb (5E3) has been developed, characterized and used for *in vivo* pharmacology and safety studies in CD-1®mice. The data generated with 5E3 were used in combination with a package of *in vitro* studies in human systems to support the entry of NI-0101 into the clinic. As NI-0101 is being developed for chronic indications, a long term treatment toxicology study was necessary. Chronic studies with intravenous dosing in mice are both technically and logistically challenging due to the number of animals involved and the skilled personnel required to perform the dosing procedure. Here, we describe the setup and results from a combination study that involved treatment of mice for either 16 or 26 weeks, at 3 dose levels, with satellite groups to assess toxicokinetic and immunotoxicology parameters. The separate sub-chronic and chronic arms allowed an interim evaluation of the data and expedited reporting via an audited interim report suitable for regulatory submission. Although challenging both for Sponsor and CRO, this strategy allows small companies to manage risk, optimize data delivery and shorten the duration of generally long studies in order to meet ambitious development timelines.

ABSTRACT FINAL ID: 3665 Poster Board: P357
TITLE: Nonclinical Safety Assessment of Ryanodex®

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: B. Chanas, M.D. Smith. *Eagle Pharamceuticals, Inc., Woodcliff Lake, NJ.*
KEYWORDS: Pharmaceuticals; Safety Pharmacology; Nanotechnology; Dantrolene

ABSTRACT BODY: Ryanodex® (dantrolene sodium, for injectable suspension) is a novel nanoparticle suspension approved for the treatment and prevention of malignant hyperthermia. Ryanodex rapidly dissolves after iv dosing. Reformulation of dantrolene sodium (a peripherally acting muscle relaxant) from a 0.3 mg/mL aqueous solution to a 50 mg/mL suspension allows for examination of any dantrolene toxicity at a higher Cmax than previously assessed. Safety pharmacology and toxicology studies were conducted to characterize the safety of Ryanodex and support the initiation of a clinical trial. The systemic hemodynamic effects were studied in anesthetized Yorkshire pigs; doses were administered as a single iv injection (10 mg/kg) or multiple injections of 2.5 to 15 mg/kg/dose every 5 min (total dose of 30, 125 or 150 mg/kg). Blood pressure, heart rate, femoral blood flow and cardiac output were monitored and anatomic pathology was examined. No treatment-related microscopic findings were observed. Doses ≤30 mg/kg had no significant effect on systemic hemodynamics. Non-adverse decreases in blood pressure and peripheral vascular resistance and increases in heart rate and blood flow were noted after 125 or 150 mg/kg and were attributed to the expected pharmacologic effects of dantrolene. In a repeat-dose study, conscious minipigs were dosed with Ryanodex for 14 days at 10, 30 or 70 mg/kg/d via bolus iv injection and with dantrolene aqueous solution (0.3 mg/mL) at 10 mg/kg/d via 1 hr iv infusion. Doses of ≤30 mg/kg/d of Ryanodex resulted in reversible and non-adverse immediate loss of muscle tone, emesis, salivation, and decreased activity; there were no significant microscopic changes. Clinical signs after dosing with dantrolene aqueous solution were limited to emesis. Due to overt clinical toxicity, Ryanodex dosing at 70 mg/kg/d was discontinued after 8 days. The NOAEL for Ryanodex was 30 mg/kg/d (AUC0-24 219 ug.hr/mL; Cmax 64.5 ug/mL). In summary, iv administration of Ryanodex demonstrated effects consistent with the well-known safety profile of dantrolene, despite the substantially higher Cmax and faster rate of administration, as compared with dantrolene sodium aqueous solution.

ABSTRACT FINAL ID: 3666 Poster Board: P358
TITLE: High Content Analysis for Prediction of Human Drug-induced Liver Injury Across Several Pharmaceutical Companies Within the Innovative Medicines Initiative MIP-DILI Consortium

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KEYWORDS: Cytotoxicity; Predictive Toxicology; Hepatic; High Content Imaging and Analysis

ABSTRACT BODY: High content analysis (HCA) for cell health is a commonly used technique in the pharmaceutical industry to assess molecular and cellular toxicological properties of small molecules, and to predict human drug induced liver injury (DILI). Several companies in the European Federation of Pharmaceutical Industries and Associations (EFPIA) routinely use HCA assays in their primary safety screens, but since the EFPIA's legacy assays are different from each other, several questions stand out. What is the general performance of the assays across companies? What are the most relevant endpoints and cell types? And perhaps most importantly; are companies making similar decisions when testing the same compounds? An HCA-focused group within the Innovative Medicines Initiative (IMI) Mechanism-based Integrated Systems for Prediction of Drug-induced Liver Injury (MIP-DILI) consortium aims to address these questions. A training set of compounds (14) and a test set of compounds (69 DILI positive and 22 DILI negative drugs) have been tested in the assays that the various companies employ, and across different cell models and endpoints, using the same test concentrations to allow comparisons. Data for the training and test set of compounds show that the various assays identify roughly half of the DILI positive compounds (~50% sensitivity), with only 10% false positives (~90% specificity). Endpoints such as mitochondrial membrane potential and oxidative stress boost predictivity compared to cell death alone. There is good correlation of results across companies and across assays with an overall scoring concordance of 85%. These results highlight the value of using HCA for cell health analysis during drug discovery, and that, despite variations in the HCA setups, pharmaceutical companies make similar decisions for the same compounds. This work forms part of the IMI MIP-DILI project through Grant Agreement number 115336.

ABSTRACT FINAL ID: 3667 Poster Board: P359
TITLE: A Systematic Assessment of Human Druggable Target Genes Identifies Absent Orthologues in Mouse and Rat

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M.A. Miller[1], P.M. Bradley[1], G.S. Baxter[1], J.E. Sidaway[2]. *[1]Instem, Conshohocken, PA; [2]Phenotox Ltd, Bollington, Cheshire, United Kingdom.*
KEYWORDS: Safety Evaluation; Bioinformatics; Pharmaceuticals; Translational Toxicology

ABSTRACT BODY: Target-mediated toxicity is a significant cause of project attrition during drug discovery. Consequently the absence of the orthologous target gene in the preclinical toxicology test species can undermine accurate risk assessment. The mouse and rat are the most commonly used species for the initial toxicological evaluation of candidate drugs. Here, we have systematically identified the absent orthologues in mouse and rat for over 3000 druggable human genes from the Drug Gene Interaction Database (DGIdb). Species orthologue absences were determined with scripts in the R and BASH languages using theoretical models and empirical sequence similarity: 1) no orthologue in Metaphors 2014 beta with consistency score >= 0.9 & tree count >= 3 AND 2) no UniProt BLAST hit with AA identity > 80% or query coverage >= 80%. DGIdb and Metaphors are consensuses of highly regarded sources including IUPHAR's GTP, DrugBank, PharmGKB, PhylomeDB, Ensembl and OrthoMCL. There were 170 absent orthologues in mouse and 172 in rat, with 134 in common to both species. Absent orthologues in mouse and rat included genes from major pharmacological target classes and several targets of current drug discovery and development programmes, for example G-protein coupled receptors (motilin receptor, melanin-concentrating hormone receptor 2, purinergic receptors P2Y 8 and P2Y11), enzymes (cholesteryl ester transfer protein, matrix metallopeptidase 26, D-amino acid oxidase activator and poly (ADP-ribose) polymerase family member 15) and ion channels (ionotropic serotonin receptors 3C and 3E). This assessment has shown that orthologues for most druggable targets are present in the mouse and rat. Species differences in similarity and expression of orthologues may still impact on pharmacological and toxicological relevant ligand interactions for some targets but this could be addressed by checking domain architecture and by incorporating gene expression data. Our approach has systematically identified the subset of druggable human target genes that is absent in the mouse and rat. This work flow would allow the selection of a more appropriate species for improved translational toxicology when evaluating candidate drugs that act on these targets. The work flow could also be used for optimised translational toxicology in other research areas such as immunotoxicity and environmental toxicity.

ABSTRACT FINAL ID: 3668 Poster Board: P360
TITLE: A Systematic Comparison of Genetic Intervention and Antibody Studies for the Immune Check Point Inhibitors Supports a Strategy for Predicting Novel Target-mediated Toxicities

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: P.M. Bradley[1], G.S. Baxter[1], H.R. Mellor[2], J.E. Sidaway[3]. *[1]Instem, Conshohocken, PA; [2]Vertex Pharmaceuticals (Europe) Ltd, Abingdon, Oxfordshire, United Kingdom; [3]Phenotox Ltd, Bollington, Cheshire, United Kingdom.*
KEYWORDS: Safety Evaluation; Bioinformatics; Biotech Products; Immune Checkpoint

ABSTRACT BODY: The immune checkpoint family are promising oncology targets for monoclonal antibody (mAb) therapy. Here we used immune checkpoint proteins as a test study to assess the value of genetic intervention data (e.g. mouse knock out and human genetic analysis studies) and systematic text mining for predicting novel target-mediated toxicities by comparing to the adverse outcome profile of antibody interventions (e.g. blockade experiments and clinical studies). A corpus of 3446 abstracts referring to cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and programmed cell death 1 (PD1) and their clinically used mAbs (ipilimumab, nivolumab and pembrolizumab) was extracted from Medline. Abstracts were text mined for sentences that asserted that the target or mAb caused an adverse event, which then was mapped to Medical Dictionary for Regulatory Activities (MedDRA) system organ level classification (SOC). Abstracts with assertions were then automatically sorted into categories relating to genetic and antibody interventions. Finally, the SOC categorisation and assertion accuracy was curated by manual review. MedDRA SOCs were consistently represented between genetic and mAb intervention studies: 13/13 for CTLA4 and 10/13 for PD1. For CTLA4, metabolism, endocrine, immune system, gastrointestinal and hepatobiliary disorders formed 86.7% of all adverse events associated with genetic interventions and 68.0 % of all mAb adverse events. For PD1, infections, immune system, cardiac, hepatobiliary, metabolism and respiratory disorders formed 69.7 % and 54.2% of genetic and mAb adverse events, respectively. The relationship between genetic and antibody interventions was less well conserved at the more descriptive adverse event level, e.g. endocrine disorders such as Grave's Disease featuring strongly for CTLA4 genetic interventions, whereas inflammation of the pituitary gland (hypophysitis) was prominent for CTLA4 mAb interventions. However, for both CTLA4 and PD1 each adverse event was clearly related to altered autoimmune function. In conclusion, systematic text mining analysis of genetic intervention studies is a useful tool for predicting novel target-mediated toxicities.

ABSTRACT FINAL ID: 3669 Poster Board: P361
TITLE: Intra and Interlaboratory Analysis of Sens-is: A 3-D Reconstituted Epidermis Based Model for Quantifying Chemical Sensitization Potency

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KEYWORDS: Rt-Pcr; Chemical Allergy; Cytokine, Signalling; Sensis; Sensitizer

ABSTRACT BODY: The mechanism behind skin sensitization and the elicitation of Allergic Contact Dermatitis (ACD) has been investigated for many years and is documented by the OECD as an Adverse Outcome Pathway (AOP) (OECD, 2012). Actually, 6 main test methods are currently used. A range of different *in vitro* chemistry-based like DPRA or GSH reactivity as well as cell-based methods like hCLAT or Keratinosens have been developed and allow evaluating the sensitization potential of cosmetic ingredients, however these methods are still limited: Metabolism as well as bioavailability of ingredients are not taken into account. The SENS-IS method classifies sensitizers according to potency categories based on the expression profiles of 61 genes, which are grouped in one gene set for irritancy and two (SENS-IS and ARE) for sensitisation (Cottrez, 2015). EpiSkin tissues (Skinethic, France) are exposed to the test substances using the protocol for determining *in vitro* skin irritation described in OECD Guideline 439. The validation of the method was carried out in two phases, a first training step of 8 ingredients tested blindly to assess transferability, followed by a blind study test on 19 ingredients to assess reproducibility and reliability. Phase I showed a very good transferability to the naïve lab and within laboratory reproducibility, leading to correct prediction of 100% for Sensitizer/Non Sensitizer hazard determination, and of 87,5% (7/8) for the determination of potency class according to ECETOC (weak, moderate, strong and extreme). Phase II was performed on 19 blindly tested chemicals (performance standards for SENS-IS). Again good results were obtained with Between laboratory reproducibility that reached the target value of 90% and reliability on both hazard and potency class determination of more than 85%. These results confirm the good transferability and reproducibility of the SENS-IS protocol and its ability to correctly classified sensitizing potency in 5 classes similarly to those of the LLNA.

ABSTRACT FINAL ID: 3670 Poster Board: P362
TITLE: Development of a Targeted Gene Array for Identification of Major Pathway Inducers

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: E.-J. Poulton, Z. Jayyosi. *Sanofi, Framingham, MA*.
KEYWORDS: Methods/Mechanism; Toxicogenomics

ABSTRACT BODY: Gene upregulation caused by compound transcription factor binding can have a number of consequences affecting the toxicity profile of a drug candidate. Drug metabolizing enzyme induction driven by AHR or PXR can lead to drug/drug interactions, NRF2 induction can be a result of oxidative stress, NFKb induction can point to an inflammatory response, or ER receptor induction can signal possible endocrine effects. Some of these effects, particularly drug metabolizing enzyme induction, are screened for during drug discovery programs, but others are assessed less routinely. We have attempted to develop an array that can monitor for the induction of multiple transcription factor driven pathways simultaneously, using reproducible and relatively high throughput methods to develop an assay that is suited for use during the early drug development process. We focused on 4 pathways; PXR/CAR, AHR, PPARa, and NRF2. Cryopreserved rat hepatocytes were used as the model system. Compounds were incubated with the rat hepatocytes for 6 or 24 hours, RNA isolated and cDNA prepared using commercially available kits before being analyzed. Treatments with compounds were performed in duplicate or triplicate, and data was statistically analyzed by comparing the fold-induction of genes in one pathway to the fold-induction of all other genes on the array. P-values were generated demonstrating if a pathway was statistically significantly up regulated. Data was also subjected to scientific scrutiny to ensure that the statistical methods represented biologically meaningful results; for example following treatment with flutamide, an AHR inducer, PPARa was statistically significantly changed with a p value <.01, however, examination of the data showed that this was due to lack of fold change for genes in this pathway, leading to a statistically significant result that was not biologically meaningful. We were able to correctly identify positive controls for all pathways with P values of <.05, including WY4896 and fenofibrate as PPAR inducers, flutamide as an AHR inducer, CPDT and D3T as NRF2 inducers, and PCN as PXR/CAR inducer. Further validation efforts are underway. Additional work will study *in vivo* samples and human hepatocytes.

ABSTRACT FINAL ID: 3671 Poster Board: P363
TITLE: Can *In Vivo* Preclinical Toxicity be Predicted Using the Glu/Gal Mitochondrial Assay?

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KEYWORDS: Safety Evaluation; Pharmaceuticals; *In Vitro* and Alternatives; Mitochondria

ABSTRACT BODY: Mitochondria are the powerhouse of almost all mammalian cells, making them crucial for many cellular processes. Many drugs withdrawn from the market, or with labelling restrictions imposed, have been shown to disrupt mitochondria. These converging observations have resulted in years of research yielding many mitochondrial assays, several of which are utilised by pharmaceutical companies today. Despite a wealth of *in vitro* data, information linking *in vitro* mitochondrial disruption with (pre)clinical exposures, and toxicological outcome, is limited. This data gap makes it difficult to quantify mitochondrial risk pre-clinically, meaning that compounds are either discarded unnecessarily during the discovery phase or enter clinical development with inherent mitochondrial liabilities which might contribute to sub-optimal therapeutic windows and late stage attrition. AstraZeneca employ a high-throughput mitochondrial "Glu/Gal" toxicity screen in the discovery phase (>6,700 compounds analysed to date). We cross referenced this dataset with our preclinical toxicology database to identify all *in vitro* mitochondrial toxic compounds where preclinical *in vivo* toxicology data existed. We assessed *in vitro* mitochondrial toxicity potency (Gal IC50), *in vivo* dose-limiting toxicity (DLT) and Cmax to evaluate whether *in vitro* Gal IC50 had the potential to predict the Cmax at which significant toxicity might be observed *in vivo*. Cytotoxic compounds were excluded from the analysis (THP1 cell viability assay). A key finding of our analysis was that, when Cmax exceeded Gal IC50, no difference was observed in the number of groups where significant toxicity was recorded compared with No Adverse Effect Level (NO(A)EL) groups. A greater proportion of toxic groups were expected in this cohort. Interestingly, where Cmax was below the Gal IC50, significantly more NO(A)EL groups were observed than toxic groups, warranting further investigation on a larger set of compounds. Although we were unable to predict the Cmax at which DLT was observed *in vivo* for these compounds using our Glu/Gal data, predictivity might be improved by combining data from multiple assays. Cross-pharma data sharing might provide the data required to determine the most appropriate panel of assays and endpoints, as well as enabling use of existing (pre)clinical data sets, required to build our translational understanding of mitochondrial toxicity.

ABSTRACT FINAL ID: 3672 Poster Board: P364
TITLE: Analysis of Solvents Used in Preclinical Oral Toxicity Studies for Drug Candidates

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KEYWORDS: Pharmaceuticals; Toxicity; Acute; Formulations

ABSTRACT BODY: The selection of the vehicle employed in preclinical animal oral toxicology studies can be a daunting task to the toxicologist when developing new drug candidates, particularly when there is limited information available on vehicle/drug interactions. Questions may be asked in determining the choice of vehicle would include: which vehicle would best formulate the proposed test material, what concentration should be used, and should the vehicles be used in combination or alone. Based on the above questions, vehicles from 308 recently conducted preclinical oral toxicity studies at our facility were investigated. Animal species included monkey, dog, rabbit, pig, rat, guinea pig, and mouse; study durations ranged from single dose to 39-week repeated dose studies. A total of 24 vehicles (not including water) were used in the 308 studies, and the results of the survey showed that 1 or 2 vehicle components (or water only) were usually sufficient for formulation preparation (96.43% studies). The most preferred vehicle for oral administration was 0.5% (ranging from 0.1% to 4% concentration) methylcellulose or its derivatives (sodium carboxymethylcellulose and hydroxy propyl methyl cellulose) (56% of the vehicles used). Other vehicles in the order of preference included 20% (ranging from 10% to 40% concentration) cyclodextrin derivatives (Hydroxypropyl-β-Cyclodextrin and sulfobutylether-β-cyclodextrin), 10% polyethylene glycol (ranging from 10% to 60% concentration), 0.2% Tween 80 (ranging from 0.1% to 5% concentration), 0.1% sodium-dodecyl sulphate (ranging from 0.1% to 0.5% concentration), and 20% solutol (ranging from 5% to 20% concentrations). Tween 80 and sodium-dodecyl sulphate were usually used as solubilizers and generally in combination with other vehicle components. The preference for 0.5% methylcellulose as attributed to its ability to produce homogeneous and stable formulations/suspension over a wide variety of test materials; was used in low concentration; and produced no adverse side effects. All of the 24 vehicles used were well tolerated. As a general use vehicle for oral administration, when no other vehicle information is available, 0.5% methylcellulose is recommended, and 0.2% Tween 80 could be added to increase the solubility and bioavailability when necessary or changed to use 20% cyclodextrin derivatives or 10% polyethylene glycol. Such information will hopefully aid the toxicologist in selecting the appropriate vehicle/constituents for their test materials.

ABSTRACT FINAL ID: 3673 Poster Board: P365
TITLE: *In Vitro* Assessment of the Mixture Toxicity of West Virginia Chemical Spill Compounds, 4-Methyl Cyclohexane Methanol and Propylene Glycol Phenyl Ether

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: A. Han, E. Fabyanic, M. Prediger, J. Boyd. *West Virginia University, Morgantown, WV.*
KEYWORDS: Exposure Assessment

ABSTRACT BODY: Gallons of industrial chemicals, crude 4-methylcyclohexane methanol (MCHM) and propylene glycol phenyl ether (PPH), leaked from industrial tanks into the Elk River in Charleston, West Virginia in 2014. Consequently, a considerable number of people were reported to exhibit symptoms of chemical exposure and an estimated 300,000 residents were advised not to use or drink water for several weeks. At the time of the chemical spill, the existing toxicological data of the involved chemicals were insufficient for appropriate evaluation of the health risks. Since then, a considerable number of studies regarding MCHM and PPH toxicity have been published and released to provide additional information to a limited toxicological data set. There is, however, a gap in understanding the mixture effects and potential additive toxicity of the two chemicals. In this study, we analyzed cell viability, following a 24 hour co-exposure to varying concentrations (0-1000 μM) in an effort to assess the additive effects of the mixture. Individually, cell viability of the observed cell lines, HEK 293 (human-derived kidney), HepG2 (human-derived liver), H9C2 (rat-derived heart), and GT17 (mouse-derived brain), was not reduced for any cell lines except at the highest dose of MCHM (1000 μM), while PPH had no significant effect across all doses measured. Exposures that included binary combinations of MCHM and PPH further decreased cell viability, as compared to single exposures, in GT17, HEK293, and H9C2 cells. Co-exposures in HEPG2 cells did not elicit any differential toxicity when compared to individual compounds. Theoretical cell viability of mixture exposures, calculated by Bliss Independence criterion, was significantly higher than the observed viability for select MCHM and PPH combinations in GT17, HEK293 and H9C2 cells, indicating an additive, or greater than additive, decrease in cell viability due to mixture exposures. These findings stress the importance of examining co-exposures in order to fully understand the potential toxic effects.

ABSTRACT FINAL ID: 3674 Poster Board: P366
TITLE: Neuroproteomic Profiling of the Sleep-Restricted Aged Female Rat after Ischemic Stroke Using iTRAQ LC-MS/MS

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KEYWORDS: Proteomics; Epidemiology; Shift Work

ABSTRACT BODY: The economic and social demands of a 24 h society has many benefits, including work schedule flexibility and increasing employment opportunities. However, the need for 24 h staffing has resulted in deleterious health effects. Shift work is a risk factor for stroke as well as other circulatory diseases and is known to interfere with circadian rhythms, sleep quality and duration. Alterations in the sleep-wake cycle, such as sleep restriction, have been shown to alter the biological response to immune or toxicant challenge. While the relationship between shift work, sleep, and disease has been a recent focus in epidemiological and surveillance studies, limited characterization of both the shift work and sleep variables in these studies has hampered our understanding of how shift work influences stroke. Utilizing well controlled and characterized animal studies of ischemic stroke and sleep restriction can expand our understanding. Approximately 80% of strokes are ischemic with age being a key factor in patient recovery. Most result from middle cerebral artery occlusion (MCAO) causing damage to striatum and cortex resulting in long-term disability. Here we use our well-characterized stroke model in aged female rats subjected to repeated daily sleep restriction (8 h/day) or normal sleep for the 6 days prior to MCAO to better characterize how chronic sleep restriction exposure impacts stroke. To this end we analyzed the proteomic profile of cortex and striatum at 2 and 12 h following occlusion via isobaric tag for relative and absolute quantification (iTRAQ) with liquid chromatography-tandem mass spectrometry (LC-MS/MS). Our proteomic analysis identified several key proteins altered by ischemia in a time-related fashion as well as by repeated sleep restriction exposure. Upon further pathway analysis, many of the identified proteins (e.g., ATP5a1, CKB, DPYSL2, ENO1/2, GAPDH, and MDH1/2) are involved in glycolysis, metabolism, cell death, and transport. This proof of concept study opens the door to include sleep restriction as an exposure component for future studies concerning workplace exposure toxicity.

ABSTRACT FINAL ID: 3675 Poster Board: P367
TITLE: Effect of Different Concentrations of Sodium Arsenite on Expression of MRP2 and MRP4 in Rat Liver

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: X. Shen[1], Y. Zhou[1], Y. Li[1], Y. Zou[1], W. Zheng[2] .*[1]School of Public Health, Zunyi Medcial University, Zunyi, China; [2]School of Health Science, Purdue University, West Lafayette, IN.*
KEYWORDS: Exposure, Environmental; Liver

ABSTRACT BODY: Arsenic(As) is an environmental toxicant. Upon exposure, liver is the predominant site for methylation of inorganic arsenic, followed by biliary and urinary excretion. Multidrug resistance protein(MRPs) is an important transport protein in the liver cells. Previous studies have indicated that MRP2 and MRP4 play an important role in excretion of arsenic and its derivatives. The present study was designed to investigate the effect of sodium arsenite exposure on the liver function in rats, as well as the expression of MRP2 and MRP4 in liver. Adult SD rats (n=10 per group) received sodium arsenite(NaAsO2) by oral gavage once daily for 30 days, at the dose of 2.2, 6.7 and 20 mg/kg as the low-, mid-, and high- dose groups, respectively. The control group received the equal volume of distilled water. Data showed that As exposure significantly reduced the body weight and liver weight only in the high exposure group($p<0.05$).The concentrations of total As in serumand liver were increased by 19.7-69 fold and 3-9.4 fold, respectively as compared to controls ($p< 0.001$).Quantitative RT-PCR showed an increased expression of mRNAs encoding MRP2 and MRP4 in the liver of As-exposed rats in comparison to controls. Further studies using immunohistochemical analysis also confirmed a significantly higher expression of MRP2and MRP4 proteins in As-exposed liver than the controls($p<0.01$). These results indicate that oral exposure to sodium arsenite causes arsenic accumulation in hepatocytes and results in liver injury. This could be due to an altered expression of MRP2 and MRP4 in liver, which may distort the excretion of arsenic species from the liver and increase arsenic retention in the body.

ABSTRACT FINAL ID: 3676 Poster Board: P368
TITLE: Development of an Acute Renal Safety Model in Sprague Dawley Rats for Relative Evaluation of Platinum-based Therapeutics

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M.A. Abernathy[1], E. Goka[2], C. Carter[3], A. Aulbach[3], T. Baird[1]. *[1]Safety Pharmacology and Neurobehavioral Sciences, MPI Research, Mattawan, MI; [2]Geneyus, LLC., Miami, FL; [3]Clinical Pathology, MPI Research, Mattawan, MI.*
KEYWORDS: Renal; Pharmaceuticals; Methods/Mechanism; Cisplatin

ABSTRACT BODY: ICH guidance S7A outlines considerations for the conduct of safety pharmacology studies, which are typically performed utilizing a range of acute doses of an experimental compound to characterize potential off-target, adverse pharmacology. When conducting safety studies, ICH S7A suggests the use of positive control treatments to demonstrate the sensitivity of the model. The objectives of this experiment were to identify the optimal timing and magnitude of effects on serum and urinary endpoints following a single dose of Cisplatin in male Sprague Dawley rats against which alternative platinum based agents could be evaluated for relative safety. Twelve 8-week old male Sprague Dawley rats were equally divided into two groups receiving intravenous injections of either saline or 6 mg/kg Cisplatin. Paired serum and urine samples were collected for chemistry evaluations, including, but not limited to, urine volume, fractional excretion of electrolytes in urine, urine proteins, urine pH, urine glucose concentration, urinary markers of tubular injury (N-acetyl-beta-D-glucosaminidase (NAG) and Gamma-glutamyl transpeptidase (GGTs), and serum creatinine and urea nitrogen at 2, 4, and 6 days following cisplatin administration. As expected, Cisplatin administration produced signs of functional deficits relating to progressive renal tubule injury characterized by a maximal increase in serum urea nitrogen and creatinine of 255% and 194% at 6 days following administration, respectively. Urinary GGT increased by 166% at 4 days following dosing but began to decrease by day six. Urinary NAG concentrations progressively increased following dosing and reached 167% of control values at 6 days following dosing. The greatest effects were observed in urine quantitative glucose values which increased to 6987% of controls at 6 days following dosing. This experiment suggests that single dose renal safety studies using platinum-based chemotherapeutic should be at least six days in duration so as to allow for detection of the largest toxicological impact. Additionally, this experiment demonstrated the suitability of the selected serum and urinary markers for detection of Cisplatin-induced renal toxicity in a range suitable to allow characterization of comparator platinum-based therapeutics.

ABSTRACT FINAL ID: 3677 Poster Board: P369
TITLE: Evaluation of Three Alternative Surgical Placements for a Minimally Invasive Telemetry Device Implant in the Cynomolgus Monkey

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: T.J. Baird, D. Holdsworth, J. Gesaman, K. O'Donohue, M. Abernathy, D. Gauvin, J. Dalton, C. Papagiannis. *MPI Research, Mattawan, MI.*
KEYWORDS: Safety Pharmacology; Safety Evaluation; Cardiovascular System

ABSTRACT BODY: The use of jacketed external telemetry (e.g., DSI-JET™) monitoring of the ECG in combination with use of a minimally invasive blood pressure telemetry implant to allow continuous recording of arterial blood pressure has been described in relative detail in recent years, particularly in the context of support for high quality cardiovascular data collection as part of repeated dose drug safety studies. Potential problems associated with surgery are related to differential recovery or development of post-surgical complications, which can be dependent upon particular circumstances of the surgical procedure employed. This study investigated three alternative surgical placements (femoral artery catheterization with subcutaneous transmitter placement, femoral artery catheterization with sub-muscular transmitter placement, and iliac artery catheterization with intraperitoneal transmitter placement) of the DSI TA11PA-C10-TOX-SA telemetry unit in Cynomolgus monkeys in an attempt to understand possible differential outcomes and potential best practices from the standpoint of surgical recovery, data quality, and device longevity *in vivo*. When data collected periodically over 4 months post-surgery were analyzed, there were no differences in qualitative aspects of surgical site observations or absolute duration of healing time associated with each technique, though surgical procedure-related differences in data quality and device longevity were observed. The most stable implant procedure involved the internal iliac artery pressure catheter placement, which evidenced higher overall pulse height and less deviation over time in absolute (systolic, diastolic, and mean) arterial pressure determinations. Although the implications of these data with regards to preferred surgical methodology depend upon individual study circumstances, there are clear indications that implant methods resulting in a relatively more static position of the telemetry device are associated with better overall outcomes in terms of data quality and longevity of *in vivo* device functionality.

ABSTRACT FINAL ID: 3678 Poster Board: P370
TITLE: The Irwin Test: A CNS Comparison of Individual vs. Social-Housed Rats

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M.S. Coffee. *WIL Research, Ashland, OH.*
KEYWORDS: Safety Evaluation; Safety Pharmacology

ABSTRACT BODY: The purpose of safety pharmacology is to investigate effects of the test substance on vital functions, including CNS. ICH S7A guideline (2001) specifies that the effects of test substance on motor activity, behavioral changes, coordination, sensory/motor reflex responses and body temperature should be evaluated through the use of a modified Irwin, or other appropriate test. In 2011, the Guide for the Care and Use of Laboratory Animals (8th Edition) stated that members of a social species should be socially housed whenever possible, including rodent species. WIL Research adopted this policy for rodent Irwin studies conducted to meet the ICH S7A guideline in 2014. Recent studies have evaluated space needs and the effects of social housing, group size, density, age, and housing conditions for rodents, and have reported varying effects on behavior (such as aggression) and experimental outcomes. Based on this work, an effort was conducted at WIL Research to determine the differences in behavior (CNS effects) when comparing results from single-housed vs. social-housed animals. Male Sprague Dawley rats were individually or socially housed. Animals were orally dosed with vehicle or positive control (Chlorpromazine at 5 mg/kg). Irwin assessments were performed prior to dosing and at 30, 90, 150, and 300 minutes post-dosing. Data (individually-housed and socially-housed) were compiled from the vehicle and positive control results of multiple client and in-house studies, and are part of the WIL Research Historical Control Database. All observations were performed blind to dose designations to eliminate any potential observer bias. The vehicle and positive control data were then compiled and presented as a heat graph. One main difference noted between housing conditions was socially-housed animals exhibited less CNS behaviors than individually-housed rats. Most notably, paralysis, abnormal body carriage, loss of righting reflex, decreased corneal reflex, and vocalization were not observed in the socially-housed animals and were predominant in the individually-housed animals. Based on this and comparing to the vehicle response, this was thought to be related to the more satisfying housing arrangements social housing affords. Our conclusions indicate that social housing animals for the Irwin test does not hinder the quality of data. Instead, it enhances the natural state of the rodents, creating a more valuable environment for neurological assessments.

ABSTRACT FINAL ID: 3679 Poster Board: P371
TITLE: Assessing Potential Herb-Drug Interactions Using a Common Framework Approach: Boswellia serrata as a Case Study

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KEYWORDS: Natural Products; Disposition; Cytochrome P450; Herb-Drug Interactions; Boswellia Serrata

ABSTRACT BODY: As the use of dietary supplements increases, herb-drug interaction (HDI) potential should be evaluated using a systematic approach. Considerations such as history of safe use, literature data, and extract/constituent characterization may guide whether to assess HDIs experimentally. Boswellia serrata extract (BSE) is traditionally used for inflammatory diseases (e.g., arthritis). Potent *in vitro* inhibition (≥65%) across major drug metabolizing enzymes (e.g, CYP3A4/5, CYP2C9) using pooled human liver microsomes (PHLM) have been reported in the literature for levels of BSE. This potent inhibition contrasted with a history of safe use of BSE, led us to question the relevance of the results using PHLM. We chose to study the *in vitro* inhibition potential of BSE against CYP3A4/5 and CYP2C9, using a sandwich-cultured human hepatocyte (SCHH) system, and compare generated IC50 values between SCHH and PHLM. In SCHH, direct inhibition of CYP3A4/5 by BSE was observed resulting in an IC50 = 17.2 µg/mL, versus IC50 = 1.4 µg/mL with PHLM. IC50 values for CYP2C9 inhibition by BSE were >75 µg/mL versus 11 µg/mL using SCHH or PHLM; respectively. Analytical characterization of BSE used in these *in vitro* studies has been conducted which includes identification of the major boswellic acids. Further work is underway to analyze SCHH cell lysates to determine intracellular concentrations of key boswellic acids (e.g., β-boswellic acid and 11-Keto-β-boswellic acid). An overall prediction of HDI relevant to BSE used in dietary supplement products may be further defined by combining data from *in vitro* studies, analytical characterization of extracts, published clinical data, and product formulation considerations. In summary, our proposed integrated and sophisticated approach for assessing HDI potential of dietary supplement ingredients can be consistently applied across herbal extracts.

ABSTRACT FINAL ID: 3680 Poster Board: P372
TITLE: Pharmacokinetics of Oseltamivir Across the Pregnancy Stages of Rhesus Monkeys

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: L. Loukotkova[1], A. Lumen[1], D. Williams[1], M. Basavarajappa[1], J.J. Chen[1], R. Roberts[2], X. Yang[1], M.G. Paule[1], W. Slikker[1], D. Mattison[3], S.M. Morris[1], J. Fisher[1], F.A. Beland[1], G. Gamboa da Costa[1]. *[1]US FDA/National Center for Toxicological Research, Jeffereson, AR; [2]US FDA/Center for Drug Evaluation and Research, Silver Spring, MD; [3]Risk Sciences International, Ottawa, ON, Canada.*
KEYWORDS: Pharmacokinetics; Rhesus Monkey; Oseltamivir

ABSTRACT BODY: Oseltamivir is an antiviral drug approved to treat influenza in humans. Although the dosing regimen for this drug is well-established for non-pregnant patients, it is not clear if the significant physiological alterations associated with pregnancy affect the pharmacokinetics of oseltamivir and, thus, warrant different dosing regimens to assure efficacy. In order to clarify this matter, we compared the pharmacokinetics of oseltamivir and its pharmacologically-active metabolite oseltamivir carboxylate in rhesus monkeys (n=8) after oral and intravenous (IV) administration of 2.5 mg/kg BW of oseltamivir given prior to and during the 1st, 2nd, and 3rd trimesters of pregnancy. We report here a summary of the initial key findings. Considerable biological variability among the monkeys was observed at each stage of pregnancy, probably reflecting the genetic variability of this outbred animal model. Compared to the nonpregnant condition, the clearance rate (CL) of oseltamivir was decreased by 35-57% ($p < 0.05$) and the AUC was increased by 58-161% ($p < 0.05$) in the 3rd trimester in animals treated IV or orally. The AUC of oseltamivir carboxylate was reduced 20-25 % ($p < 0.05$) during all three trimesters of pregnancy in animals dosed IV. The elimination half-lives of oseltamivir and its metabolite were not affected by pregnancy for either mode of administration; likewise, the bioavailability of oseltamivir was not affected by pregnancy. Physiologically-based pharmacokinetic modeling is currently being conducted to assess the significance of these pharmacokinetic alterations as they may pertain to humans.

ABSTRACT FINAL ID: 3681 Poster Board: P373
TITLE: P-glycoprotein Induction by Newly Synthetized (thio)xanthones Prevents Paraquat Cytotoxicity

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: R. Silva[1], A. Palmeira[2], H. Carmo[1], D. Barbosa[1], M. Gameiro[1], E. Sousa[2], M. Pinto[2], M. Lourdes Bastos[1], F. Remião[1]. *[1]Biological Sciences - Lab Toxicology, Faculty of Pharmacy - University of Porto, Porto, Portugal; [2]Chemical Sciences - Lab of Organic and Pharmaceutical Sciences, Faculty of Pharmacy - University of Porto, Porto, Portugal.*
KEYWORDS: Xenobiotic Transporters; Pesticides; Disposition

ABSTRACT BODY: In this presentation, the induction and/or activation of P-glycoprotein (P-gp) for limiting the toxicity caused by the substrates of this efflux pump is presented. Thus, the aim of this study was to screen ten newly synthetized (thio)xanthonic derivatives, a group known to interact with P-gp, as potential inducers of the pump´s expression and/or activity, and to evaluate whether they would afford protection against Paraquat (PQ)-induced toxicity in Caco-2 cells. An additional study was performed *in vivo* to evaluate effects in digoxin disposition. All tested (thio)xanthones (20 µM) caused a significant increase in both P-gp expression and activity, as evaluated by flow cytometry using the UIC2 antibody and rhodamine 123, respectively. Additionally, it was demonstrated that the tested compounds rapidly caused an activation of P-gp. Moreover, when simultaneously incubated with PQ, nine of the tested compounds significantly reduced the cytotoxicity of the herbicide, and these protective effects were reversed upon incubation with a specific P-gp inhibitor. *In silico* studies evaluating the interactions between (thio)xanthones and P-gp in the presence of PQ suggested that a co-transport mechanism may be operating. Based on the *in vitro* activation results, a pharmacophore model for P-gp activation was built. Also, a QSAR model was developed and validated, and the maximal partial charge for an oxygen atom was the descriptor predicted as being implicated in P-gp activation by the dihydroxylated xanthones. *In vivo*, the most potent P-gp activator, TX5, was tested to evaluate its effect on the pharmacokinetic of digoxin, a known P-gp substrate. Simultaneous administration of TX5 (30 mg/kg) and digoxin (0.25 mg/kg) per os resulted in a significant reduction on digoxin intestinal absorption, with the consequent reduction in its AUC. In conclusion, these studies demonstrate that effective antidotal pathways can be achieved by efficiently promoting the P-gp-mediated efflux of deleterious xenobiotics, resulting in a significant reduction in their intracellular levels and, consequently, in a significant reduction of their toxicity.

ABSTRACT FINAL ID: 3682 Poster Board: P374
TITLE: Lactational Transfer of Fentanyl Citrate in Rats

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KEYWORDS: Pharmaceuticals; Toxicokinetics; Developmental Toxicity; Post-Natal; Lactational Transfer; Fentanyl Citrate

ABSTRACT BODY: In this study we determined plasma and milk concentrations of fentanyl and its major metabolite norfentanyl in rats following treatment with fentanyl citrate during pregnancy and lactation. A control and two treatment groups of 12 time-mated female CD® [Crl:CD®(SD)] rats/group received once-daily subcutaneous (S.C.) doses at respective dose levels of fentanyl free base of 0, 25, and 50 µg/kg/day at a dose volume of 1 mL/kg from Gestation Day 6 through Lactation Day (LD) 20. Blood samples for determination of the plasma concentrations of fentanyl were collected at 0.25, 0.5., 1, 2, 3, 8, and 24 h on LD 12 and 20. Likewise, milk was collected 3-4 h postdose on LD 12 and 20 for analysis. Following once-daily S.C. administration of nominal doses of 25 or 50 µg/kg of fentanyl as fentanyl citrate, peak plasma concentrations of fentanyl on LD 12 were achieved at approximately 30 minutes after dosing. Systemic exposure to fentanyl increased in a dose-related manner, with composite C_{max} values of 8695 and 21,822 pg/mL for the 25- and 50-µg/kg/day dose groups, respectively, and composite AUC_{0-∞} values of 16,366 and 37,899 pg·h/mL, respectively. The peak plasma concentrations of norfentanyl on LD12 were achieved at approximately 2 hours after dosing. Systemic exposure to norfentanyl also increased in a dose-related manner, with composite C_{max} values of 1376 and 2348 pg/mL for the 25- and 50-µg/kg/day dose groups, respectively., and composite AUC_{0-∞} values of 6117 and 11,936 pg·h/mL, respectively. Fentanyl and norfentanyl were detected in milk samples and were several fold higher than respective concentrations in maternal plasma; hence, nursing pups would received greater fentanyl exposure via the milk when treated during lactation.

ABSTRACT FINAL ID: 3683 Poster Board: P375
TITLE: Developmental PFOA Exposure Causes Morphological and Transcriptomic Alterations in Zebrafish

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: K. Thompson[1], S. Wirbisky[1], L. Lee[2], M. Sepulveda[2], J. Freeman[1]. *[1]School of Health Sciences, Purdue University, West Lafayette, IN; [2]Purdue University, West Lafayette, IN.*
KEYWORDS: Perfluoronated Agents; Exposure, Environmental

ABSTRACT BODY: Perfluorooctanoic Acid (PFOA; CF3(CF2)6COOH), a fully fluorinated eight-carbon carboxylic acid chain used in industry, is emerging as a persistent environmental contaminant. PFOA is ubiquitously detected worldwide in the environment as well as the general human population. Human exposure may result from contact with contaminated drinking water, food, fire-fighting foam, or consumer products. Within those highly exposed, epidemiology studies report that serum PFOA levels display positive associations with kidney and testicular cancers, which led the US EPA to state that the evidence of PFOA is suggestive of carcinogenicity. Also, PFOA has been detected in human cord samples and breast milk, indicating probable maternal transfer to child. Although several companies are voluntarily working with the US EPA to eliminate the use of PFOA, exposure is still occurring, and developmental toxicity is limited. The zebrafish model is ideal for developmental and genetic toxicity studies. We hypothesized that developmental exposure to PFOA will alter morphology and gene expression in zebrafish. Zebrafish were exposed to control or 4, 40, or 400 ppb of PFOA throughout embryonic development from 1-72 hours post fertilization (hpf). Morphology measurements of body length, head length, and head width were recorded of zebrafish larvae, and significant differences were found to occur at $p<0.05$ for all measurement types. A custom zebrafish microarray platform was then used to determine global gene expression profiles. The number of human homolog genes altered were 26, 66, and 55 in the 4, 40, and 400 ppb PFOA treatments, respectively. Categories of gene function and diseases were identified for altered genes using Ingenuity Pathway Analysis. Cancer was the top disease identified among the treatments. Pathway analysis revealed a correspondence with findings of current literature. Our findings will further the current understanding of the effects caused by PFOA developmental exposure as well as its mechanisms of action.

ABSTRACT FINAL ID: 3684 Poster Board: P376
TITLE: Placental Nitrosative Stress and Exposure to Ambient Air Pollution During Gestation: A Population Study

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: H.A.C. Roels, N.D. Saenen, K. Vrijens, B.G. Janssen, N. Madhloum, M. Peusens, W. Gyselaers, T.S. Nawrot. *Centre for Environmental Sciences, Hasselt University, 3950 Diepenbeek, Belgium.*
KEYWORDS: Developmental Toxicity; Prenatal; Exposure, Environmental; Epidemiology; 3-Nitrotyrosine

ABSTRACT BODY: The placenta plays a crucial role in fetal growth and development through adaptive responses to perturbations of the maternal environment. We investigated the association between placental 3-nitrotyrosine (3-NTp), a biomarker of oxidative stress, and exposure to air pollutants during various time windows of pregnancy. We measured placental 3-NTp of 330 mother-newborn pairs, enrolled in the ENVIRONAGE birth cohort (2010 to 2013). Daily particulate matter ≤ 2.5 μm (PM2.5), black carbon (BC), and nitrogen dioxide (NO2) concentrations were interpolated for each mother's residence using a spatiotemporal interpolation method. Placental 3-NTp levels, adjusted for covariates, increased by 35.0% (95% confidence interval (CI): 13.9, 60.0%) for an interquartile range increment in entire pregnancy PM2.5 exposure. The corresponding estimate for BC exposure was 13.9% (95% CI: -0.21, 29.9%). These results were driven by the first [PM2.5: 29.0% (95% CI: 4.9, 58.6%); BC: 23.6% (95% CI: 4.4, 46.4%)] and second gestational exposure window [PM2.5: 39.3% (95% CI: 12.3, 72.7)]. This link between placental nitrosative stress and exposure to fine particle air pollution during gestation is in line with experimental evidence on cigarette smoke and diesel exhaust exposure. Further research is needed to elucidate potential health consequences later in life through particle-mediated nitrosative stress during fetal life.

ABSTRACT FINAL ID: 3685 Poster Board: P377
TITLE: Postnatal Development of Hematopoietic System in Rats

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J.F. Figueiredo, M. Mattix, T.L. Papenfuss. *WIL Research, Ashland, OH.*
KEYWORDS: Liver; Hematopoiesis, Bone Marrow, Spleen

ABSTRACT BODY: The hematopoetic sites vary during the postnatal period in rats as most tissues are not fully developed at birth. The aim of this study was to describe postnatal hematopoiesis in rats, including intramedullary and extramedullary, from PND 0 to 42. To characterize the developmental changes in the hematopoietic system of juvenile rats, bone marrow, liver and spleen from male and female Sprague Dawley rats on postnatal days 1 through 42 were collected, processed, paraffin-embedded, stained with hematoxylin and eosin, and examined microscopically. Additionally, bone marrow smears of sternum and femur were performed and the slides were stained with modified Wright-Giemsa. Relevant hematopoietic changes in the bone marrow, spleen and liver histologic sections were described. The myeloid to erythroid ratio and the proportion of lymphoid population were estimated by evaluating the bone marrow smears. Selected changes included gradually decreased hepatic hematopoiesis from PND 0 through PND 28, marked splenic hematopoiesis from PND 0 to 7 and minimal bone marrow hematopoiesis at PND 0 and 1. Our findings indicate a physiological change in the hematopoietic organs in rats during the postnatal period that needs to be considered when interpreting toxicologic studies.

ABSTRACT FINAL ID: 3686 Poster Board: P378
TITLE: Utility of Juvenile Animal Studies: CDER's Experience

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: I. Khan, F. Basso, D. Fegley, P. Espandiari, T. Mcgovern, B. Hayes, I. Elayan, E. Fisher, E. Hausner, O. Dina, C. Wrzesinski, A. Ravindran, A. Bourgois, M. Tassinari, P. Brown, K. Davis Bruno. *US Food and Drug Administration, Silver Spring, MD.*
KEYWORDS: Juvenile Toxicity; Regulatory/Policy

ABSTRACT BODY: An initial assessment of the utility and appropriateness of juvenile animal studies (JAS) in drug and biologics development was addressed through a review of internal JAS data submitted to CDER between 2009 and 2014. The rationale for requesting the JAS and endpoints (EP), as well as whether the study results had a regulatory impact, were evaluated. The predictive role of previously available data from adult animal toxicity studies, clinical data, or the pharmacology of the drug (both primary and secondary) on JAS outcome was also evaluated. Among ~400 applications, 81 did not require studies, while 281 required studies based on prior data and uncertainty. The agency reviewed 107 out of the 281 studies that also included 25 sponsor initiated studies (not requested by the Agency). The majority had central nervous system, gastrointestinal, cardiovascular, or endocrine indications across CDER review divisions. Most studies requested EP for General toxicity (GT), developmental landmarks (DL), neurodevelopment (ND), bone, and reproductive toxicity (RT). Positive findings were identified in 75% of studies out of which ~70% showed unique toxicity or increased sensitivity in juvenile animals compared to the adult. Drug related toxicity with regulatory impact was observed with bone, DL, ND, GT, and RT end points. Seventy two percent of studies correlated with regulatory outcomes in NDAs, while 38% correlated with safety outcomes in INDs. Prior adult animal toxicity data, human data, and pharmacology of the drug correlated with JAS outcome in 50%, 30%, and 61% of studies, respectively whether prior data or uncertainty was the rationale for EP selection. Overall, ND, GT, DL and bone are EPs that correlated with identification of new toxicities and/or increased sensitivities in juvenile animals and they also led to safety and/or regulatory outcomes. Prior toxicity signals in adult animals, clinical data and pharmacology may associate with a positive drug related toxicity response in a JAS.

ABSTRACT FINAL ID: 3687 Poster Board: P379
TITLE: Inhibition of Endocannabinoid Metabolizing Enzymes in Peripheral Tissues following Developmental Chlorpyrifos Exposure

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: R.W. Buntyn, R.L. Hybart, C.A. Nail, M.K. Ross, G.C. Parker, R.L. Carr.
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KEYWORDS: Pesticides; Juvenile Toxicity; Undergraduate Student; Chlorpyrifos

ABSTRACT BODY: We previously reported that developmental exposure to low levels of the organophosphate insecticide chlorpyrifos (CPF) inhibits brain fatty acid hydrolase (FAAH) activity. At slightly higher exposure levels, CPF can also inhibit brain monoacylglycerol lipase (MAGL) activity. FAAH and MAGL are responsible for the hydrolysis of the endocannabinoids anandamide (AEA) and 2-arachidonylglycerol (2-AG), respectively. AEA and 2-AG have physiological roles in peripheral tissues, including promotion of fat accumulation and antiinflammatory effects. However, it is not clear if repeated exposure to low levels of CPF will alter the hydrolysis of these endocannabinoids in peripheral tissues. The goal of the present study was to investigate the effects of developmental exposure of rats to low levels of CPF on endocannabinoid metabolism in the spleen and liver and compare to that in brain. Ten day old rat pups were exposed orally to either 0.5, 0.75, or 1.0 mg/kg CPF daily for 7 days. At 12 hrs post-exposure, the activities of MAGL and FAAH were determined. For the three dosages of CPF, FAAH activity was inhibited in a dose dependent manner [% inhibition: liver (52%, 74%, and 83%, respectively), brain (28%, 47%, and 66%) and spleen (30%, 47%, and 57%)]. Thus, FAAH activity in liver was more potently inhibited than that in brain and spleen. For the three dosages of CPF, the inhibition of MAGL was only significant for the highest dosage in liver (31%). These data suggest that in addition to impacting brain endocannabinoid metabolism, low level CPF exposure might also affect endocannabinoid metabolism in the liver and spleen. Funded by NIH R15ES023162 and R15ES015348-02.

ABSTRACT FINAL ID: 3688 Poster Board: P380
TITLE: The Comparative Evaluation of Gonadotoxic Activity of Two λ-cyhalothrin Generics

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M. Prodanchuk, G. Prodanchuk, Y. Kolyanchuk. *L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety, Kyiv, Ukraine.*
KEYWORDS: Pesticides; Reproductive And Developmental Toxicology; Safety Evaluation; Gonadotoxic Activity; λ-cyhalothrin

ABSTRACT BODY: The aim of the study was to study the gonadotoxic activity of generic λ-cyhalothrin produced by *Makhteshim chemical works ltd* (test substance LC1) and *Red sun group corporation* (test substance LC2) and to compare the results. The study of gonadotoxic activity was conducted on Wistar Han rats of both sexes using segmented approach in the design of the experiment with detection of functional state of the gonads and evaluation of the reproductive ability. The state of the estrous cycle, duration and frequency of each stage were evaluated in females. The motile sperm, the total number and the number of abnormal forms of sex cells were examined and evaluated in males. As the result of the study it was found that both substances LC1 and LC2 have reproductive toxicity in the maximum dose (3 mg/kg). The effect of the test substance LC1 was manifested in significant changes in reproductive parameters only in males. The effect of the test substance LC2 in its turn was manifested in significant changes in reproductive parameters of both males and females. No negative effects on reproductive function were found in minimal exposed dose (0.3 mg/kg) for both test substances. In comparative evaluation of the study results it was showed that investigated test substances have different signs of reproductive toxicity despite the fact that they are generics of the same active substance with similar composition and have the same no effect level in the dose 0.3 mg/kg.

ABSTRACT FINAL ID: 3689 Poster Board: P381
TITLE: Developmental Exposure to Chemicals Associated with Unconventional Oil and Gas Extraction Alters $CD4^+$ T Cell Populations in Multiple Immune Responses

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: B.P. Lawrence. *Environmental Medicine, University of Rochester School of Medicine & Dentistry, Rochester, NY.*
KEYWORDS: Immunotoxicity; Developmental Toxicity; Prenatal; Endocrine Disruptors; Hydro-Fracking

ABSTRACT BODY: Chemicals associated with oil and gas extraction by hydraulic fracturing have the potential to cause adverse biological effects, but this has yet to be extensively evaluated *in vivo*. A notable knowledge gap is the impact of these chemicals on the development and function of the immune system. Recently it was reported that prenatal exposure of mice to a mixture of 23 chemicals used and/or produced by hydraulic fracturing adversely affected offspring. Developmental exposures to other chemicals have been linked to altered immune function later in life. To examine the effects of developmental exposure to chemicals associated with hydraulic fracturing on the immune system, pregnant and lactating mice were exposed via drinking water to an equimolar mixture of 23 chemicals used in hydraulic fracturing. Immune function in their adult offspring was examined using 3 different disease models: experimental autoimmune encephalomyelitis (EAE, a mouse model of multiple sclerosis), house dust mite-induced allergic airway inflammation, and influenza virus infection. While several aspects of the immune response were unchanged, in all 3 model systems developmental exposure shifted the regulatory:conventional $CD4^+$ T cell ratio, such that there were fewer Tregs ($Foxp3^+$ $CD25^+$ $CD4^+$ cells) and more Th17 ($ROR\gamma t^+$ $CD4^+$) cells. Thus, developmental exposure to this mixture skews $CD4^+$ T cell populations in multiple immunological contexts. Moreover, in the EAE model, disease onset occurred earlier and was more severe in exposed mice. Since the Treg:Th17 ratio correlates with altered disease progression in EAE, the skewed $CD4^+$ T cell differentiation may impact overall disease progression. Together, these observations suggest that developmental exposure to this mixture has durable effects on T cells, which could contribute to more exacerbated clinical outcomes due to imbalances in immune function.

ABSTRACT FINAL ID: 3690 Poster Board: P382
TITLE: Establishment of a Novel *Caenorhabditis elegans* Obesogen Screen

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: A.K. Killeen. *Molecular Medicine, USF Health, Tampa, FL.*
KEYWORDS: Developmental Toxicity; Prenatal; Endocrine Disruptors; Reproductive And Developmental Toxicology; Bisphenol-A

ABSTRACT BODY: Obesity is a pervasive disease in the United States, with 35% of all adults in the United States clinically obese. Additionally, over a third of our nation's children are currently overweight or obese (CDC & NIH). One explanation for the rise in obesity is the widespread presence of environmental obesogens. Similar to a teratogen, an obesogen is an agent acting during the early development of an embryo, predisposing this offspring to preferentially store fat that can escalate into obesity in later life. Many putative obesogens span a variety of chemical classes, including estrogen mimics (bisphenol-A, diethylstilbestrol), heavy metals (cadmium, arsenic), biocides (triclosan, tributyltin), pesticides (fenthion), and even voluntary exposures like cigarette smoke (nicotine, benzo-α-pyrene). *Caenorhabditis elegans* is a soil nematode with a short lifecycle and conserved pathways for lipid synthesis and metabolism that make it an ideal model organism to study developmental exposures. We hypothesize that obesogens act during early embryonic development to alter energy efficiency in adult *C. elegans*. After parental exposure to obesogens, the resultant adult offspring (F1) are stained with Oil Red-O dye to assess lipid content. Pharyngeal pumping and body thrashing assays are also performed to assess energy intake and expenditure, respectively. We report with a proof-of-principle that 100μM bisphenol-A acts during development to induce increased F1 lipid storage and higher pharyngeal pumping rates with no significant difference in body thrashing rate, suggesting their energy intake is greater than their expenditure. We also report that developmental exposure to sub-embryonic lethal doses of the previously highlighted obesogens increases F1 lipid storage while inducing similar positive shifts in energy balance. We currently plan to use an enzymatic triglyceride assay to quantify total fat stores. In future work we plan to evaluate the role of key bioinformatics-identified molecular players using RNA interference.

ABSTRACT FINAL ID: 3691 Poster Board: P383
TITLE: Rat Sperm mRNA Transcript Levels Are Sensitive Indicators of Testicular Dysfunction

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: E. Dere[1,2], A. Altemus[3], J. Smith[3], J. Phillips[3], K. Blanchard[3], K. Boekelheide[2]. *[1]Division of Urology, Rhode Island Hospital, Providence, RI; [2]Department of Pathology and Laboratory Medicine, Brown University, Providence, RI; [3]Nonclinical Drug Safety, Boehringer Ingelheim Pharmaceuticals, Inc, Ridgefield, CT.*
KEYWORDS: Reproductive and Developmental Toxicology; Biomarkers; Sperm

ABSTRACT BODY: The human testis is sensitive to toxicant-induced injury, yet the available tools for detecting exposure effects are limited, insensitive and unreliable. Animal studies use sensitive histopathological endpoints to assess toxicity, but these endpoints require testicular tissue that is not available during human clinical trials. More sensitive and reliable molecular biomarkers of testicular injury that can be used to monitor testicular toxicity in both a clinical and nonclinical setting are needed. In this study, adult male Wistar Han rats exposed for 4 weeks to either cisplatin (0, 0.2, 0.3, or 0.4 mg/kg/day), BI665915 (0, 20, 70, 100 mg/kg/d), BI665636 (0, 20, 100 mg/kg/d) or BI163538 (0, 70, 150, 300 mg/kg/d) to evaluate reproductive toxicity and assess changes in sperm mRNA abundance. None of the compounds resulted in any significant changes in body, testis or epididymis weights, nor were there decreases in testicular homogenization resistant spermatid head counts. Histopathological evaluation of the testes found that only BI665915 treatment caused any testicular effects, including minor germ cell loss and disorganization of the seminiferous tubule epithelium, and an increase in the number of retained spermatid heads. A custom PCR-array panel was used to assess changes in sperm mRNA induced by each compound. BI665915 resulted in both significant increases and decreases of each of the mRNAs measured on the PCR array panel; Abi2, Alox15, Gimap4 and Sod3 levels were all elevated by treatment while Clu, Ptgds and Tmeff1 levels were decreased. BI66536 resulted in a significant decrease in Ptgds and Tmeff1 sperm transcript levels relative to vehicle control. These results demonstrate that sperm mRNA levels are sensitive molecular indicators of testicular injury that can potentially be translated into a clinical setting.

ABSTRACT FINAL ID: 3692 Poster Board: P384
TITLE: Transgenerational Effects of Di-(2-ethylhexyl) Phthalate on Anxiety and Cognition in Female Cd-1 Mice

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: C. Chiang[1], J. Willing[2], S. Rattan[1], K.M. Hatcher[1], J.M. Juraska[2], J.A. Flaws[1], M.M. Mahoney[1]. *[1]Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL; [2]Psychology, University of Illinois at Urbana-Champaign, Urbana, IL.*
KEYWORDS: Phthalates; Behavioral; DEHP

ABSTRACT BODY: Phthalates are chemicals that are commonly used as plasticizers in many everyday products. Di-(2-ethylhexyl) phthalate (DEHP) is one of the most commonly used phthalates and has known endocrine disrupting properties. Very little, however, is known about the transgenerational effects of DEHP on female behavior. Thus, we hypothesized that prenatal exposure to DEHP negatively affects anxiety and behavior in the F3 generation of female mice. Pregnant CD-1 mice were orally dosed with corn oil (vehicle control) or DEHP (20 and 200 µg/kg/day; 500 and 750 mg/kg/day) daily from gestational day 10.5 until birth. Female offspring were bred with untreated male mice until the F3 generation was produced. F3 adult female mice underwent behavioral tests including novel object recognition (NOR) (n=4-14 individuals/treatment), the elevated plus maze (EPM) (n=9-14 individuals/treatment), and the Morris water maze (MWM) (n=4-14 individuals/treatment). DEHP exposure did not affect NOR, indicating prenatal exposure to DEHP does not affect recognition memory in the F3 generation. DEHP exposure (200 µg/kg/day; 500 and 750 mg/kg/day) significantly increased time spent in the open arms of the EPM, but it did not affect total arm entries compared to controls, indicating that the increased open arm time was not due to a decrease in overall activity. Further, although DEHP exposure did not affect time to initial location of the platform in the MWM, it (500 and 750 mg/kg/day) caused a trend towards increased latency with platform reversal compared to controls. Collectively, these data show that prenatal DEHP exposure in the F1 generation results in transgenerational alteration of anxiety-like behavior and spatial cognition, but not recognition memory, in the F3 generation of female mice. Supported by NIH P01 ES022848, EPA RD-83459301, and T32 ES007326.

ABSTRACT FINAL ID: 3693 Poster Board: P385
TITLE: Mechanism of Chromium-VI Toxicity on the Cell Death Pathways of Rat Placenta

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Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX
KEYWORDS: Endocrine Disruptors; Developmental Toxicity; Prenatal; Metals; Placenta; Hexavalent Chromium

ABSTRACT BODY: Hexavalent chromium (CrVI), one of the most toxic heavy metals, is widely used in more than 50 industries. As one of the world's leading producers of Cr compounds, the U.S. is facing growing challenges in protecting human health against multiple adverse effects of CrVI. Placenta plays a very significant role for the nutrition of the embryo and development of secretory and regulatory functions for the maintenance of pregnancy. It also acts as a filter reducing the passage of harmful substances, protecting the fetus from exposure to endocrine disrupting chemicals (EDCs). However, several EDCs including heavy metals were detected in the placental tissues, amniotic fluid and umbilical cord blood. However, not much studies were carried out to determine the toxic effects of CrVI on the placental structure and function. The current study is focused to identify the molecular mechanism behind the effects of CrVI on the placental survival and/or cell death pathways. Pregnant rats were given 50 ppm CrVI (potassium dichromate) from gestational day (GD) 9.5 to 14.5 through drinking water, placentae were removed on GD18, and various analyses were performed. Results showed that gestational exposure to CrVI: 1) increased Cr accumulation in the placenta; 2) increased apoptosis in both maternal and fetal compartments by increasing BAX, p53, PUMA and NOXA; and decreasing BCL2; 3) Increased cleaved caspase-3 in maternal compartment but not in the fetal compartment; 4) increased AIF and p27 and decreased XIAP in both the compartments; 5) Interestingly, Beclin-1, a major regulator of autophagy, was not detected either in the maternal or in the fetal compartment. Taken together, our results indicate for the first time that gestational CrVI exposure directly targets the placental cell survival and increases the cell death of the placenta through selective activation of p53-dependent and caspase-3 dependent as well as caspase-3-independent cell death pathways in a spatio-temporal pattern.

ABSTRACT FINAL ID: 3694 Poster Board: P386
TITLE: Microfluidic Platform Supports Mouse Ovarian Follicle Development and *Reca*pitulates Human 28 Days Menstrual Cycle

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KEYWORDS: Ovary; Reproductive And Developmental Toxicology; Microfluidic System

ABSTRACT BODY: The main female reproductive organs include ovary, fallopian tubes, uterus, cervix, and vagina. These organs function in relation to one another to provide hormonal support and the anatomical structure through which gametes travel for the embryo to undergo development, transport, implantation and placentation. Our objective is to mimic human 28 days menstrual cycle *in vitro* by culturing mouse ovarian follicles in the microfluidic platform, and develop an *ex vivo* female reproductive tract that can be used for reproductive toxicology and therapeutic discovery based on the ovarian hormone production and their effects on the downstream gynecologic tissues. Mouse primary follicles were isolated from day 12 CD-1 mice, multiply encapsulated in 0.5% alginate hydrogels, and cultured in the microfluidic platform for 14 days with follicle-stimulating hormone (FSH), which was followed by human chorionic gonadotropin (hCG) administration on day 14 and an additional 14 days of culture without FSH. Results indicated that encapsulated *in vitro* follicle growth (eIVFG) was supported from primary to antral stage in the microfluidic system. After hCG treatment, follicles produced mature metaphase II oocytes with bipolar spindles and tightly aligned chromosomes. During the follicular phase, estradiol production increased and peaked on day 14 when follicles reached maturity. During the luteal phase, the progesterone levels increased significantly and peaked 2 days after the hCG treatment; and, the histologic analysis indicated that follicles initiated luteinization at the cellular level based upon granulosa cell hypertrophy. Taken together, our results demonstrate that the microfluidic platform provides a dynamic environment in which hormone production is maintained over a 28 day *in vitro* hormone cycle. This tool provides great potential to monitor rapid ovarian hormone changes and their effect on downstream reproductive tissues *in vitro*, and to provide a model to study the reproductive toxicology and therapeutic discovery (Funded by NIH UH3TR001207).

ABSTRACT FINAL ID: 3695 Poster Board: P387
TITLE: The Immediate Effects of Gonadotoxic Agents on the Viability of the Mammalian Ovary—Are Multidrug Resistant Transporters Protective?

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KEYWORDS: Alkylating Agents; Ovary; Xenobiotic Transporters; Cyclophosphamide

ABSTRACT BODY: Annually 810,170 women in the United States will be diagnosed with some form of invasive cancer. Approximately 8% of these women are under the age of 40. Current treatment regimens utilize aggressive gonadotoxic chemotherapy such as cyclophosphamide aimed at cure. This has led to chemotherapy-induced gonadotoxicity becoming a leading cause of ovarian insufficiency. Multidrug resistant transporters are found in ooctyes and have been reported to have a protective mechanism in the cell. To determine if multidrug resistant transporters (MDR) have an important role in the overall ovarian protection following administration of cyclophosphamide and then to maximize their utility to minimize gonadotoxicity. Design: Experimental laboratory study Setting: University and Academic Center Animals: FVBN, mdr1a/b ko, and mdr1a/b/bcrp (breast cancer resistance protein) ko female mice ages 5-6 weeks Intervention: Standard chemotherapy administration. Main Outcome measure(s): Granulosa cell death in the presence or absence of MDR expression. Mice were injected intraperitoneally (IP) with saline or cyclophosaphamide 75 mg/kg or 150 mg/kg. The ovaries were then removed and incubated with Live/Dead Mammalian Cytotoxicity Assay at 37° C (calcein-AM and ethidium homodier 1). Ovaries were imaged using a multiphoton confocal microscope. Dead/Live Ratios were calculated by creating a macro in ImageJ to measure red (non-viable) particles: area of green ovary. Three images were taken per ovary and the average Dead/Live ratio was taken and SD calculated. Ovaries with wild type MDR expression have less cell death of granulosa cells compared to triple knockouts for mdr1a/b/bcrp 24 hours after IP cyclophosphamide 75 mg/kg (p=0.0112) and 150 mg/kg (p=0.0159). Wild type and mdr1a/b knockouts had similar cell death rates for both dosages. MDR -1 may work in conjunction with BCRP to protect the mammalian ovary from gonadotoxic chemothearapy. Our future focus is to determine if oocyte demise by chemotherapy is direct, or indirect through the compromised granulosa cells.

ABSTRACT FINAL ID: 3696 Poster Board: P388
TITLE: Investigation of Immunotoxicity and Thyroid Disrupting Effects after Developmental Exposure to Perfluorohexane Sulfonate (PFHxS) in Rats

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KEYWORDS: Perfluoronated Agents; Immunotoxicity; Endocrine Disruptors; Perfluorohexane Sulfonate

ABSTRACT BODY: Perfluorinated alkylates have been in use since the 1950s because of their water- and fat repellant properties. They are used as surfactants in many industrial and consumer products from food contact materials to furniture and fire-fighting foams. The compounds are persistent and widespread in the environment, and they bioaccumulate in the human body. Furthermore, compounds such as PFOA and PFOS are toxic due to their adverse effects on the immune system, the liver and the endocrine systems. Perfluorohexane sulfonate (PFHxS) is a shorter chained perfluorinated compound, which in epidemiological studies has been strongly correlated to reduced number of antibodies in response to vaccinations (*). Experimental animal studies which could confirm these correlations and cast light upon other toxic effects due to developmental exposure to PFHxS are however presently lacking. In a developmental toxicity range-finding study, time-mated Wistar rat dams (n=8) were dosed by oral gavage with PFHxS from gestation day 7 through postnatal day (PND) 22 with doses of 0, 25, and 45 mg/kg/day. At sacrifice on PND 22 serum thyroxine (T4) levels were measured in the dams, and T4 and triiodothyronine (T3) levels were measured in one male and one female pup per litter (n = 5-7). Liver, thymus, and spleen were excised from male pups on PND 16. No signs of overt toxicity were observed in the dams, and dam liver weights were not affected. Dam serum T4 levels were reduced to app. 40 % of control values. Exposure to PFHxS also dose-dependently reduced pup serum T4 levels to 60 % and 50 % of control levels, in the two dose groups respectively. Male pup liver weights were increased dose-dependently on PND 16. No significant effects on male pup thymus and spleen weights were seen. This range-finding study is to our knowledge the first to show effects in pups after developmental exposure to PFHxS. A large study presently underway will provide more results on possible endocrine disruption and immunological endpoints. In this range-finding study no effects were seen on thymus and spleen weights at this early developmental stage. However, functional immune studies are needed to cast light upon the severe effects found in humans due to exposure to perfluorinated compounds as PFHxS. (*) Grandjean et al., JAMA, 2012, 307(4); Mogensen et al., Environmental Health (2015) 14:47.

ABSTRACT FINAL ID: 3697 Poster Board: P389
TITLE: Exposure to Cigarette Smoke During Pregnancy Decreases Offspring and Placental Weights and Alters mRNA Expression of Genes Associated with Nutrient Transport

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KEYWORDS: Reproductive And Developmental Toxicology; Cigarette Smoke, Placenta

ABSTRACT BODY: Even after decades of warnings about the hazards of smoking cigarettes, it is estimated by the WHO that 24-27% of child-bearing age women worldwide continue to smoke and many continue to smoke during pregnancy. In the U.S. alone more than 1.5 million babies are born each year after having been exposed *in utero* to either active or passive (or both) cigarette smoke (CS). Studies have identified CS constituents in placentas and fetal blood of actively-smoking mothers showing that many CS constituents reach and cross the placental barrier. On average, babies born to women who smoke during pregnancy are 200 g (as much as 8%) lighter than unexposed babies. This depression in birth weight is likely a result, in part, from depressed nutrient transfer by the placenta which provides a semi-permeable barrier between the mother and her offspring allowing for nutrient and gas transfer to the offspring, while transporting waste products back to the mother for excretion. Toxicants, such as CS, may impact some of these functions affecting transport of glucose, amino acids (AA), and lipids necessary for normal development and growth of the offspring *in utero*. The hypothesis for this study is to determine if low birth weight associated with maternal exposure to inhaled CS results from changes in nutrient transport pathways. Timed-pregnant B6C3F1 mice were exposed to CS generated from Univ. of Kentucky 3R4F research cigarettes using an automated CS generation system (Baumgartner-Jaeger CSM 2070) for a total of four hours daily from gestational day (GD) 0.5 to GD15.5 and euthanized on GD16.5. During exposures, mice were exposed to a mean daily chamber concentration of 19.9 µg particles/m^3 and 26.8 ppm carbon monoxide. The mean urinary cotinine level of the mice was 19.4 ng/ml. Litter size was unaffected by CS exposure, but fetal bodyweight was significantly decreased (~9%). Placental weight was also significantly decreased (~5.6%) as a result of CS exposure. Examination of mRNA expression of nutrient transporters showed that the glucose transporter, Slc2a3, was decreased by 80% and fatty-acid transporters FatP1 and FatP6 were reduced by 55% and 32%, respectively. Conversely, AA transporters Slc38a1 and Slc38a2 were increased by 4.5- and 2.7-fold, respectively. These changes demonstrate that CS may affect how nutrients are transferred to the offspring and possibly increasing disease susceptibility later in life. Supported by ES000260.

ABSTRACT FINAL ID: 3698 Poster Board: P390
TITLE: Cigarette Smoke Condensate Induces Epithelial-Mesenchymal Transition in Ectocervical Cells: Correlation with Pathogenesis of CIN, Cervical Cancer and Twist1/Snail Up-Regulation

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KEYWORDS: Environmental Toxicology; Reproductive Tract; Female; Exposure, Environmental

ABSTRACT BODY: Numerous studies have demonstrated that cigarette smoking is an important risk factor for cervical cancer, albeit the underlying mechanism(s) remains unclear. Tobacco components and a tobacco-specific carcinogen have been found in cervical mucus of women smokers. The aim of our study was to examine potential effects of cigarette smoke condensate (CSC) on a human ectocervical cell line immortalized with human papillomavirus 16/E6E7 (Ect1/E6E7). Ect1/E6E7 cells were exposed to 10 µg/mL CSC for 24h, 72h and 90d. Results show CSC did not significantly induce cell proliferation by MTS assays; however, by using light microscopy and TEM we observed that cells exposed to CSC lost their “cobblestone” morphology and became progressively “spindle-like” at 24h, 72h and 90d. Interestingly by TEM, CSC-treated cells were significantly enlarged nearly 1.5 times that of controls (329.48±278.1 vs. 237.2±182.5 µm2/cell, respectively, p≤0.05). Additionally, these cells had decreased surface filapodia, cytoplasmic swelling, single membrane vacuoles, and variable-sized mitochondria, which are ultrastructural changes compatible with EMT. E-cadherin and vimentin, markers of Epithelial-Mesenchymal Transition (EMT), are critical for the progression of cervical and other cancers. A considerable reduction in E-cadherin- and a significant increase in vimentin-positive fluorescence signals were found in CSC-treated cells, compared to controls at 72h. These data were confirmed by western blot and RT-PCR. The expression levels of Twist1 and Snail were up-regulated by RT-PCR. Our data suggest that CSC induces EMT in human cervical epithelial cells, and Twist1/Snail may be involved in the pathogenesis of cervical intraepithelial neoplasia (CIN) and cervical cancer. EMT may be a novel molecular mechanism of early cellular transformation and implicates cigarette smoking as a risk factor for cervical cancer.

ABSTRACT FINAL ID: 3699 Poster Board: P391
TITLE: Effect of Vinclozolin on Integrity of the Blood-Testis Barrier in Male Adult Rat

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KEYWORDS: Endocrine Disruptors; Testis; Reproductive Tract; Male; Fungicide; Vinclozolin

ABSTRACT BODY: Vinclozolin (V) is a fungicide with anti-androgenic properties by interacting with the androgen receptor. Many studies have shown that V causes abnormal male reproductive tract and alters sperm quality and testosterone balance. The blood-testis barrier (BTB) is a susceptible structure to be affected by anti-androgenic xenobiotics. The BTB plays a crucial role in spermatogenesis, since it seals the paracellular route between Sertoli cells and protects post meiotic germ cells from the immune system present in the systemic circulation. It is constituted by several types of junctions including tight junctions (TJ). ZO-1, ZO-2, occludin, and claudin-11 are proteins present in TJ and their expression and localisation are androgen-dependent. There is no available information about the effect of V on the integrity of the BTB. The aim of this study was determine the effect of V on the BTB integrity in male adult rats. V (100 mg/kg/d) was orally administered to male adult Wistar rats for 7 days. Eight hours after dosing animals were anesthetized and sacrificed. Immunofluorescence was used to identify function (biotin) and integrity (ZO-1, ZO-2, occludin, and claudin-11) of the BTB. V and its metabolites testes levels were evaluated by HPLC/DAD. V treatment induced the appearance of a leaky BTB by using the biotin tracer. Occludin was diminished and disturbed by numerous breaks in the BTB circumscribed each Sertoli cell near the basement membrane. A delocalization in claudin-11 filaments was observed but there was non-observable effect on ZO-2 and ZO-1. Western blots analysis showed increases on occludin, claudin-11 and ZO-1. M5 was the main metabolite detected and only trace levels of M1 and M2 metabolites, and V were observed. These results suggest that V alters the integrity and function of the BTB. In addition, M5 metabolite could play an important role in the development of anti-androgenic effects on male reproductive system associated to V exposure.

ABSTRACT FINAL ID: 3700 Poster Board: P392
TITLE: Fetal Bisphenol A Exposure Alters Body Weight and Male Urogenital Tract Morphology at Birth: Findings from the NIEHS/FDA Clarity-BPA Research Program

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KEYWORDS: Endocrine; Estrogens; Endocrine Disruptors; Reproductive System; BPH, FeBAD, Fetal Reprogramming

ABSTRACT BODY: Bisphenol A (BPA) is a chemical used in the production of consumer products resulting in widespread exposure to humans. Reports by the FDA and others suggest that BPA is safe at current exposure levels. However, a large number of other studies have questioned the safety of BPA. A collaboration of the National Toxicology Program, National Institute of Environmental Health Sciences, U.S. Food and Drug Administration, and academic research universities was established to help bring consensus to the role of BPA in health related issues. This collaboration is known as the Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA). Here we utilized postnatal day 1 male NCTR-CD-SD rats that were exposed to, BPA (0, 2.5, 25, 250, 2500, 25000 ug/kg/day) or ethinyl estradiol (EE; 0.05, 0.5 ug/kg) as positive controls for estrogenic activity, via maternal gavage from gestation day 6 - parturition. We conducted a computer based morphometric analysis of serial sectioned-3-D reconstruction of the postnatal day 1 male urogenital sinus (UGS). We observed a significant increase in body weight in male pups prenatally exposed to the 2.5, 25 and 250 ug/kg/day BPA doses as well as the two EE doses, but not the two highest BPA doses. Analysis of 3-D reconstructions of UGS showed several significant ($p<0.05$) or trending ($p<0.1$) changes in collicular and urethral tissues due to low but not high doses of BPA similar to EE. These data suggest that BPA mediates its effects on the fetal UGS via estrogenic pathways. Interestingly, at the highest BPA dose, a significant difference from control (0 ug/kg) rats was not observed for any outcome. Additionally, consistent with studies in mice, we observed a non-monotonic dose response for body weight and width within the UGS-urethra/lumen. The observed increase in body weight at birth is interesting because NCTR CD-SD rats have rapid growth and susceptibility to obesity, which is likely exacerbated by BPA and EE. However, the effects of BPA in older rats from this collaborative study may be undetectable due to repeated stresses beginning at birth in the form of daily restraint and intra-gastric gavage for which there were no non-gavage controls.

ABSTRACT FINAL ID: 3701 Poster Board: P393
TITLE: Bisphenol A Alternatives Can Effectively Substitute for Estradiol in Promoting Cell Growth through Estrogen Receptors in Human Breast Cancer Cells

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KEYWORDS: Endocrine Disruptors; Cell Culture; Cell Proliferation; Bisphenol A

ABSTRACT BODY: Plasticizers with estrogenic activity, such as bisphenol A (BPA), have been reported to have potential adverse health effects in humans, especially in fetal and infant stages. Due to mounting evidence and public pressure BPA is being phased out by the plastics manufacturing industry and is being replaced by other bisphenol variants in “BPA-free” products. We have compared estrogenic activity of 7 bisphenol analogues (BPA; bisphenol S, BPS; bisphenol F, BPF; bisphenol AP, BPAP; bisphenol AF, BPAF; bisphenol Z, BPZ; bisphenol B, BPB). Activation of estrogen receptors was determined using a mammary T47D cell line harbouring a stably integrated copy of a luciferase reporter gene under control of a promoter containing estrogen response elements. Cell proliferation induced by the activation of estrogen receptors was determined in three breast cancer cell lines (MCF-7, T47D, and MDA-MB-231) by the conventionally used E-screen assay. The natural hormone estradiol was used as a reference. All bisphenols were able to substitute for the natural hormone in promoting cell growth through estrogen receptors. In both assays, BPAF was most potent bisphenol (50% effective concentration for receptor activation=0.08µM) followed by BPB (0.3µM) < BPZ (0.4µM) ~ BPA (0.4µM) < BPF (1µM) ~ BPAP (1µM) < BPS (3µM). The addition of ICI 182,780 (100 nM) antagonized the activation of estrogen receptors by both estradiol and bisphenols. Our results show that BPA-free products are not necessarily safer. Three bisphenols (BPAF, BPB, BPZ) were more estrogenic than BPA. The clinical relevance of human exposure to BPA alternatives in hormone-dependent breast cancer progression should be investigated.

ABSTRACT FINAL ID: 3702 Poster Board: P394
TITLE: Thyroid Hormone Disruption Induced by Bisphenol A and Its Alternatives in Zebrafish Embryo

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KEYWORDS: Endocrine; Thyroid; Aquatic Toxicology; Bisphenol

ABSTRACT BODY: Bisphenol A (BPA) has been employed in various commercial products such as food containers, toys and thermographic and pressure sensitive receipt paper. Owing to growing concerns on adverse health outcomes associated with BPA, alternatives such as BPA structural analogues, e.g., bisphenol F (BPF), bisphenol S (BPS), bisphenol Z (BPZ), have been used in growing amount. Accordingly, BPA analogues were detected in human biological samples and in various environment media such as indoor dust and wastewater. However, toxicity of them has not yet been well understood, especially on thyroid hormone (TH) system and development. This study was conducted to examine effects of BPA and its analogues on TH system and development using zebrafish embryo/larvae model. Three BPA analogues (BPF, BPS and BPZ) were selected as study chemicals based on the detected environment level along with BPA. Zebrafish embryos (<4 hr after fertilization) were exposed to BPA, BPF, BPS, or BPZ until 120 hpf. Then, whole-body level of TH (T3, T4) and gene transcriptions related to TH (*crh*, *tshβ*, *tshr*, *nkx2.1*, *pax8*, *tpo*, *tg, hhex, trα, trβ*, *ttr, dio1*, *dio2*, and *ugt1ab*) were analyzed. Embryo/larvae survival, hatching and malformation were also observed as development endpoints. Significant increase in T3 or T4 were observed after exposure to BPA, BPF and BPS. In addition, BPA, BPF and BPS commonly induced gene transcription related to thyroid development (*hhex*, *tpo*, or *tg*), TH transport (*ttr*) and TH metabolism (*ugt1ab*). Time to hatch was delayed by exposure to BPA and all the tested analogues at non-lethal concentrations. Both BPA and its structural analogues stimulate TH production, which may cause developmental delay, although the test concentrations were several orders of magnitude higher than environmental concentrations. Thyroid related health consequences of their use deserve further investigation.

ABSTRACT FINAL ID: 3703 Poster Board: P395
TITLE: Inhibition of Islet Amyloid Polypeptide Aggregation and Associated Cytotoxicity by Non-Steroidal Anti-Inflammatory Drugs

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KEYWORDS: Pancreas; Endocrine; Other; Pharmaceuticals

ABSTRACT BODY: Non-steroidal anti-inflammatory drugs (NSAIDs) constitute an important pharmacotherapeutic class which, over the past decade, have expanded applications for a panoply of medical conditions. They have been tested for neurodegenerative diseases such as Alzheimer's to reduce the inflammation and also in the attempt to abrogate amyloid deposition. However, the use of NSAID as aggregation inhibitors has not been extensively studied in pancreatic amyloid deposition. Pancreatic amyloidosis involves the misfolding of islet amyloid polypeptide (IAPP) and contributes to the progression of type 2 diabetes in the human and feline. To ascertain their anti-amyloidogenic activity, several NSAIDs were tested *in vitro* using fluorometric ThT assays, circular dichroism, photo-induced cross-linking assays and cell viability assays. Celecoxib, diclofenac, indomethacin, meloxicam, niflumic acid, nimesulide, phenylbutazone, piroxicam, sulindac and tenoxicam reduced fibrillization at a molar ratio of 1:10. Diclofenac, niflumic acid, nimesulide, phenylbutazone and tenoxicam delayed the conversion of the secondary structure into a beta sheet. The oligomerization of human IAPP was abrogated with diclofenac and sulindac at a molar ratio of 1:5. The cytotoxic effects of pre-incubated human IAPP on cultured INS-1 cells were noticeably reduced in the presence of diclofenac, meloxicam, phenylbutazone, sulindac and tenoxicam at a molar ratio of 1:10. Our results demonstrate that NSAIDs can provide chemistry scaffolds to generate new promissing anti-amyloidogenic agents that can be used alone or as a coadjuvant therapy.

ABSTRACT FINAL ID: 3704 Poster Board: P396
TITLE: Evaluation of the Endocrine Disruptor Potential of Fenbutatin Oxide Technical in Wistar Rats

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KEYWORDS: Endocrine Toxicology; Endocrine Disruptors; Pesticides; Fenbutatin Oxide Technical

ABSTRACT BODY: Potential of fenbutatin oxide technical (FBT) at 10 and 75 mg/kg/day for endocrine disruptor activity following US EPA guidelines was evaluated. For male and female pubertal assay, juvenile wistar rats of PND 23 and 22 were used at the time of initiation of dosing, respectively. For Hershberger and Uterotrophic bioassay, castration and ovariectomy was performed, respectively on PND 42 and 47. Study design (including number of animals/group, number of groups, study specific positive controls, frequency of observation and duration of treatment) was as per the guideline. Organs as defined in test guidelines were excised and weighed from all animals. In all the assays, endpoints of FBT groups treated at 10 mg/kg/day were comparable with concurrent control group. Significant reduction in body weight, body weight gain and feed consumption was observed in all animals treated with FBT at 75 mg/kg/day. Delay in onset of puberty was observed in male and female animals treated with FBT at 75 mg/kg/day. In male pubertal assay and androgen antagonist bioassay, statistically significant reduction was observed in androgen dependent organs in animals treated with FBT at 75 mg/kg/day. In female pubertal assay, significant decrease in pituitary, kidney, adrenal, ovaries, wet uterus, blotted uterus and thyroid weight was observed in animals treated with FBT at 75 mg/kg/day. Corpus luteum was not present in ovaries of thirteen female animals treated with FBT at 75 mg/kg/day. In pubertal assays, thyroid hormone level in serum was comparable in all the groups whereas testosterone level was significantly reduced in the male animals treated with FBT at 75 mg/kg/day. In androgen agonist bioassay, all androgen dependent organ weights of animals treated with FBT was comparable with control group. In estrogen agonist bioassay, uterus weight (wet and blotted) of FBT treated groups was comparable with control group. Hence, it is concluded that fenbutatin oxide technical does not alter pubertal development nor it affects androgen, estrogen and thyroid pathway at 10 mg/kg/day.

ABSTRACT FINAL ID: 3705 Poster Board: P397
TITLE: Evaluation of a Novel Standardized *In Vitro* Human Islet Model System for Assessment of Drug-Related Pancreatic β Cell Functional Perturbation

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KEYWORDS: Pancreas; Safety Evaluation; Pharmaceuticals; Islets

ABSTRACT BODY: The availability of *in vitro* models that reliably assess drug-related human endocrine pancreatic functional perturbation remains a significant challenge in drug development. To address this unmet need, 3D Insight™ Human Pancreatic Microislets were evaluated for functionality and responsiveness to compounds with known effects on β cell function. 3D Insight™ Human Pancreatic Microislets are standardized microtissues reaggregated from dispersed islets and cultured in GravityTRAP™ 96-well plates at 1 islet/well of equal size & cellular composition allowing for high throughput data acquisition with low intra-assay variability. Glucose-stimulated insulin secretion (GSIS) was 0.059 ± 0.009 nM versus 0.442 ± 0.123 nM at low (2.8mM) and high (16.7mM) glucose, respectively in 8-11 day cultures (n=3 donors) and 0.030 ± 0.012 nM versus 0.802 ± 0.172 nM at low (2.8mM) and high (16.7mM) glucose, respectively in 18-36 day cultures (n=3 donors). GSIS was significantly suppressed by exposure to two marketed drugs associated with inhibiting insulin secretion. Octreotide, a somatostatin analogue ($p<0.05$, n=3), and Tacrolimus, an immunosuppressive agent that impaired insulin secretory capacity by 60% after 72 h exposure at 1 µM. In addition a proprietary ER stress modulator impaired GSIS in human microislets with an IC50 of 3-6 µM (n=4 donors) after 72 h treatment, with a reduction in viability only seen at 12.5 µM ($p<0.05$). Finally, to assess human microislets for testing of long-term compound effects on GSIS, both Tacrolimus & Rapamycin (another marketed drug known to inhibit GSIS) were added to microislets over 11 days. Tacrolimus inhibited GSIS by 50% at 10 nM, 100 nM and 1 µM, while Rapamycin blunted GSIS by over 80% at 1 nM, 10 nM and 100 nM (n=6 replicates from 1 donor) with minimal impact on viability. 3D InSight™ Human Pancreatic Microislets represent a robust model for *in vitro* risk assessment of β cell function as drugs associated with beta cell perturbation in patients correlated to decreased GSIS in acute or chronic exposure culture conditions. The initial procurement of high-quality human islets is a critical prerequisite to this model's reproducibility and represents a significant challenge.

ABSTRACT FINAL ID: 3706 Poster Board: P398
TITLE: Severe Hypothyroxinemia, But No Behavioural or Auditory Effects in Rats, After Developmental Exposure to a Brominated Flame Retardant

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KEYWORDS: Endocrine; Thyroid; Neurotoxicity; Developmental; PBDE

ABSTRACT BODY: In all mammalian species, correct levels of thyroid hormones are important for proper brain development. In humans, a number of studies have shown that even mild maternal hypothyroxinemia during pregnancy is associated with impaired fetal brain development. Therefore exposure to chemicals that reduce human thyroid hormone levels could have detrimental consequences, both for the affected individuals and for society as a whole. In rats, developmental hypothyroidism induced by exposure to potent anti-thyroid drugs like propyl thiouracil (PTU), has been shown to causes adverse effects on brain development. Here we investigated whether exposure to a brominated flame retardant, known to act as a thyroid disrupter in rats, would also cause neurobehavioral changes. Time-mated Wistar rat dams (n=20) were dosed with a polybrominated diphenyl ether (PBDE) called DE-71. Exposure was from gestation day (GD) 7 through postnatal day (PND) 16 at doses of 0, 40, and 60 mg/kg/day. Serum thyroxine (T4), triiodothyronine (T3) and thyroid-stimulating hormone (TSH) were measured at various time points in dams and offspring, along with thyroid gland weights and histopathology. In the offspring, motor activity was determined on PND 21 and PND 79, cognition was assessed in a Morris water maze at 19 weeks of age, and hearing was assessed at 6 months of age by test of otoacoustic emission. In the dams, T4 and T3 levels were significantly decreased on GD 15 in both dose groups. Furthermore, the pups from both dose groups showed T4 reductions of ~70% on PND 16. However, postnatal body weights, TSH levels, thyroid gland weights and histopathology were unaffected by DE-71 treatment, as were motor activity levels, learning and hearing ability. This lack of effects in a validated battery of behavioral tests testing was surprising, because similar T4 reductions had previously caused significant effects on activity, learning and hearing ability in our PTU study. Further studies, investigating structural abnormalities in the brains of developmentally hypothyroid animals, could be used to elucidate why compounds showing T4 decreases of similar magnitudes cause such different effect on behavior. However, the present results indicate that the mode of action of a thyroid disrupting chemical is important with regards to its adverse effects on rodent brain development.

ABSTRACT FINAL ID: 3707 Poster Board: P399
TITLE: The Use of HepaRG as a Human Hepatocyte Model to Study NASH Development in Class III Obese Patients and the Consequent Activation of TLR4 Signaling Pathway by the Cytotoxic Effect of Palmitic Acid

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KEYWORDS: Cell Lines, Transfected; Liver; Endocrine Toxicology; HepaRG, NASH, NAFLD; Palmitic Acid, LPS

ABSTRACT BODY: Nonalcoholic fatty liver disease occurs frequently in the setting of metabolic syndrome, but the factors leading to nonalcoholic steatohepatitis (NASH) are not fully understood. This study investigated Toll-like receptor 4 (TLR4) signaling in human liver with the goal of delineating whether activation of this pathway segregates those with nonalcoholic fatty liver from those with NASH. Experiments were performed using liver biopsy tissue obtained from class III obese subjects undergoing bariatric surgery, and extended to an immortalized human hepatocyte HepaRG cell line and primary human hepatocytes. The bacterial endotoxin lipopolysaccharide (LPS) and total free fatty acid levels were significantly increased in plasma of NASH patients. TLR4 mRNA levels were significantly increased in subjects with NASH compared with NAFL as was interferon regulatory factor (IRF) 3 in the myeloid differentiation factor 88 (MyD88)-independent signaling pathway. In HepaRG cells, nuclear factor-κB (NF-κB) nuclear translocation and functional activity increased following treatment with the fatty acid, palmitate, and following exposure to LPS compared with hepatocytes stimulated with a lipogenic treatment that induced *de novo* lipogenesis. Palmitate and LPS induction of NF-κB activity was partially attenuated by chemical- or small-interfering RNA (siRNA)-mediated inhibition of TLR4. Expression of TLR4 and its downstream mediators was upregulated with palmitate and LPS. Similar results were observed using primary human hepatocytes from a lean donor. Interestingly, NF-κB activity assays showed obese donor hepatocytes were resistant to chemical TLR4 inhibition. In conclusion, TLR4 expression is upregulated in a large cohort of NASH patients, compared with those with NAFL, and this occurs within the setting of increased LPS and fatty acids. Moreover, our results demonstrate also that, in HepaRG cells, LPS stimulation increased NF-κB through both MyD88-dependent and -independent mechanisms. Taken all that into consideration, HepaRG cells seem to open new horizons to study novel aspects in toxicology , hence demystifying mechanisms of important hepatic pathophysiologies such as NAFLD and NASH.

ABSTRACT FINAL ID: 3708 Poster Board: P400
TITLE: Determination of Thyroxine (T4) in Mouse Plasma by Microsampling

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KEYWORDS: Endocrine; Thyroid; Pharmacokinetics; Endocrine Toxicology

ABSTRACT BODY: Multiple blood sampling times over a short period are often required in preclinical studies. Bioanalytical methods typically use blood volumes that are too large for repeated sampling in mice. The objective of our study was to improve blood sampling and bioanalytical techniques in order to allow multiple sampling of each mouse over the full duration of the study. The experiment included 20 untreated CD1 mice sampled for T4 levels (6 timepoints over 24 hr) on 2 occasions separated by 6 weeks and at one additional timepoint every 2 weeks in between. The blood collection site was the saphenous vein. Fifty µL of blood/timepoint was collected using a heparinised capillary. The capillary was placed in a conical tube for centrifugation, and spun to separate the plasma from the blood. Ninety percent of the plasma samples were recovered. The sample processing consisted of a solid phase extraction followed by mass spectrometric detection. Overall, T4 plasma levels ranged from 8-45 ng/ml. The results indicate that a slight decrease in T4 concentrations appears at the beginning of the nocturnal cycle. Levels of T4 showed both individual variation throughout the day and inter-individual variations during the study. In conclusion, our laboratory has developed a successful approach to sampling blood in mice on multiple occasions over a 24-hour period combined with a bioanalytical method with volumes as low as 10 µL of plasma. The results are indicative of circadian variation in T4 levels in mice between age 6-13 weeks. In addition, this approach is in line with 3R principles and could reduce the number of animals utilized for bioanalysis in toxicology studies without limiting the statistical interpretation of the data.

ABSTRACT FINAL ID: 3709 Poster Board: P401
TITLE: A Novel Cell Panel Based Real Time Cellular Assay to Detect and Differentiate Endocrine Disruptors

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KEYWORDS: Endocrine Disruptors; Endocrine Toxicology

ABSTRACT BODY: Endocrine disruptors mimic endogenous hormones, alter the homeostasis of the endocrine systems, leading to health problems including development and cancer. To date, due to the complexity of endocrine pathways, there is an unmet need to identify these chemicals, especially those affecting estrogen receptor (ER), androgen receptor (AR) and thyroid hormone receptor (THR). Here, we describe a method utilizing a panel of three mammalian cell lines to identify modulators of these hormones. Modulation of endocrine pathways leads to changes in cell number, morphology and adhesion which can be detected by gold microelectrodes embedded in the bottom of the well of a specialized microtiter plate. Human and rat cell lines expressing ER, AR, and THR were stimulated with representative agonists and antagonists. Preliminary test is promising based on different sensitivities and unique kinetic response profiles of each cell line. Human breast cancer cell line T47D selectively detects two distinct nuclear hormone responses: ER and AR. T47D cells showed sensitive response to estrogen agonists such as 17beta-estradiol and bisphenol A. These responses were inhibited by ER antagonists ICI182780 and Tamoxifen. Additionally, T47D cells displayed different kinetic response profiles to androgen agonists R1881 and DHT at nM range, which were abolished by AR antagonists bicalutamide and vinclozolin, but not by ER antagonists. Unlike T47D cells, which showed no response to THR agonists triiodothyronine (T3) and thyroxine (T4), rat pituitary tumor cell line GH3, showed sensitive (EC50= 20pM for T3) response leading to 5- fold growth in 4 days. THR antagonists 1-850 and desethylamiodarone effectively blocked T3/T4 effect in GH3 cells. The human prostate cancer cell line LNCAP was added to the study for its unique kinetic response to AR agonists, which were greater than 10 times more sensitive than that observed in T47D cells. In summary, this method collectively uses mammalian cells with different tissue and species origins, to detect 3 distinctive classes of endocrine hormone disruptors within a single cellular assay.

ABSTRACT FINAL ID: 3710 Poster Board: P402
TITLE: A High-Throughput High-Content Screen for the Detection of Androgen Receptor Modulators

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KEYWORDS: Endocrine Toxicology; Endocrine Disruptors; High-Throughput High-Content

ABSTRACT BODY: Environmental contaminants such as industrial and agricultural chemicals have been shown to alter androgen receptor (AR) function through agonistic or antagonistic modulation. Here we demonstrate use of a 384 well high throughput, high content fluorescent screening assay (AR Redistribution Assay, ThermoScientific) that can differentiate between AR agonists and antagonists through AR localization with spot detection algorithms (ThermoScientific). This assay is image based, so secondary compound effects such as cytotoxicity or auto-fluorescence can also be identified to determine false positives or negatives. This versatile screen can also be run in agonist mode in the presence of dihydrotestosterone to determine compounds which block AR translocation or activation. Here we demonstrate the validation of this assay using known modulators of AR which act on different aspects of its activation pathway. Dihydrotestosterone treatment results in translocation and activation of AR. Flutamide and 17-AAG are used to show the loss of translocation of the fluorescently tagged AR to the nucleus. Bicalutamide and mifepristone are tested to demonstrate AR antagonistic effects while still translocating to the nucleus. This assay was then used to run a high throughput assay on a commercially available compound library where it performed with Z' greater than 0.5 for each of the reported endpoints.

ABSTRACT FINAL ID: 3711 Poster Board: P403
TITLE: Prediction of Estrogenic Bioactivity of Environmental Chemical Metabolites

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KEYWORDS: Endocrine; Estrogens; Metabolic Activation; QSAR

ABSTRACT BODY: The US Environmental Protection Agency's (EPA) Endocrine Disruptor Screening Program (EDSP) is using *in vitro* data generated from ToxCast/Tox21 high-throughput screening (HTS) assays to assess the endocrine activity of environmental chemicals. Considering that *in vitro* assays may have limited metabolic capacity, inactive chemicals that are biotransformed into metabolites with endocrine bioactivity may be missed for further screening and testing. Therefore, there is a value in developing novel approaches to account for metabolism and endocrine activity of both parent chemicals and their associated metabolites. We used commercially available software to predict metabolites of methoxychlor and 18 other parent compounds known to have estrogenic metabolites. Three ER QSAR models were used to determine potential estrogen bioactivity of the parent compounds and predicted metabolites, and the chemicals were then ranked based on the total estrogenicity of the parent chemical and metabolites. The metabolite prediction software correctly identified known estrogenic metabolites for 18 out of 19 parent chemicals, and 30 out of 36 estrogenic metabolites were predicted as potential biotransformation products derived from the parent chemical. The ER QSAR models estimated stronger estrogenic activity for the majority of the known estrogenic metabolites compared to their parent chemicals. Finally, the three ER QSAR models identified the same parent compounds (mestranol, methoxychlor, 2,2-diphenylpropane, benzophenone, benzophenone 3 and diphenyl) as top ranked chemicals based on the estrogenicity of all predicted metabolites. This proposed *in silico* approach is an inexpensive and rapid strategy for the detection of chemicals with estrogenic metabolites and may reduce potential false negative results from *in vitro* assays.

ABSTRACT FINAL ID: 3712 Poster Board: P404
TITLE: Kinetic Studies of Fluoride Inhibition of Rabbit, Liver, Kidney, and Brain Arginase

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KEYWORDS: Toxicity; Chronic; Environmental Toxicology; Toxicity; Chronic; Drinking Water Floridation, Kinetic; Fluoride

ABSTRACT BODY: The first community drinking water fluoridation program began in 1945 in Grand Rapids, Michigan and spread across US to other parts of the world. Over the past three decades, there has arisen evidences showing that the harmful effects of drinking water fluoridation may outweigh the benefit in preventing dental caries. Tormanen demonstrated in 2004 that fluoride caused substrate inhibition of rat liver arginase at concentrations of 4mM. and lower concentration of fluoride inhibited Rat kidney arginase. The purpose of this work is to study the mechanism and the nature of fluoride inhibition of rabbit liver, kidney and brain arginase. There are no reports of such a kinetic study involving the liver, kidney and brain arginase in rabbit. The effects of supernatant concentrations of rabbit liver, kidney and brain arginase on the production of L-ornithine from arginine were studied according to standard methods. IC50s and kinetic studies of fluoride inhibition of rabbit liver, kidney and brain arginases at pH 7.4 and at pH 9.5 were also determined according to standard methods. The plots of the effects of supernatant concentrations of rabbit liver, kidney and brain arginase at pH 7.4 and at pH 9.5 against L-ornithine concentration were linear. Rabbit liver, kidney and brain arginases were inhibited by fluoride at pH 7.4 and at pH 9.5. A stronger inhibition was found at pH of 7.4. The IC50 of fluoride inhibition of liver arginase were 1.75mM and 1.37mM at pH 7.4 18mM at pH 9.5; the IC50 of fluoride inhibition of kidney arginase was 1.68mM at pH 7.4 and 158mM at pH 9.5. The IC50 of fluoride inhibition of rabbit brain arginase were 0.95mM at pH 7.4, and 78.3mM at pH of 9.5. The range of concentration of fluoride in drinking water in the United States is 0.7 to 1.2 mg/L, the IOM recommended daily water intake of 3.7 liter, this implies a total daily intake of 4.44 mg of fluoride from drinking water, thus the finding of IC50 of fluoride inhibition of liver, kidney and brain arginases at pH 7.4 to be 1.75Mm, 1.68Mm and 0.95Mm respectively calls for a change in the policy of drinking water fluoridation around the world.

ABSTRACT FINAL ID: 3713 Poster Board: P405
TITLE: Oxidative DNA Damage of Peripheral Blood Polymorphonuclear Leukocytes, Selectively Induced by Chronic Arsenic Exposure, Is Associated With Extent of Arsenic-Related Skin Lesions

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KEYWORDS: Exposure, Environmental; Arsenic, Skin Lesion, Endemic Arsenic Poisoning, 8-Hydroxy-2'-deoxyguanosine (8-OHdG), Polymorphonuclear Leukocytes (PMNs), Monocytes (MNs)

ABSTRACT BODY: There is increasing evidence that oxidative stress is an important risk factor for arsenic-related diseases. Peripheral blood leukocytes constitute an important defense against microorganisms or pathogens, while the research on the impact of chronic arsenic exposure on peripheral blood leukocytes is much more limited, especially at low level arsenic exposure. The purpose of the present study was to explore whether chronic arsenic exposure affects oxidative stress of peripheral blood leukocytes and possible linkages between oxidative stress and arsenic-induced skin lesions. 75 male inhabitants recruited from an As-endemic region of China were investigated in the present study. The classification of arsenicosis was based on the degree of skin lesions. Arsenic levels were measured in drinking water and urine by Atomic Fluorescence Spectroscopy. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) was tested by Enzyme-Linked Immunosorbent Assay. 8-OHdG of peripheral blood leukocytes was evaluated using immunocytochemical staining. 8-OHdG-positive reactions were only present in polymorphonuclear leukocytes (PMNs), but not in monocytes (MNs). The 8-OHdG staining of PMN cytoplasm was observed in all investigated populations, while the 8-OHdG staining of PMN nuclei was frequently found along with the elevated amounts of cell debris in individuals with skin lesion. Urinary arsenic levels were increased in the severe skin lesion group compared with the normal group. No relationship was observed between drinking water arsenic or urine 8-OHdG and the degree of skin lesions. These findings indicated that the target and persistent oxidative stress in peripheral blood PMNs may be employed as a sensitive biomarker directly to assess adverse health effects caused by chronic exposure to lower levels of arsenic.

ABSTRACT FINAL ID: 3714 Poster Board: P406
TITLE: Combining Risk Assessment and Epidemiology Approaches to Interpreting Polycyclic Aromatic Hydrocarbons Soil and Air Data for Public Health

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KEYWORDS: Risk Assessment; Epidemiology; Polycyclic Aromatic Hydrocarbons

ABSTRACT BODY: The city of Sault Ste. Marie in Ontario, Canada is home to a steel manufacturing industry that is more than 100 years old. The manufacturing process involves distilling coal to produce coke and generates air emissions containing polycyclic aromatic hydrocarbons (PAHs), among other substances. Since 1998, one neighbourhood downwind of the steel mill has been the focus of soil and ambient air testing to investigate the extent of PAH deposition. These data showed elevated concentrations of PAHs in the neighbourhood, and were communicated to residents amid escalating public concerns about lung, colon and rectal cancer and childhood leukemia incidences in their community. To assist the local health authority in addressing public concerns, a two-pronged approach was conducted to assess PAH soil and air data and potentially associated cancer outcomes. First, toxicological and epidemiological data were used to identify possible health effects associated with PAHs and how and when people may be exposed. This information was used to estimate the incremental cancer risk associated with PAH exposures from air and soil. The second approach used Ontario cancer registry data to compare local incidence rates to comparator geographic areas. While it is clear from the measurements of PAHs in soil and air that there are elevated concentrations of PAHs present in the neighbourhood, likely originating from the steel mill, the results of the risk assessment predicted that less than a single PAH-related cancer would be expected to occur in neighbourhood residents exposed over 76 years. While cancer statistics at the neighbourhood level were not available, review of cancer statistics for the smallest geographic area available, Sault Ste. Marie, did not reveal evidence of an excess of cancers potentially related to PAHs. The conclusions from both approaches are similar and neither found evidence that the concentrations of PAHs in the neighbourhood have resulted in PAH related cancers. These results were provided to the local health authority and used in addressing community concerns.

ABSTRACT FINAL ID: 3715 Poster Board: P407
TITLE: The PhenX Toolkit: Consensus Measures for E-Cigarettes Research

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KEYWORDS: Bioinformatics; Respiratory Toxicology; Regulatory/Policy; E-Cigarettes

ABSTRACT BODY: The impact of the increasing use of e-cigarettes on public health is unknown. With studies ongoing, Federal research and regulatory agencies are seeking to expand the depth and breadth of tobacco-related measures that can enhance cross-study analysis. We report the results of the selection of consensus measures to enable data comparison, validation, replication, meta-analysis and collaboration in e-cigarette research. A Tobacco Regulatory Research Panel (TRRP) provided guidance to several expert working groups who collectively selected 51 measures in support of the HAVE model: Host, Agent, Vector and Environment. These measures standardize data collection of interpersonal factors that influence product use; characterize product use, exposures, and outcomes; assessment of tobacco products, industry and retailer activities, and environmental factors influencing tobacco use. The identification of standard measures these represents an important first step; use of these measures by industry, academics, and others in their research will lead toward greater understanding of the health impact of tobacco-related products. These measures are included in the PhenX Toolkit (consensus measures for Phenotypes and eXposures) as a publicly available, Web-based resource (www.phenxtoolkit.org) for the research community.

ABSTRACT FINAL ID: 3716 Poster Board: P408
TITLE: Associations Between Urinary Mercapturic Acids Derived From Volatile Organic Compounds and Liver Function Indexes in Young Men

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KEYWORDS: Epidemiology; Liver; Biomarkers; Volatile Organic Compounds

ABSTRACT BODY: Acrolein, acrylonitrile, benzene, and 1,3-butadiene are hazardous volatile organic compounds in tobacco smoke, which can be metabolized to 3-Hydroxypropylmercapturic acid (3-HPMA) , N-acetyl-S-(2-cyanoethyl)-cysteine (CEMA), S-phenylmercapturic acid (SPMA), and dihydroxy-butyl-mercapturic acid (DHBMA), separately. These mercapturic acids (MAs) can be regarded as specific biomarkers for evaluating exposure to these carcinogenic volatile organic compounds. Animal studies have shown that exposure to theses volatile organic compounds may affect liver function. However, human studies on associations between biomarkers of these volatile organic compounds and liver function are limited. We therefore recruited 147 male participants aged among 21 and 25 years old from Zhengzhou, China. Plasma and 24 hour urine samples, as well as questionnaires were collected. After excluded drinkers and hepatitis patients, 121 participants were involved in this study. Urine samples were analyzed for creatinine and four MAs. Concentrations of MAs were normalized by creatinine. Plasma samples were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total protein (TP), albumin (Alb), globulin (Glo) and albumin/globulin ratio (A/G). 3-HPMA, CEMA, SPMA, and DHBMA were detected in 95.0%, 95.0%, 23.1%, and 92.6% samples, separately. The results indicated that exposure to acrolein, acrylonitrile, and 1,3-butadiene was widespread in young men from Zhengzhou, China. There was significant positive association between normalized urinary CEMA and plasma ALP. Normalized urinary CEMA and DHBMA were found positively associated with plasma Alb. No significant association was found between urinary MAs and other liver function indexes. Among the 121 participants, 10, 28, and 18 subjects had abnormal TP, Alb, and Glo, separately. Binary logistic regression analyses showed that there were significant positive associations between normalized urinary CEMA, DHBMA and abnormal plasma Alb. Overall, our findings hint an effect for acrolein and 1,3-butadiene exposure on liver function. Although we did not show a causal associations due to the cross-sectional nature, our results provided clues for future epidemiologic and toxicology researches.

ABSTRACT FINAL ID: 3717 Poster Board: P409
TITLE: Associations Between Urinary Bisphenol-A Concentrations and Inflammation and Oxidative Stress Biomarkers Using Repeated Measures During Pregnancy

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KEYWORDS: Endocrine Disruptors; Reproductive and Developmental Toxicology

ABSTRACT BODY: Bisphenol-A (BPA) has been linked to an array of adverse health effects in both animal studies and in humans. While endocrine disrupting mechanisms have been investigated most thoroughly, the induction of oxidative stress and inflammation may be important pathologic responses as well. In this epidemiologic study we examine the relationship between urinary BPA concentrations and biomarkers of oxidative stress and inflammation using repeated measures taken from women across pregnancy. Pregnant women in the present analysis were part of a nested case-control study of preterm birth (N=130 cases, 352 controls), with subjects selected from a prospective birth cohort in Boston, MA (USA) between 2006 and 2008. At four study visits mothers provided urine and plasma samples for biomarker measurement (median 10, 18, 26, and 35 weeks gestation). We assayed urine samples for total BPA concentrations, as well as two biomarkers of oxidative stress, including 8-hydroxydeoxyguanosine (8-OHdG) and 8-isoprostane. In plasma, we measured C-reactive protein and cytokines IL- 1β, IL-6, IL-10, and TNF-α as markers of inflammation. Associations between exposure and outcome biomarkers were assessed using repeated measures models, which were weighted for case-control status so as to make results generalizable to the parent cohort population. BPA concentrations were associated with increases in both biomarkers of oxidative stress in fully adjusted models. An interquartile range (IQR) increase in BPA was associated with a 5.09% increase in urinary 8-OHdG (95% confidence interval [CI]=0.54, 9.84). For 8-isoprostane, an IQR increase in BPA was associated with an 8.79% increase (95% CI=1.66, 16.4). For biomarkers of inflammation, urinary BPA was associated with a significant increase in IL-6 (percent change in association with an IQR increase in BPA=10.2, 95% CI=3.88, 16.8), and this association persisted after inclusion of correlated phthalate metabolites in the same model (percent change=9.72, 95% CI=3.45, 16.4). In conclusion, maternal exposure to BPA in pregnancy was associated with increases in biomarkers of both inflammation and oxidative stress. These systemic changes may mediate adverse birth outcomes and/or fetal development.

ABSTRACT FINAL ID: 3718 Poster Board: P410
TITLE: Ambient Asbestos and Long-Term Trends in Pleural Mesothelioma Incidence Between Urban and Rural Areas in the United States (1973 To 2012)

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KEYWORDS: Exposure, Environmental; Lung; Pulmonary Or Respiratory System; Epidemiology; Asbestos

ABSTRACT BODY: Over the past 40 years, measured ambient asbestos concentrations in the U.S. have been reportedly higher in urban vs. rural areas. The purpose of this study was to compare U.S. pleural mesothelioma (PM) trends in historically occupationally exposed (male) and generally unexposed (female) populations to determine whether variations in ambient asbestos concentrations have influenced disease risk. Annual age-adjusted PM incidence rates from 1973 to 2012 were obtained from the Surveillance, Epidemiology, and End-Results (SEER) 9, 13, and 18 databases, and standardized rate ratios were compared for gender-specific urban and rural rates. Additionally, annual percent changes in PM incidence were calculated, and years with statistically significant changes in trend were identified. Male urban PM rates were elevated over rural rates for nearly all years examined and were statistically significantly elevated for 22 of the 40 years. In contrast, female rural rates exceeded urban rates in almost half of the years analyzed, although the increases were not statistically significant. Trend analyses demonstrated increases in male urban and rural incidence into the early 1990s followed by a decrease/leveling off, whereas trends for females remained relatively constant over time. The results suggest that measured differences in ambient asbestos concentrations do not appear to have influenced the risk of PM over time. These results are inconsistent with theoretical increased cancer risks calculated using current linear low-dose regulatory risk assessment models for asbestos.

ABSTRACT FINAL ID: 3719 Poster Board: P411
TITLE: Hair Mercury Level is Associated with Anemia and Increased Vitamin B12 in Children Living Near ASGM in the Peruvian Amazon

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KEYWORDS: Metals; Exposure, Environmental; Children'S Health; Methylmercury

ABSTRACT BODY: The Madre de Dios (MDD) region of the Peruvian Amazon has experienced rapid artisanal small-scale gold mining (ASGM) expansion over the past 10 years. ASGM releases inorganic mercury (Hg) directly into soil and river sediment and burns Hg off into the atmosphere. Anaerobic bacteria bio-methylate Hg to form methylmercury (MeHg), which biomagnifies in fish. Communities near ASGM are exposed to high levels of MeHg via consumption of contaminated fish. We examined health impacts of ASGM in ~4,000 individuals in ~1500 households across 19 MDD communities. Here, we report our analysis of total hair Hg, hemoglobin (Hb), and serum micronutrient levels in children <12 years of age (n=83). In our sample, 46% (n=33) of those aged 0-5 years (n=71) were anemic (<11 g/dL), as well as 58% (n=7) of those aged 5-11 years (n=12; <11.5 g/dL). Only three children were vitamin B12-deficient and none were folate-deficient, suggesting anemia was not due to malnutrition. Average total hair Hg, which represents primarily MeHg over the prior 2 months, was 2.2 μg/g (range: 0.054-9.7 μg/g). In contrast to lead, MeHg is not known to cause anemia. However, MeHg is linked to folate deficiency, which can lead indirectly to anemia. We found an inverse association between Hg and Hb (β =-0.122 g/dL, p=0.065), but no association between Hg and folate (p=0.40). However, Hg was positively associated with vitamin B12 (β =19.7 pmol/L, p=0.045, adjusted for age and sex). Hg and Hb remained inversely associated (p=0.08) in multiple linear regression of data clustered by community and adjusted for age, sex, supplement intake, and vitamin B12. MeHg inhibits methionine synthase (MS), which interacts with vitamin B12 and folate to regenerate methionine from homocysteine. Severe inhibition of MS by MeHg can lead to anemia. In addition, vitamin B12 alone or with folate increased *in vivo* Hg methylation in guinea pigs, which can compound MS inhibition. This work supports a novel link between MeHg exposure and anemia, and highlights the importance of epidemiology in environmental toxicology.

ABSTRACT FINAL ID: 3720 Poster Board: P412
TITLE: Influence of Thiopurine Methyltransferase (TPMT) Gene Polymorphisms on the Efficacy and Toxicity of 6-Mercaptopurine in Turkish Children with Acute Lymphoblastic Leukemia (ALL)—Including a Patient with a Rare Allele of *14

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KEYWORDS: Genetic Polymorphisms; Carcinogenesis; MALDI-TOF MS; 6-Mercaptopurine (6Mp)

ABSTRACT BODY: 6-Mercaptopurine (6MP), an anti-cancer prodrug, has been succesfully used in the treatment of childhood ALL. However, efficacy and/or toxicity of therapy is significantly influenced by inactivation of polymorphic enzyme, TPMT. Patients with low activity experience severe fatal hematological toxicity with standard 6-MP doses. First, the frequencies of four common alleles, TPMT*2, TPMT*3B, TPMT*3C, TPMT*3A, were investigated in 156 children with ALL by allele specific PCR and PCR-RFLP methods. Two patients were heterozygous with phenotypes of TPMT*1/3C and ,TPMT*1/3A and one patient was compound heterozygous carrying both *3A and *3C. For patients with genotypes of (*1//*3A), (*1/*3C) and (*3C/*3A) maintainance therapy had to be withheld 15%, 28% and 42%, respectively, because of febrile neutrapenia and infections. In addition, there were two other cases with similar hematological toxicities that could not be explained by their genotype. Thus, frequencies of common and rare TPMT alleles (TPMT*4,-*23) known for their low activity were also determined by MALDI-TOF Mass Spectrometry. In terms of rare alleles, MALDI-TOF analysis identified one heterozygous patient (TPMT *1/*14) with rare allele of * 14 and she succesfully completed therapy with slide side effects. Since 2007, she is in remission and not taking any anti-cancer drugs. Upto date, TPMT*14 allele was identified only in one patient and in her father in Sweden. In conclusion, TPMT*3A, 3C and*14 were the only deficients alleles detected in this study with the low allele frequences of 0.6%, 0.6% and 0.3%, respectively. In patients with common TPMT alleles, the side effects observed during 6 MP therapy and genotypes matched perfectly. On the other hand, in the patient with rare *14 allele and two patients with wild type genotype, the observed clinical histories cannot be explained by their genotypes. Furher research is needed in this respect.

ABSTRACT FINAL ID: 3721 Poster Board: P413
TITLE: BDDCS Classification Does Not Support BSEP Inhibition as Being DILI Causative

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KEYWORDS: Systems and Integrative Toxicology; Liver; Biotransformation And Toxicokinetics

ABSTRACT BODY: Drug-induced liver injury (DILI) is a leading cause of drug failure in clinical trials and a major reason for drug withdrawals from the market. Some primary compound-specific mechanisms have been linked to DILI including: cytotoxicity, reactive metabolite formation, inhibition of the bile salt export pump (BSEP), and mitochondrial dysfunction. The present study examined the clinical impact of the Biopharmaceutics Drug Disposition Classification System (BDDCS) in evaluating the severity of DILI warning in drug labels approved by the Food and Drug Administration (FDA), the withdrawal status due to adverse drug reactions (ADRs), and the role of BSEP inhibition. FDA drug labels for 182 registered drugs have been assessed for their BSEP inhibition using an *in vitro* membrane vesicle BSEP inhibition assay. The distribution of BSEP inhibition in each FDA hepatic liability category and BDDCS class were evaluated, as was, the correlation of all sources of FDA hepatic liability and BDDCS Class. Results were analyzed using chi-square tests; followed by post-hoc tests to identify which pairs were significant. For 73 of the 182 drugs identified as BSEP inhibitors, the compound was assigned to an “Adverse Reactions” category. In the same manner, 61 drugs were found to be in the “Warning and Precautions” category, 2 drugs in the “Withdrawn” category and 14 in the “Boxed Warning” category. The remaining 35 drugs were classified to have no mention of hepatic adverse reactions. When BSEP inhibition data were correlated with FDA drug labels of registered drugs, we observed no discernible pattern between BSEP inhibition and DILI severity assessment categories ($p = 0.41$). Yet, when FDA hepatic adverse reactions were correlated with BDDCS Class a highly significant result ($p < 0.0015$) was found. We also found a highly significant association between BSEP inhibition and BDDCS class ($p < 0.001$). We observe that the great majority of strong BSEP inhibitors are BDDCS Class 2 drugs (84.6%, n=33/39), with concomitant decreases in the percentages of BDDCS class 1 and 3 drugs as BSEP inhibition increases. It appears that an apparent correlation of BSEP inhibitors with DILI is not related to the BSEP inhibition process, but due to the fact that the great majority of BSEP inhibitors are BDDCS Class 2 compounds, which are highly correlated with DILI independent of the BSEP process.

ABSTRACT FINAL ID: 3722 Poster Board: P414
TITLE: Novel Selective Aryl Hydrocarbon Receptor Modulators and Their Properties *In Vitro* and *In Vivo*

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KEYWORDS: Receptor; Aryl Hydrocarbon; Dioxin; Cell Culture

ABSTRACT BODY: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the toxicity of dioxins, but also plays important physiological roles. Selective AHR modulators, which elicit some effects imparted by this receptor without causing the marked toxicity of dioxins, are presently under intense scrutiny. Two recently introduced such compounds are *N-acetyl-N-phenyl-4-acetoxy-5-chloro-1,2-dihydro-1-methyl-2-oxo-quinoline-3-carboxamide* and *N-acetyl-N-(4-trifluoromethylphenyl)-4-acetoxy-1,2-dihydro-5-methoxy-1-methyl-2-oxo-quinoline-3-carboxamide,* which as di-acetyl prodrugs represent *N*-hydrogen metabolites of the drug compounds laquinimod and tasquinimod, respectively, intended for the treatment of autoimmune diseases and cancer. Here, their selected *in vivo* properties were assessed in Sprague-Dawley rats. In addition, their de-acetylated active metabolites were studied *in vitro* using the H4IIE cell line. *In vivo*, both after a single dose and with repeated dosing for 5 consecutive days, there were no overt clinical signs of toxicity even at the highest doses achievable, limited by the solubility of the substances. However, both the absolute and relative weights of the thymus were found to be significantly decreased. The only marked alteration in serum biochemistry was a reduction in triglycerides. *Cyp1a1* gene expression, a sensitive marker for AHR activation, was substantially increased in all examined tissues: Liver, duodenum, kidney, testis and lung. *In vitro*, the compounds exhibited low if any tendency to cytotoxicity, as established by measurement of LDH leakage. Also, they were not mutagenic in the Ames test. Similarly to the *in vivo* outcome, they were very potent activators of *Cyp1a1*, in fact comparable to TCDD, as assessed by a luminescent assay. Thus, it appears that these novel compounds are potent activators of the AHR both *in vivo* and *in vitro*, but lack some major characteristic toxicities of dioxins, such as the wasting syndrome. They therefore represent promising new selective AHR modulators.

ABSTRACT FINAL ID: 3723 Poster Board: P415
TITLE: Glutamate Release Inhibitor Reduces Exosome Release in Metabotropic Glutamate Receptor 1 (GRM1) Expressing Melanoma

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KEYWORDS: Receptor; G-Protein Coupled; Signal Transduction

ABSTRACT BODY: GRM1, a G-protein coupled receptor whose ligand is glutamate, is normally expressed in the central nervous system and involved in neuronal signaling. It has been demonstrated that GRM1 mediated glutamatergic signaling is a key player in numerous cancers including melanoma. Aggressive cancers are characterized by the ability to invade surrounding tissues, and in some cases, metastasize to distal organs, which is the major cause of death in all cancers, including melanoma. Exosomes, nano-sized vesicles released by cancer cells in high quantities, have been postulated to promote metastasis. We investigate the putative involvement of exosomes in melanoma metastasis using melanoma cells and our melanoma-prone transgenic mouse model with two approaches: genetic and pharmacological inhibitors. Using an inducible siRNA to GRM1 we showed that including the inducer in the growth media reduced GRM1 expression and also exosomal production by 40%. Similar results were obtained with riluzole, an inhibitor of glutamatergic signaling. Daily treatment of our melanoma-prone mice with riluzole for 18 weeks showed no alteration in liver histology, liver or body weight. Blood was sampled periodically throughout treatment, exosomes were isolated, and immunoblots for the exosomal marker CD63 were performed as a qualitative analysis of circulating exosome load. A significant reduction (~50%, $p<0.001$) in CD63 was seen in the riluzole treated mice at week 18 compared to vehicle controls. Immunohistochemical analysis of the lung shows a 10% reduction in a marker for premetastatic niche formation in the treated group when compared to vehicle. Quantification of pigmented skin lesions also indicated a decrease of 10-20% in treated mice compared to vehicle. These preliminary results suggest that a reduction in GRM1 may lead to decrease in exosomal production and disease progression.

ABSTRACT FINAL ID: 3724 Poster Board: P416
TITLE: Further Evaluation of the CAR/PXR MOA and Human Relevance of Silthiofam-Induced Rat Liver Tumors

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KEYWORDS: Knockouts; Hepatocytes; Receptor; Nuclear Hormone; Silthiofam

ABSTRACT BODY: Silthiofam, a fungicide used as a seed treatment for wheat, caused an increase in liver tumour incidence in a 2-year rat study. The mode of action (MOA) and human relevance of these tumors were previously evaluated in a 2-week rat dietary study and *in vitro* studies in male rat and human hepatocytes. Results suggested that the MOA was via the activation of constitutive androstane receptor (CAR) and/or pregnane X receptor (PXR) and the proliferative response in rats is not relevant to humans. Additional studies have now been conducted in double knockout rat hepatocytes and female human hepatocytes. Hepatocytes isolated from male wild type (WT), CAR/PXR double knockout (CARKO/PXRKO) rats and cryopreserved human hepatocytes were cultured in the presence of silthiofam for 96 hr. Cell proliferation was evaluated by BrdU incorporation. CAR and PXR activation were assessed indirectly by evaluating mRNA expression and enzyme activity for CYP2B and CYP3A. In WT rat hepatocytes, silthiofam increased PROD, BROD and BQ enzyme activities (~2-5X); CYP2B1, CYP2B2 and CYP3A1 mRNA expression (~11-97X); and cell proliferation (~2X). However, exposure of CARKO/PXRKO rat hepatocytes to silthiofam only resulted in a slight increase in CYP2B1 mRNA expression (~5X). Most importantly, there was no increase in cell proliferation. Weak CAR and PXR activation, as indicated primarily by increases in CYP2B6 and CYP3A4 mRNA expression (~2-5), but no increase in cell proliferation was observed in female human hepatocytes exposed to silthiofam. Similar results were previously reported in male human hepatocytes. These results provide strong evidence that the MOA for silthiofam-induced hepatocellular proliferation in rats is CAR and/or PXR-mediated. The absence of a proliferative response in human hepatocytes indicates that silthiofam would be highly unlikely to elicit liver tumors in humans. Therefore, the increased incidence of liver tumors in the 2-year silthiofam rat study is not considered to be of human relevance.

ABSTRACT FINAL ID: 3725 Poster Board: P417
TITLE: Multi-Spheroid Imaging Analysis of Human Induced Pluripotent Stem Cell-Derived Cardiomyocyte by Cellvoyager CV7000 System for the Assessment of *In Vitro* Caridiotoxicity of Anti-Cancer Kinase Inhibitors

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KEYWORDS: *In Vitro* and Alternatives; Safety Evaluation; Kinase; Cardiomyocyte; Sunitinib

ABSTRACT BODY: Throughput screening is extremely important for early lead optimization process. Human induced pluripotent stem cell-derived cardiomyocyte (iPS-CM) are especially attractive because they express ion channels and demonstrate spontaneous mechanical and electrical activity. Actually, many publications showed that electrophysiological assessment with multi-electrode array system has excellent correlations between field potential duration and QTc prolongation. In the present study, we would like to introduce new *in vitro* cardiotoxicity assay method in iPS-CM by live-cell imaging analysis and present assay results with several anti-cancer kinase inhibitors. Human iPS-CM (iCell® Cardiomyocytes) was seeded into 96-half well plate which has 250 of fibronectin-coated spots in each well and spheroids were formed on all spots after 7 days culture in a plate. Calcium (Ca) sensing fluorescence dye and tested kinase inhibitor solutions were added and the dynamic changes in fluorescent intensity of all spheroids were measured at 24 hour after compounds incubation using Cellvoyager CV7000 system. From 30 seconds live-Ca image capture for each well, beat rate, minimum and maximum cellular Ca levels and peak Ca amplitude were analyzed. Spheroid size, nuclear intensicty and mitochondoria function were also checked at the same time. Human iPS-CM was incubated 24 hour with a concentration of 0.01, 0.1, 1.0 and 10 µmol/L of each kinase inhibitors, such as sunitinib, sorafenib, erlotinib, nilotinib, pazopanib and afatinib, respectively. Spontanious beating of iPS-CM spheroids were reduced by addition of sunitinib at 0.1 µmol/L and above. At 10 µmol/L, arrest of cardiomyocyte spheroids was observed by sunitinib. Sorafenib and nilotinib also slowed beat rate and sorafenib increased maximum cellular Ca levels. On the other hand, erlotinib, pazopanib and afatinib were not found significant effect on iPS-CM beating upto 10 µmol/L. More detail analyzed results including changes in nuclear intensicty and mitochondoria function will be presented.

ABSTRACT FINAL ID: 3726 Poster Board: P418
TITLE: An Automated 3D High-Content Screen for Migration of Neural Crest Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: B. Cai, S. Feng, K. Kordestani, R. Ingermanson, P. McDonough. *Vala Sciences, San Diego, CA.*
KEYWORDS: Neurotoxicology; Neural Crest Cells, Migration, 3D, Automation

ABSTRACT BODY: Cell motility is fundamental to development of neural-crest-derived structures in the embryo, as well as regeneration in certain animals, making it an attractive candidate for cell-based therapies, drug discovery and disease modeling. Using neural crest stem cells (NCSCs) derived from human parthenogenetic stem cells (hpSCs), we developed a scalable 3D high content screening (HCS) assay to measure the effect of chemicals on NCSC migration. In this work, cells were first seeded onto matrigel coated 384 well plate, a Teflon-coated pin tool was then used to create a “scratch” on a monolayer of confluent cells. In order for the assay to be routinely performed on hundreds of test chemicals, the pin tool incorporates 384 elements so that each well is scratched in parallel and reproducibly. The scalability of this assay enabled us to rapidly screen the NCC2 library of FDA-approved drugs as well as several reference compounds such as cytochalasin D and paclitaxel. Our experiment showed that the scratch produced a cell free area in the center of the wells, but at the same time damaged the matrigel coating and prevented cells migration. In order to allow cell migration, a matrigel and fibronectin mixture was add into the wells after the scratch to fill in the damaged coating area. This added matrix also embedded all the cells in a 3D mixture to mimic more closely the *in vivo* microenvironment for cell migration. After 7-10 days of incubation, cell migration was visualized by nuclear staining and evaluated by measuring the size of the remaining scratches. In the neutral control wells, the cells migrated into and closed the scratched area. This migration of cells was inhibited by cytochalasin D and paclitaxel treatment, while BMP4 treatment promoted cell migration. Not only did the imaging-based format of this assay enabled large-scale screening via fixed-endpoint screening, but it also allowed follow-up screens that incorporated staining for markers such as Sox10, and in some cases live-cell imaging to measure alterations in directional persistence for individual cells.

ABSTRACT FINAL ID: 3727 Poster Board: P419
TITLE: Conditions That Influence Functional Synaptogenesis in Commercially Available Human Stem Cell-Derived Neurons

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KEYWORDS: Cell Culture; Chemical and Biological Weapons; Embryonic Stem Cells

ABSTRACT BODY: Neurons derived from human pluripotent stem cells (hPSCs) have the potential to provide a physiologically relevant model for investigating human responses to biological and chemical neurotoxins. While many publications describe the differentiation of hPSCs into stem cell-derived neurons (hSNs) that exhibit molecular markers of neurotypic identity, most fail to functionally demonstrate the onset of synaptic currents and emergence of network activity. Therefore, we sought to develop conditions that accelerate the functional maturation of hPSCs into networked cultures of hSNs. hSNs were differentiated using commercially available hPSCs, culture reagents and protocols from Axol Biosciences and MTI-Globalstem. Morphological and functional maturation of hSNs were longitudinally evaluated using immunocytochemistry and whole-cell patch-clamp electrophysiology. Differentiated hSNs exhibited morphological markers of post-mitotic neurotypic identity (e.g., MAP2, Tau and NeuN) within weeks. In both cases, miniature post-synaptic currents were detected within 7 weeks, characterized by excitatory AMPA receptor-mediated events that were eliminated by treatment with CNQX. The onset time and frequency of spontaneous post-synaptic events were time- and density-dependent and developed subsequent to the maturation of intrinsic electrical behaviors. Neuronal culture in a defined electrophysiological buffer accelerated the onset of synaptic activity. Addition of botulinum neurotoxin serotypes A or B resulted in SNARE protein cleavage and eliminated spontaneous events, confirming that they represent synaptic neurotransmission. Collectively, these data suggest that careful differentiation of human-induced pluripotent stem cell-derived neurons using commercially available reagents may provide a physiologically relevant, synaptically active platform appropriate for mechanistic and therapeutic studies.

ABSTRACT FINAL ID: 3728 Poster Board: P420
TITLE: Bleomycin-Induced Cytotoxic Effect on Human Bronchial/Tracheal Epithelial 2D & 3D Model *In Vitro*

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KEYWORDS: Cytotoxicity; Lung; Pulmonary or Respiratory System; Cell Proliferation; Bronchial; Bleomycin

ABSTRACT BODY: Bleomycin (BLM) is an antibiotic that has been used in clinical cancer chemotherapy it's been more than 40 years. Approximately 46 % pulmonary damage and may be fatal in 1-2 % of the patients has been reported. Bleomycin Sulfate-Induced Pulmonary Fibrosis has been extensively described in mice. However, to our knowledge few *in vitro* human models have been implemented before. Here we investigated the differences of pathogenic/inflammatory potential of Bleomycin exposure on human Tracheal/Bronchial Epithelial cells between 2D cell and 3D tissue culture model. Dose dependent cytotoxicity curve was obtained by subjecting Normal Human Bronchial Epithelial (NHBE) cells to varying doses of BLM (0.1 µM to 400 µM). Lactate Dehydrogenase (LDH) tests were done to measure drug toxicity. Approximately 20% cells were dead at dose 400 µM. The cell proliferation decreased 40% compared to control culture at dose 0.1 µM BLM. NHBE Tissue were challenged via varying doses of Bleomycin Sulfate (0.1 µM and 10 µM) as well. Tissues were followed for 3 weeks for further observations. Transepithelial Resistance test was performed weekly. The experiments were completed by staining for tight junction, nuclear staining and mass spectrometry. No TEER changes has observed comparing to control tissues. The tight junction formation was similar across treated and non-treated tissues. LDH data showed tissue that were treated with 10µM showed 50% more cytotoxicity than the tissue treated with 0.1 µM. These findings suggest that Bleomycin induced toxicity on 3D tissue culture model and this might prove to be a prerequisite for translational research.

ABSTRACT FINAL ID: 3729 Poster Board: P421
TITLE: *Cyp2b6* Genetic Variability and Bupropion Metabolism in Pregnant Women

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KEYWORDS: Genetic Polymorphisms; Xenobiotic Transporters; Clinical Toxicology; Developmental Toxicity, Prenatal; Bupropion

ABSTRACT BODY: Bupropion (BUP) is indicated to promote smoking cessation in males and non-pregnant patients. However, data are still needed to support its potential use during pregnancy. A pilot study on the safety and efficacy of BUP sustained release as an aid for smoking cessation during pregnancy is underway at UTMB. CYP2B6 is the primary enzyme that metabolizes BUP to its active metabolite hydroxybupropion (OH-BUP). The differential response to BUP smoking cessation treatment is thought to be associated with the rate of CYP2B6-mediated formation of OH-BUP. The expression and activity of CYP2B6 enzyme shows large interindividual variability, partially due to genetic variants in the *CYP2B6* gene. These variants are arrayed into 6 major haplotypes: *CYP2B6**2 (C64T), *3(C777A), *4 (A785G), *5 (C1459T), *6 (G516T and A785G) and *7 (G516T, A785G and C1459T). The effect of *CYP2B6* haplotypes on BUP metabolism in pregnant women has not been evaluated. Here we evaluate the effect of these 6 CYP2B6 haplotypes on the rate of OHBUP formation in 70 pregnant women treated with BUP at steady state. A total of 13 haplotypes combinations were observed in these patients, including *1/*1 (30%), *1/*2 (5.7%), *1/*3 (2.9%), *1/*4 (1.4%), *1/*5 (5.7%), *1/*6 (30%), *1/*7 (1.4%), *1/*9 (4.3%), *2/*3 (1.4%), *2/*5 (1.4%), *2/*6 (2.9%), *2/*9 (2.9%), *6/*6 (10%). We then determined the plasma concentrations of OHBUP and BUP in pregnant women treated with BUP. The activity of CYP2B6 was estimated by the ratio of area under the curve (AUC) of OH-BUP to AUC of BUP. We observed a lower ratio of the mean of AUC OH-BUP/AUC BUP in carriers of the *6 than in the wild type *1/*1 carriers (24.7±10 and 12.1±10, respectively, $p<0.05$), suggesting that the *CYP2B6* *6 allele decreases the rate of BUP hydroxylation during pregnancy. The results are consistent with the reported functional effect of the *CYP2B6* *6 allele and suggest that the effect of *CYP2B6* polymorphisms on the BUP metabolism may be independent of pregnancy-associated maternal physiological changes (supported by R01 DA030998-01).

ABSTRACT FINAL ID: 3730 Poster Board: P422
TITLE: Hydroxyapatite Coatings Deposited by High Velocity Oxygen Fuel on CoCrMo Microimplants Promotes Positive Ossification and Biocompatibility of Intraoseal Implants *In Vivo*

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: A.De Vizcaya-Ruiz[1], V. Escamilla-Rivera[1], L. Rodriguez[2], M. Esquivel-Gaon[1], A. Giraldo[2], J. Hermann[2], M. Uribe-Ramirez[1], B. Chavez-Alvarez[3], C. Sam-Miranda[3], J. Fernández-Hernández[3], J. Muñoz-Saldaña[2]. [1]*Toxicology Department, Cinvestav-IPN, Distrito Federal, Mexico;* [2]*Materials-Queretaro, Cinvestav-IPN, Queretaro, Mexico;* [3]*UPEAL, Cinvestav-IPN, Distrito Federal, Mexico.*
KEYWORDS: Metals; Bone; Hip Prostheses

ABSTRACT BODY: Success of prosthetic implants depends on various intrinsic and biological factors mainly related to the surface exposed to the tissue. Biocompatible coatings, such as Hydroxyapatite (HAp), which is a bioactive ceramic deposited on metallic prosthetic implants substrates by thermal spray techniques interact directly with hard and muscle tissues increasing bone healing. We previously demonstrated that HAp coating deposited by High Velocity Oxygen Fuel (HVOF) on metallic surfaces has the potential to increase biocompatibility in human osteoblasts *in vitro*. In the present contribution the biocompatibility of HAp-coated intraoseal microimplants *in vivo* in male Wistar rats during 12 weeks was evaluated. Two types of implants (with and without HAp coating) were surgically placed inside the left femur in contact with bone marrow. After 2, 4, 6 and 12 weeks of recovery, rats were euthanized and biochemical, clinical and histopathological analyses were performed. Results showed that the presence of implants did not affect weight gain or general health of the animals during the study. No changes in hematological parameters (hemoglobin, red cells, and white cells subpopulations) were observed at any time point for both implants. Moreover, cytokines profile did not reveal signs of systemic inflammation or infections. Histological analyses showed that as soon as 2 weeks, HAp coated implants promote an increment of endochondral ossification with a minimal local inflammatory response compared to non-coated implant. None of the two implants induced osteonecrosis or affected hematopoiesis. Our results suggest that HAp coating by HVOF on metallic implants promoted an increment in osseointegration in the femur and favored the recovery of animals compared with uncoated ones, at 12 weeks. Deposition of HAp by HVOF emerges as a viable and low risk alternative to be considered in the development of prosthetic implants in medicine (Funding: Conacyt-PEI 221791).

ABSTRACT FINAL ID: 3731 Poster Board: P423
TITLE: Fast Track Towards Safety Assessment of New Therapeutic Targets in Neurodegenerative Diseases

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: B. Rakic[1], V. Veljovic[1], A. Stelkic[1], G. Apic[1], R.B. Russell[2] . *[1]Cambridge Cell Networks, Cambridge, United Kingdom; [2]University of Heidelberg, Heidelberg, Germany.*
KEYWORDS: Computational Toxicology; Systems And Integrative Toxicology; Safety Evaluation

ABSTRACT BODY: Good functional characterization of any therapeutic target is essential in order to minimize its side effects, maximize selective target activity and its clinical application. In our study, we focused on neurodegenerative diseases, and manually extracted and processed molecular information about those diseases from the public domain (PubMed articles and FDA reports). These data were used to populate a database module containing a complete biological network of all human proteins with their functions and associations to various neuronal pathologies. By applying proprietary algorithms, we were able to analyze mechanistic networks and biological pathways involving human proteins related to neurodegenerative processes. In order to test the system, we analyzed three potential targets such as LRRK2, PDE and Cathepsin B, to get quick insight into their biological functions and a list of possible on- and off- target effects, as an essential aspect for characterization as valid and safe therapeutic targets for Parkinson's, Alzheimer's and Huntington's disease. Application of developed database module and software tool granted fast and accurate target safety assessment for neurodegenerative diseases, saving time and increasing the coverage of traditional safety reports.

ABSTRACT FINAL ID: 3732 Poster Board: P424
TITLE: A QSAR Model for Thyroperoxidase Inhibition and Screening of a Large Set of Environmental Chemicals

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KEYWORDS: QSAR; Endocrine; Thyroid; Predictive Toxicology

ABSTRACT BODY: Thyroid hormones (THs) are critical modulators of a wide range of biological processes from neurodevelopment to metabolism. Well regulated levels of THs are critical during development and even moderate changes in maternal or fetal TH levels produce irreversible neurological deficits in children. The enzyme thyroperoxidase (TPO) plays a key role in the synthesis of THs. Inhibition of TPO by xenobiotics leads to decreased TH synthesis and, depending on the degree of synthesis inhibition, may result in adverse developmental outcomes. Recently, a high-throughput screening assay for TPO inhibition (AUR-TPO) was developed and used to screen the ToxCast Phase I and II chemicals. In the present study, we used the results from the AUR-TPO screening to develop a Quantitative Structure-Activity Relationship (QSAR) model for TPO inhibition in Leadscope®. The training set consisted of 898 discrete organic chemicals: 134 positive and 764 negative for TPO inhibition. A 10 times two-fold 50% cross-validation of the model was performed, yielding a balanced accuracy of 78.7% within its defined applicability domain. More recently, an additional ~800 chemicals from the US EPA Endocrine Disruption Screening Program (EDSP21) were screened using the AUR-TPO assay. This data was used for external validation of the QSAR model, demonstrating a balanced accuracy of 85.7% within its applicability domain. Overall, the cross- and external validations indicate a model with a high predictive performance. Next, we used the QSAR model to screen 32,197 environmental chemicals to which humans are potentially exposed. The model could predict 15,391 (47.8%) of the chemicals within its applicability domain, and of these 3786 (24.6%) chemicals were predicted to be positive for TPO inhibition. Predictions from this screening can be used in a tiered approach to prioritize putative thyroid disrupting chemicals (TDCs) for further evaluation. This abstract does not necessarily reflect US EPA policy

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ABSTRACT FINAL ID: 3733 Poster Board: P425
TITLE: Evaluation and Comparison of Bisphenol A Analog Activity Using ToxCast Data

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KEYWORDS: Alternatives To Animal Testing; ToxCast; Bisphenol A

ABSTRACT BODY: Bisphenol A (BPA) is used in consumer products and industrial applications, primarily in plastics, and has been detected in the environment, human urine, blood, and breast milk. Mainly studied as an endocrine disruptor, other toxicities, including obesity, metabolic conditions such as diabetes, and neurodevelopmental effects have also been associated with exposure to BPA, indicating that its effects may not be limited to estrogenicity. In addition, a number of BPA analogs are in use and may exhibit other additional toxicities. To address these unknowns, we examined the bioactivity of 21 BPA analogs across a selection of ToxCast/Tox21 assays grouped by 7 gene sets including estrogen receptor (ER), androgen receptor (AR), thyroid receptor (TR), peroxisome proliferator-activated receptor (PPAR), pregnane x receptor (PXR), aromatase (AROM), and aryl hydrocarbon receptor (AHR). The most active compounds were bisphenol AF (BPAF) (ER, AR, AROM, AHR), bisphenol A glycidyl methacrylate (TR), 3,3',5,5'-tetrabromobisphenol A (PPAR) and bisphenol B (BPB) (PXR). We used these data to produce toxicological prioritization index (ToxPi) scores and images to integrate and visually compare the toxicity profiles across all gene sets. The compounds with highest ToxPi scores were BPAF, BPA and BPB. We also mapped the intended gene targets for all ToxCast assays to their associated KEGG BRITE protein families in order to characterize their toxicity profiles on a broader spectrum. The compounds with the highest ToxPi scores were again BPAF, BPA and BPB, all of which were particularly enriched in the nuclear receptor, ion channel, transporter, and transcription factor protein families. Finally, we explored the structure-activity relationships of the analogs to the gene sets in order to determine how predicted models of their activity compares to our *in vitro* data. Using the OASIS Pipeline Profiler, we found good predictivity for ER and AR (17/21 and 13/21 correct) but not AHR or AR (3/21 and 8/21). These broad-based screening approaches allowed us to identify a wide spectrum of potential biological targets and build more comprehensive toxicity profiles of BPA and its analogs in order to better evaluate their potential health effects. This abstract may not necessarily reflect official Agency policy.

ABSTRACT FINAL ID: 3734 Poster Board: P426
TITLE: A Systematic Evaluation of Analogs for the Read-Across Prediction of Estrogenicity

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KEYWORDS: Risk Assessment; Endocrine; Estrogens; Computational Toxicology; Read-Across

ABSTRACT BODY: Read-across is a data gap filling technique widely used within category and analog approaches to predict a biological property for a target data-poor chemical using known information from similar (source analog) chemical(s). Potential source analogs are typically identified based on structural similarity. Although much guidance has been published for read-across, practical guiding principles for the identification and evaluation of the scientific validity of source analogs, which is a critical step in deriving a robust read-across prediction, remains largely lacking. This case study explores the extent to which 3 structure descriptor sets (Pubchem, Chemotyper and MoSS) and their combinations are able to identify valid analogs for reading across Estrogen Receptor (ER) activity for a specific class of chemicals: hindered phenols. For each target chemical, two sets of analogs (hindered and non-hindered phenols) were selected using each descriptor set with two cut-offs: (1). Minimum Tanimoto similarity (range 0.1 - 0.9), and (2). Closest *N* analogs (range 1 - 10). Each target-analog pair was then evaluated for its agreement with measured ER binding and agonism. Subsequently, the analogs were filtered using physchem properties ($LogK_{ow}$ & Molecular Volume) and the resultant agreement between each target-analog pair was evaluated. The data set comprised 462 hindered phenols and 296 non-hindered phenols. The results demonstrate that: (1) The concordance in ER activity rises with increasing similarity, (2) none of the 3 descriptor sets are clearly superior to the others for analog identification for ER read-across, (3) selecting hindered versus non-hindered phenols as analogs does not significantly improve concordance in ER activity, and (4) filtering of analogs using physchem properties improves overall concordance. As an example, selecting hindered phenols as analogs with a similarity cut-off of 0.9, the maximum concordance observed is 76% (Chemotyper+MoSS) for ER binding and 93% (Pubchem+MoSS) for agonism. Filtering with physchem properties increases these values to 78% (Chemotyper+Moss) for ER binding and 95% (Chemotyper alone) for agonism. This case study demonstrates how biologically-relevant chemical descriptors can be used to identify valid analogs for read-across. This abstract does not necessarily reflect US EPA policy.

ABSTRACT FINAL ID: 3735 Poster Board: P427
TITLE: Evaluation of Sequencing Approaches for High-Throughput Toxicogenomics

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: R. Thomas[1], A. Karmaus[2], P. Kothiya[2], M. Martin[1]. *[1]NCCT, US EPA, Research Triangle Park, NC; [2]ORISE Fellow at US EPA/ORD/NCCT, Research Triangle Park, NC.*
KEYWORDS: Computational Toxicology; Bioinformatics; Toxicogenomics

ABSTRACT BODY: Whole-genome *in vitro* transcriptomics has shown the capability to identify mechanisms of action and estimates of potency for chemical-mediated effects in a toxicological framework, but with limited throughput and high cost. We present the evaluation of 3 toxicogenomics platforms for potential application to high-throughput screening: 1. TempO-Seq utilizing custom designed paired probes per gene; 2. Targeted sequencing (TSQ) utilizing Illumina's TruSeq RNA Access Library Prep Kit; 3. Low coverage whole transcriptome sequencing (LSQ) using Illumina's TruSeq Stranded mRNA Kit. Each platform was required to cover the full transcriptome, operate directly with cell lysates, and be automatable with 384-well plates. Technical reproducibility was assessed using MAQC control RNA samples A and B, while functional utility for chemical screening was evaluated using six treatments at a single concentration after 6 hr in MCF7 breast cancer cells. All RNA samples and chemical treatments were run with 5 technical replicates. The 3 platforms achieved different read depths, with the TempO-Seq having ~34M mapped reads per sample, while TSQ and LSQ averaged 20M and 11M aligned reads per sample, respectively. Inter-replicate correlation averaged ≥0.95 for raw log2 expression values in all 3 platforms across all samples. When the ratio of MAQC samples A:B was correlated between the technologies and the reference MAQC-III Illumina results, r2 values >0.74 were observed, suggesting good technical reproducibility for each sequencing platform. When chemically-treated samples were evaluated, the inter-replicate and cross-technology correlations of fold-change values were significantly reduced. Bland-Altman plots revealed that genes with low read counts accounted for the greatest variability. Application of a minimum read-count cutoff was necessary to achieve good concordance. Finally, connectivity map (CMAP) analysis was conducted to evaluate the ability of each platform to identify modes-of-action in the chemically-treated samples. TempOSeq showed the best concordance with mechanistically similar chemical treatments; however, this may be due to the increased TempOSeq read depth. In summary, the 3 toxicogenomics platforms measured whole-genome transcript levels with good technical reproducibility and show promise for the integration of toxicogenomics into high-throughput screening. *This abstract does not necessarily reflect US EPA policy.*

ABSTRACT FINAL ID: 3736 Poster Board: P428
TITLE: Identification of Reference Chemicals for Evaluating Screening Methods for Thyroid Bioactivity

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KEYWORDS: Endocrine; Thyroid; Predictive Toxicology; Endocrine Disruptors

ABSTRACT BODY: EPA's Endocrine Disruptor Screening Program (EDSP) is incorporating High Throughput Screening (HTS) assays and computational tools into its screening and testing paradigm. To validate new HTS assays and predictive models of thyroid bioactivity, we identified reference chemicals with thyroid activity *in vivo* that span a range of potencies, are structurally diverse, and include chemicals known to interact with several key events in the thyroid pathway. Specifically, we examined results of EDSP Tier 1 assays that include thyroid-responsive endpoints (the amphibian metamorphosis, rat male pubertal, and rat female pubertal assays) from EPA and OECD validation studies, EDSP List 1 chemical data evaluation records, and 'Tier 1-like' studies in the literature. Studies were considered Tier 1-like if they were consistent with EDSP Tier 1 assay protocols for exposure routes, exposure timing, endpoints evaluated, and lacked overt toxicity at doses tested. Mechanistic studies in the literature were used to support reference chemical identification and characterization. Active reference chemicals met the following criteria: 1) the chemical had significant effects on thyroid-responsive endpoints in ≥2 independent Tier 1 or Tier 1-like studies and 2) the chemical had effects on thyroid weight/histopathology in at least one of the Tier 1/Tier 1-like studies or mechanistic evidence from at least 2 studies in the literature meeting inclusion criteria. Inactive reference chemicals met the alternate criteria: 1) the chemical must have been tested in all three Tier 1 assay types with no significant effects in any, and 2) no effects of the chemical were reported on thyroid-responsive endpoints in rodent or amphibian studies meeting minimum inclusion criteria in ToxRefDB, CEBS, or EcoToxDB databases. Using these criteria we identified 29 active reference chemicals and five inactive reference chemicals. Evidence in the literature links over half of these active reference chemicals to at least one specific key event in the thyroid pathway. The 34 candidate reference chemicals can be used to evaluate the performance of HTS assays and predictive models of thyroid bioactivity as alternatives to existing EDSP Tier 1 screening assays. *This abstract does not necessarily reflect EPA policy.

ABSTRACT FINAL ID: 3737 Poster Board: P429
TITLE: CASE Ultra Konsolidator: A Knowledge Driven Algorithm to Assist in Expert Review of ICH M7 Based (Q)SAR Analysis of Bacterial Mutagenicity of Impurities

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KEYWORDS: Genetic Toxicology; QSAR; Mutagenesis; ICH M7

ABSTRACT BODY: (Q)SAR approach has become an important *in silico* tool to predict mutagenic potential of drugs and impurities, particularly after the implementation of the International Conference on Harmonisation's (ICH) M7 guideline. The guideline recommends including predictions from both statistical and expert rule based models. In this context, it is beneficial to perform expert review of the raw output of the (Q)SAR predictions with the help of supporting information. Application of expert knowledge improves the accuracy, increases confidence, and provides rationale for the predictions. In this study, we are reporting features of Konsolidator - a knowledge driven algorithm that generates a variety of supporting evidence in a systematic and transparent fashion in order to assist those performing an expert review. Konsolidator was developed because expert review can be greatly complemented by relevant supporting evidence that can be generated rapidly and automatically. Supporting evidence provided by Konsolidator includes the structural relationship between the active drug substance and the impurity, an analysis of structurally similar analogs, an evaluation of relevance of alerts, and an evaluation of the structural features not covered by the (Q)SAR models. Konsolidator also suggests an overall call for bacterial mutagenicity assessment by combining results of statistical and expert rule based models, which resolves out of domain and inconclusive prediction calls. To validate Konsolidator, a test of 3566 chemicals (519 positives/3047 negatives, Japan NIEHS data) was performed against two bacterial mutagenicity statistical models and one expert rule based model. After applying Konsolidator, we obtained 62% sensitivity, 77% specificity and 95% coverage by using the best features of the individual models. A significant improvement of coverage was seen.

ABSTRACT FINAL ID: 3738 Poster Board: P430
TITLE: Mitigation of Bacterial Mutation (Q)SAR Alerts Using Limited-Ames and Tester Strain Data

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: K.P. Cross, C. Hasselgren, G. Myatt. *Leadscope, Inc., Columbus, OH.*
KEYWORDS: Computational Toxicology; Regulatory/Policy; Genetic Toxicology

ABSTRACT BODY: The ICH M7 guidance on pharmaceutical impurities describes how to handle impurities that are DNA reactive, including performing bacterial mutation testing. The guidance allows for deviations from fully adequate protocols and compliance with Good Laboratory Practices for practical reasons whereby “the selection of bacterial tester strains may be limited to those proven to be sensitive to the identified alert”. This poster describes a bacterial mutagenicity screening process using (Q)SAR predictions with follow-up limited-Ames testing to qualify impurities as negative. Bacterial tester strains were analyzed for sensitivity to particular Leadscope structural alerts using a large database of over 10,000 compounds with genetic toxicity data. 69% of the correlating alerts were highly sensitive in either Salmonella TA98 or TA100 and 14% to Salmonella TA102 or E. coli. Leadscope (Q)SAR predictions were run on a test set of 1633 compounds containing bacterial mutation data for 5 standard Ames strains (TA98, TA100, TA1535, TA1537, TA102 or E. coli WP2 yielding 88% specificity (935 true negatives/1067 total negatives). 231 test compounds contained at least one of 42 alerts highly sensitive in Salmonella TA98 or TA100. In a fail-fast scenario, pharmaceutical companies are running limited-Ames tests using only TA98 and TA100 strains on suspect impurities where only a limited amount of material may be available. To simulate limited-Ames testing, TA98 and TA100 experimental data was subsequently reviewed for the test compounds having alerts. This resulted in reversal of 43 of 132 false positive alert predictions (resulting when both TA98 and TA100 data were found to be negative) and confirmation of 171 true positive alert predictions (resulting when one or more of the two strains were found to be positive). This process resulted in elimination of one-third of the false positives from the M7 (Q)SAR predictions reducing further unnecessary testing while focusing additional testing on compounds of highest interest.

ABSTRACT FINAL ID: 3739 Poster Board: P431
TITLE: Assessing Interval Estimation Methods for Hill Model Parameters in a High-Throughput Screening Context

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: D.F. Kapraun, E.D. Watt, R. W. Setzer, R.S. Judson. *National Center for Computational Toxicology, Environmental Protection Agency, Research Triangle Park, NC.*
KEYWORDS: Bioinformatics; Computational Toxicology; Risk Assessment; Concentration-Response

ABSTRACT BODY: The Hill model of concentration-response is ubiquitous in toxicology, perhaps because its parameters directly relate to biologically significant metrics of toxicity such as efficacy and potency. Point estimates of these parameters obtained through least squares regression or maximum likelihood are commonly used in high-throughput risk assessment, but such estimates typically fail to include reliable information concerning confidence in (or precision of) the estimates. To address this issue, we examined methods for assessing uncertainty in Hill model parameter estimates derived from concentration-response data. In particular, using a sample of ToxCast concentration-response data sets, we applied four methods for obtaining interval estimates that are based on asymptotic theory, bootstrapping (two varieties), and Bayesian parameter estimation, and then compared the results. These interval estimation methods generally did not agree, so we devised a simulation study to assess their relative performance. We generated simulated data by constructing four statistical error models capable of producing concentration-response data sets comparable to those observed in ToxCast. We then applied the four interval estimation methods to the simulated data and compared the actual coverage of the interval estimates to the nominal coverage (e.g., 95%) in order to quantify performance of each of the methods in a variety of cases (i.e., different values of the true Hill model parameters). In general, we found that although confidence intervals produced by the various methods tended to have similar widths, certain interval estimation methods tended to be more reliable (in that actual coverage matched nominal coverage) in certain categories of situations (which we have characterized). No single method, however, tended to be more reliable than others in all situations. This work demonstrates a framework for obtaining interval estimates for potency and efficacy parameters, and thus provides a better means for quantifying uncertainty in risk decisions. (This abstract does not necessarily reflect EPA policy.)

ABSTRACT FINAL ID: 3740 Poster Board: P432
TITLE: ScrubChem: Building Bioactivity Datasets from PubChem Bioassay Data

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J.B. Harris[1], R. Judson[2]. *[1]NCCT, ORISE, Research Triangle Park, NC,[2]NCCT, US EPA, Research Triangle Park, NC.*
KEYWORDS: Endocrine Toxicology; Environmental Toxicology; Computational Toxicology; Chem-Bio Informatics

ABSTRACT BODY: The PubChem Bioassay database is a non-curated public repository with data from 64 sources, including: ChEMBL, BindingDb, DrugBank, EPA Tox21, NIH Molecular Libraries Screening Program, and various other academic, government, and industrial contributors. Methods for extracting this public data into quality datasets, useable for analytical research, presents several big-data challenges for which we have designed manageable solutions. According to our preliminary work, there are approximately 549 million bioactivity values and related meta-data within PubChem that can be mapped to over 10,000 biological targets. However, this data is not ready for use in data-driven research, mainly due to lack of structured annotations. We used a pragmatic approach that provides increasing access to bioactivity values in the PubChem Bioassay database. This included restructuring of individual PubChem Bioassay files into a relational database (ScrubChem). ScrubChem contains all primary PubChem Bioassay data that was: reparsed; error-corrected (when applicable); enriched with additional data links from other NCBI databases; and improved by adding key biological and assay annotations derived from logic-based language processing rules. The utility of ScrubChem and the curation process were illustrated using an example bioactivity dataset for the androgen receptor alpha protein. This initial work serves as a trial ground for establishing the technical framework for accessing, integrating, curating, analyzing, and making use of such massive bioactivity data. This abstract does not necessarily reflect US EPA policy. Contact: harris.jason@epa.gov (ORCID: 0000-0002-7371-0463)

ABSTRACT FINAL ID: 3741 Poster Board: P433
TITLE: SWIFT-Active Screener: Reducing Literature Screening Effort through Machine Learning for Systematic Reviews

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: K. Miller, B. Howard, J. Phillips, M. Shah, D. Mav, R. Shah. *Sciome, LLC, Research Triangle Park, NC.*
KEYWORDS: Environmental Toxicology; Bioinformatics; Systematic Review

ABSTRACT BODY: Evidence-based toxicology is an emerging discipline in which researchers within government, industry and non-profit research organizations are increasingly employing systematic review in order to rigorously investigate, analyze and integrate the evidence available in peer-reviewed publications. A critical and time-consuming step in this process is screening the available body of literature to select relevant articles for further review. To address this problem, we introduce SWIFT-Active Screener (SWIFT-AS), a web-application which uses novel statistical and computational methods to prioritize relevant articles for inclusion while offering guidance on when additional screening will no longer yield additional relevant articles. We tested SWIFT-AS on 20 diverse systematic review studies in which human reviewers have previously screened, in total, more than 115,000 titles and abstracts. When compared to a traditional screening procedure, this method resulted in a 54% reduction in screening burden, on average, while still achieving at least 95% recall; when tested on a subset of the 13 studies that contain >1,000 articles, the reduction in screening burden improved to 71%. While these results are very promising, machine-learning prioritization approaches such as this can only be deployed confidently if users are ensured that no critical article will be missed in the process. Accordingly, SWIFT-AS employs a novel algorithm to estimate recall while users work, thus providing a statistical basis for decisions about when to stop screening. Although this statistical confidence comes at a cost in terms of the total number of articles screened, the results indicate that, for large literature sets, the overall negative impact of our stopping algorithm is minimal. In SWIFT-AS, these unique methodological advancements are implemented as a user-friendly web application that allows users to manage their review, track its progress and provide conflict resolution. Together, these tools enable researchers to perform literature screening faster, cheaper and in a more reproducible manner.

ABSTRACT FINAL ID: 3742 Poster Board: P434
TITLE: High-Throughput Transcriptomics Via Select Sentinel Genes

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KEYWORDS: Toxicogenomics; Computational Toxicology; Bioinformatics; Tox21, High Throughput Transcriptomics

ABSTRACT BODY: One goal of the Tox21 high-throughput (HT) screening Phase 3 is to use transcriptomic changes as a surrogate to chemical induced toxicity to prioritize chemicals for further testing and to shed more light on the mode of action of chemical specific cellular responses. To generate HT transcriptomics profiles on large number of chemicals using a variety of cell lines or tissues, HT transcriptomics platforms capable of measuring expression levels of a set of representative or “sentinel” genes are being evaluated. To enable such HT transcriptomics screening, we developed a modular bioinformatics approach to identify the most relevant and biologically diverse set of sentinel genes that are (i) representative of highly diverse gene expression changes from publically available gene expression data sets, (ii) capable of predicting the expression changes observed across rest of the transcriptome, and (iii) represent all major biological pathways. Our bioinformatics approach is capable of utilizing expression values of approximately 2000 genes and predicting expression of rest of the transcriptome. We evaluated this extrapolation performance using data generated by Tempo-seq measurements of RNA expression levels for ~2300 rat genes in control and treated rat liver mRNA samples from the NTP DrugMatrix database which were also utilized by the SEQC consortium evaluating RNA-Seq technologies. The extrapolated gene expression and pathway enrichment scores were compared with the signal from RNA-seq and Affy array of these same RNA samples using a variety of parameters including Pearson correlation, mean squared error, concordance rate, and significance overlap. The results indicate that our approach yields accurately extrapolated gene expression resulting in mean log2 fold change with Pearson correlations (mean square error) of 0.79 (0.073) and 0.62 (0.36) when compared with Affy and RNA-seq respectively. Also, pathway level normalized enrichment scores resulted in the Pearson correlations (mean square error) of 0.75(0.75) and 0.71(0.86) when compared with that derived from the Affy and RNA-seq datasets.

ABSTRACT FINAL ID: 3743 Poster Board: P435
TITLE: Generating, Storing, and Presenting Large Quantities of Risk Data—The CRANK/Dashboard Approach

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KEYWORDS: Risk Assessment

ABSTRACT BODY: The Comprehensive Risk Analysis Kalculator (CRANK) and the "Dashboard Database" were developed for California Department of Food and Agriculture's (CDFA's) Statewide Plant Pest Prevention and Management Program as tools to generate, store, and present pesticide risk data for pesticide chemicals used to control invasive pests throughout California. This colossal project included an analysis of 8 pesticide programs, 10 human receptors, 51 terrestrial and aquatic ecological receptors, and 80 pesticide chemical ingredients. The task of producing large quantities of pesticide risk data for such a huge number of scenarios, receptors, and chemicals can seem insurmountable. However, through connecting various USEPA and other Microsoft Excel-based risk assessment models in a single workbook, the CRANK Excel workbook allowed for rapid automation and generation of risk assessment data. These features were further enhanced to manage large quantities of risk assessment data and scenarios through integration with the "Dashboard Database", a Microsoft Access database. This database was configured to serve as the reservoir for numerous risk assessment scenario inputs, to directly feed these inputs into the CRANK, and to receive, store, and display results from each individual CRANK run. In this presentation, the CRANK and "Dashboard Database" are presented as an example of how Excel-based mass workbooks and Access databases may be integrated together to generate, store, and present vast quantities of risk assessment data.

ABSTRACT FINAL ID: 3744 Poster Board: P436
TITLE: High-Throughput Sequencing of Circulating miRNAs in Human Reveals Novel Biomarkers for Drug-Induced Liver Injury, Hepatitis B, Liver Cirrhosis, and Type 2 Diabetes

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KEYWORDS: Toxicogenomics; Liver; Biomarkers

ABSTRACT BODY: Drug-induced liver-injury (DILI) is recognized as a major reason for withdrawal of drugs from the market. Since the conventional testing paradigm fails to detect about 40% of potentially hepatotoxic compounds, there is an urgent need for mechanistic biomarkers of liver injury applicable in clinical trials. Recently, cell free, serum circulating microRNAs (miRNAs) have shown a promise as biomarkers of injury in distant tissues. In this study we explored the potential of circulating miRNAs profiles (signatures) to serve as "liquid biopsies" for differentiating among a variety of liver impairments. We have used next-generation sequencing to study profiles of circulating miRNAs in serum samples taken from healthy subjects and subjects with accidental acetaminophen overdose (DILI), hepatitis B infection (HBV), liver cirrhosis (LC) and type 2 diabetes mellitus (T2DM). Compared to healthy subjects we identified a total 179 miRNAs that showed altered serum levels in subjects with liver impairments. As expected, the elevated levels of miR-122 and miR-192 were highly associated with liver injury. Interestingly, the individual disease states featured distinct miRNA signatures that were associated with their pathogenesis. For instance, the HBV signature featured miRNAs associated with immunity to HBV and the set of 61 miRNAs in T2DM signature contained several T2DM related miRNAs. Together, our study demonstrated for the first time that miRNA signatures show disease specificity and may have a potential to be used as "liquid biopsies", since the miRNAs in these signatures seems to correspond to pathogenesis of diseases or mechanisms of toxicity.

ABSTRACT FINAL ID: 3745 Poster Board: P437
TITLE: Photoreceptor Damage After Sodium Iodate Treatment Caused Plasma miR-183 Cluster and miR-124 Increase in Rats and Plasma miR-183 Increase in Cynomolgus Monkeys

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KEYWORDS: Biomarkers; Ocular Toxicity; miRNA

ABSTRACT BODY: Retinal toxicity is one of the leading causes of attrition in drug development. Derisking strategy to identify retinal toxicity early on with a retinal miRNA biomarker would benefit decision-making and reduce attrition due to retinal toxicity. To elucidate miR enrichment in rat retina, ~330 miRs were profiled using RT-qPCR. MiR-124 and miR-183 cluster (miR-183, 182 and 96) were the top 4 highly expressed miRs in rat retina. The 4 miRs were also highly differentially expressed compared with 14 other rat organs/tissues. Laser capture microdissection was used to assess the cellular expressions of these 4 miRs in rat retina. The data confirmed that miR-183 cluster was mainly enriched in photoreceptor layer and outer nuclear layer and miR-124 was highly enriched in inner nuclear layer. Our previous work indicated retina-enriched miRNAs significantly increased in plasma of a rat retinal toxicity model, reflecting retinal injuries. To validate previous findings, sodium iodate ($NaIO_3$) was used to induce retinal injury in rats. The data revealed that plasma miR-183 cluster was significantly increased 2-5 fold and miR-124 was also increased by 25 fold post repeat-dose of 30 mg/kg $NaIO_3$compared to baseline and controls, in which the severity of the retinal injury by histopathology evaluation was closely correlated with the changes of these plasma retina-enriched miRNAs. To test the translatability of the retina-enriched miRs, the retinal injury was induced with $NaIO_3$ in monkeys. In this monkey model, the plasma miR-183 was significantly increased 2-6 fold following $NaIO_3$ administration. Morphologic changes were observed in the photoreceptor layer of the monkey retina. Increases of these plasma miRNAs appeared to be dose- and time-dependent upon $NaIO_3$ treatment. Taken together, the data suggest that the retina-enriched miRs could serve as convenient and predictive biomarkers of retinal toxicity.

ABSTRACT FINAL ID: 3746 Poster Board: P438
TITLE: Pig-a Gene Mutation Frequencies in Healthy Human Subjects

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: Y. Luan, Y. Cao. *School of Public Health, Shanghai Jiaotong University School of Medicine, Shanghai, China.*
KEYWORDS: Biomonitoring; Mutation; Biomarkers

ABSTRACT BODY: The monitoring of genotoxic effects of carcinogens in humans for risk assessment is increasingly demanded. Cytogenetic methods are most often studied approaches, among them, human erythrocyte *PIG-A* assay is a novel approach for measuring *n vivo* mutation, which has been proved valuable in rodents, is promising for biomonitoring applications but lack of human data. The purpose of the current study is to determine the baseline of the erythrocyte *PIG-A* mutation frequency in healthy people, and for better understanding of how life style and common host variables may influence *PIG-A* mutation frequency. The current work used frequencies of CD59-negative red blood cells RBC^{CD59} serve as phenotypic reporters of *PIG-A* gene mutation. We determined the frequencies of spontaneous RBC^{CD59}- in 218 self-identified healthy volunteers, with age range of 18 to 92, 88 female and 130 male. The mean of RBC^{CD59}-frequencies was 5.42 4.4 10^{-6} and the median was 4.38 10^{-6} for all subjects, 14 subjects displayed mutant frequency >10 10^{-6}. Gender difference in RBC^{CD59}-frequencies was found, with mean frequencies were 6.26 and 4.16 10^{-6} in male and female subjects, respectively, showed a statistically significant difference in nonparametric tests with two independent samples ($p<0.001$). RBC^{CD59}-frequencies displayed no correlation with the age (p=0.77). The influence of smoking status was also investigated on male subjects, no significant difference was found between smoking and non-smoking subjects (p=0.63). However, RBC^{CD59}-frequencies showed a weak correlation with the pack-years cigarettes values in smoking subjects, with p value was 0.022 (two-tailed) by analysis of Pearson correlation coefficient, indicated that pack-years might be a sensitive endpoint given that *PIG-A* mutation frequencies increase with repeat exposure. The baseline level of RBC^{CD59}-frequencies in healthy people obtained in this study will be helpful for our further research to determine the assay's sensitivity in occupationally exposed individuals or hazardous environment.

ABSTRACT FINAL ID: 3747 Poster Board: P439
TITLE: Fit-for-Purpose Validation of Urinary C5b-9 As an Immune Complex-Mediated Glomerular Injury Biomarker in Cynomolgus Macaques

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M. Ko[1], A. Vitsky[1], T.P. Brown[2]. *[1]Drug Safety Research & Development, Pfizer Inc, San Diego, CA,[2]Drug Safety Research & Development, Pfizer Inc, Groton, CT.*
KEYWORDS: Biomarkers; Kidney; Immunotoxicity

ABSTRACT BODY: Non-human primates are commonly used in preclinical toxicology studies for biotherapeutic development. During such studies, animals may develop antidrug antibodies and immune complexes which can accumulate in the kidney and cause immune complex-mediated glomerulopathy. In an effort to identify a new diagnostic biomarker for immune complex-mediated glomerular injury, urinary C5b-9 was evaluated in a fit-for-purpose cynomolgus macaque validation study followed by an *in vivo* study in which immune complex-mediated glomerulopathy was intentionally induced. Using a commercial human C5b-9 assay kit, linear characteristics of the standard curve, intra- and inter-assay precision, freeze &thaw stability, dilution & urine matrix effects were assessed in a fit-for-purpose validation. Acceptable criteria were defined as a percent coefficient of variation 25%, and a recovery range of 70%-130%. Following assay validation, urine samples were collected in an *in vivo* study at baseline and then weekly from eight male monkeys administered bovine gamma globulin (BGG) daily for at least 4 weeks, and tested for urinary C5b-9. Results from the validation study showed the standard curve had linear characteristics, and that the intra- and inter-assay precision and the freeze & thaw stability met acceptable criteria. In the *in vivo* study, increases in BGG-related urinary C5b-9 occurred in all monkeys. Moreover, the urinary C5b-9 data correlated well with occurrence of mild-to-marked positive C5b-9 immunohistochemical staining in glomeruli, minimal-to-marked histologically observed glomerulopathy, and proteinuria in all monkeys. In conclusion, the results from the fit-for-purpose validation study and the *in vivo* BGG-administration study demonstrated that urinary C5b-9 could be a reliable biomarker for the occurrence of immune complex-mediated glomerulopathy in preclinical toxicology studies in cynomolgus macaques.

ABSTRACT FINAL ID: 3748 Poster Board: P440
TITLE: Comparison of Time Course of Urinary Liver-Type Fatty Acid-Binding Protein (L-FABP) and Other Urinary Nephrotoxicity Biomarkers of Drug-Induced Acute Kidney Injury in Rats

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KEYWORDS: Biomarkers; Kidney; Toxicity; Acute; L-FABP; Gentamicin

ABSTRACT BODY: L-type Fatty Acid Binding Protein(L-FABP), which is located in the cytoplasm of human renal proximal tubular cells, responses to ischemia and oxidative stress and is excreted in urine. KDIGO (Kidney Disease Improving Global Outcomes) selected L-FABP as one of the major 5 biomarkers (cystatin C, NGAL, Interleukins, Kim-1, L-FABP) in the world for detecting Acute Kidney Injury (AKI). The guideline published by JSN (Japanese Society of Nephrology) has listed urinary L-FABP as a useful urinary clinical surrogate marker following the clinical course of CKD. L-FABP ELISA kit has been approved as an IVD product in Europe and Japan. We have reported L-FABP is also very useful as a toxicological renal biomarker in experimental animals. The purpose of this research was to compare the time course of urinary L-FABP level with other urine biomarkers(Kim-1, NGAL, NAG, Cystatin C, Total protein) in AKI rat models induced by Gentamicin(GM) 50 or 200 mg/kg/day, s.c. for 4 days, Cisplatin(CDDP) 4mg/kg, i.v. single, and Amphotericin B(AMB) 50mg/kg, i.p. single. In all AKI models, mild (GM and AMB) or sever (CDDP) injury occurred in the renal proximal tubule. L-FABP and other renal biomarkers showed characteristic changes after the dosing. Only L-FABP extremely elevated (more than 3-fold) within 24 hours after the dosing in all AKI models, whereas NGAL increased greater than that of L-FABP only in the AMB model. Kim-1 did not changed in early period after the doing in all AKI models, but Kim-1 as well as L-FABP elevated significantly in later period after dosing in the CDDP model. These results suggest that the urinary L-FABP is very sensitive biomarker for detecting mild renal proximal tubule injury, while Kim-1 is a biomarker for detecting relatively severe renal proximal tubule injury.

ABSTRACT FINAL ID: 3749 Poster Board: P441
TITLE: Response of Novel Skeletal Muscle Biomarkers in Dogs following Sustained Endurance Exercise or Drug-Induced Skeletal Muscle Injury

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KEYWORDS: Biomarkers; Muscle Toxicity; Toxicity; Chronic

ABSTRACT BODY: The novel skeletal muscle (SKM) injury biomarkers, Skeletal Troponin I (sTnI), Myosin Light Chain 3 (Myl3), and Creatine Kinase Muscle Isoform (Ckm) have been shown recently to be more sensitive and specific than the conventional biomarkers, aspartate transaminase (AST) and creatine kinase (CK) enzymatic assays in rat toxicology studies. To evaluate the utility of these SKM biomarkers to translate across species, they were evaluated in two dog models: 1) before and following a 160 km endurance exercise run completed by 10 Alaskan sled dogs within 24h, and 2) before, during, and following daily dosing of 8 dogs with Merck A for 12 weeks, which resulted in Grade 1-4 skeletal muscle degeneration. In the drug-induced injury model, mean sTnI and Myl3 plasma levels were increased by 3- and 15-fold respectively over baseline and over a group of 6 control dogs as early as Study Day (SD)15, while mean plasma CK and AST levels did not increase, and biopsy samples were negative for histopathology until SD 29. Peak group mean plasma fold-change responses over baseline for sTnI, Myl3 and Ckm biomarkers were 46, 85 and 11-fold respectively, compared to 2.5-fold for AST and 4-fold for CK-enzymatic assay. In the sled dog sustained exercise model, the peak response for all biomarkers was observed at the first sampling (2h) after the 24h run. Individual dog biomarker increases varied, and correlated well with each other. The sTnI, Myl3 and Ckm mean fold peak increases compared to baseline were 82, 122 and 150-fold respectively, while AST changed 7-fold and CK-enzymatic 29-fold. These findings support the hypothesis that sTnI, Myl3 and Ckm are sensitive early tissue leakage biomarkers for monitoring SKM injury in dog, extending their utility across preclinical species beyond the rat, and provide further support to investigate their translational utility to clinical settings to monitor SKM injury and ensure patient safety against drug-induced skeletal muscle injury in early clinical trials.

ABSTRACT FINAL ID: 3750 Poster Board: P442
TITLE: Quantifying Bile Acids as Biomarkers to Elucidate Mechanistic Information on Hepatocellularcarcinoma Development

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KEYWORDS: Biomarkers; Hepatic; Carcinogenesis; Bile Acids

ABSTRACT BODY: Bile acids are being studied as biomarkers of digestive diseases as well as indicators of gut microfloral function. Bile acids may be used to elucidate mechanisms of diseases such as hepatocellularcarcinoma (HCC). Farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily with endogenous ligands as bile acids. Mice with whole-body FXR knockout (FXR KO) develop HCC spontaneously, but the underlying molecular mechanism is unclear. Through a longitudinal study, we observed that hepatocyte-specific FXR knockout mice (FXR Liv KO) did not have spontaneous HCC development (up to 24 months) while FXR KO mice did. However, these FXR Liver KO mice were susceptible to bile acid-induced liver tumorigenesis. Our aim was to determine the mechanism by which FXR Liv KO mice are resistant to HCC and whether hepatic FXR deficiency might serve only as a tumor initiator. Our hypothesis was that increased bile acids are required to promote carcinogenesis in FXR Liv KO mice. To demonstrate the dependence of bile acids in HCC development in FXR Liv KO mice, quantifying individual bile acids is required. In order to characterize the differences in individual bile acid levels in FXR Liv KO, a unique bile acid profiling method is required. For this, we have developed a high-performance liquid chromatography tandem mass spectrometry (HPLC-MS-MS) (ITMS) method for profiling of 23 unique bile acids in multiple sample types including liver, gall bladder, Ileum, and plasma. This method uses acetonitrile homogenization, followed by centrifugal separation and preconcentration under nitrogen, before injection into the LC. For this study, liver was the main tissue analyzed. It was found that FXR Liv KO mice had only slightly altered bile acid composition (<50% of bile Acids analyzed not significantly different), compared to wild type mice. The relative percentage of β-MCA and ω-MCA in the liver was increased significantly (<2% of total BA's) in FXR KO and FXR Liv KO mice. Bile acids that have lower concentration, such as CDCA, α-MCA and HDCA, were also significantly higher FXR Liv KO mice (<0.2% of total Bile Acids). Overall, the levels of ω-MCA, DCA, CDCA and α-MCA were significantly increased in FXR Liv KO mice compared to FXR KO mice, while HDCA and β-MCA also showed slight increase in FXR Liv KO mice compared to wild type mice. Bile acid profile may contribute to differential HCC development between FXR KO and FXR Liv KO mice.

ABSTRACT FINAL ID: 3751 Poster Board: P443
TITLE: From Genome-Wide Arrays to Tailor-Made Biomarker Readout—Progress Towards Routine Analysis of Skin Sensitizing Chemicals with GARD

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KEYWORDS: Immunotoxicology; Alternatives to Animal Testing; Biomarkers

ABSTRACT BODY: Allergic contact dermatitis (ACD) is caused by repeated exposure to skin sensitizing chemicals. To limit occurrence of such compounds, development of high-throughput assays for proactive risk assessment of chemicals are important to avoid outbreaks of ACD. Recently, we presented the GARD (Genomic Allergen Rapid Detection) assay as an alternative to animal testing for this endpoint. The assay uses a Support Vector Machine (SVM) to classify unknown compounds as either skin sensitizers or non-sensitizers, based on a biomarker signature of 200 genes. Using genome-wide microarrays, the accuracy of GARD is estimated to 89%. To improve performance and sample throughput, we transferred GARD procedures from microarrays to the nCounter platform, measuring only the 200 genes in the signature. We introduced the concept of including samples with known sensitizing properties into a testset, so-called benchmarks, and we challenged GARD with an external test set comprising 29 blinded chemicals previously unseen by the model. Various machine learning algorithms were evaluated to find the classification model that best fit the characteristics of the data. Algorithms were trained on nCounter measurements, calibrated on benchmarks, and applied to predict class belongings of tested samples. Using the SVM model for classification of chemicals in the test set, the accuracy was estimated to 90%, similar to the performance of the microarray platform. However, when changing to a calibrated Random Forest model (RF), all of the testing compounds could be accurately assigned to right class, reaching an accuracy of 100%. The nCounter platform validates measurements on the discovery platform, and enables a faster and more robust quantification of genes in signature. Combining high quality nCounter data and the concept of using benchmarks with a powerful RF model for classification, the GARD assay was demonstrated to have potent ability to predict sensitization, while complying with industrial demands for sample throughput and costs.

ABSTRACT FINAL ID: 3752 Poster Board: P444
TITLE: Optimization of Normalization Methods Improves Urinary miRNA Biomarker Evaluation

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: R.A. Thomas, G. Moran, C. Williams, K. Frazier, K. French, D. Ennulat.
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KEYWORDS: Biomarkers; Rt-Pcr; Toxicity; Acute; Cisplatin

ABSTRACT BODY: Ideal biomarkers are obtained through non-invasive sampling methods and are translatable across species. Urinary miRNA meet these criteria and provide the added benefit of prolonged storage stability. Adaptation of best practices across laboratories and models may aid in identification of urinary miRNA biomarkers for renal injury. The objective of this study was to use an established rat cisplatin single-dose injury model to compare urinary miRNA data normalized to an endogenous small RNA with miRNA data normalized to volume and/or urine concentration. Histopathology and urinary protein biomarker data were consistent with the dose-responsive and temporal proximal tubule injury that has been previously described. miRNAs that performed acceptably in a 5-point standard curve and demonstrated low variability in control rats were included for analysis. $4x10^7$ copies of ath-miR-159 were added per microliter urine prior to RNA extraction to enable normalization of miRNA data to urine volume. RNA was eluted into a constant volume of water and loaded into cDNA synthesis reactions. To measure urinary miRNA changes correlating with cisplatin treatment, miRNA threshold cycle (C_T) data were normalized to ath-miR-159 or an endogenous small RNA (U6), then calibrated to time-matched controls to generate Input Values (IVs). U6 was unsatisfactory as a house-keeping small RNA for this experiment because changes in expression correlating with cisplatin treatment were observed. No changes in ath-miR-159 were detected. Input values were then normalized to creatinine (Cre) to adjust for variability in urine concentration to generate Cre Normalized Input Values (CNIVs). Normalization of IVs to Cre decreased sample variability among untreated control animals when compared with IVs without normalization to Cre. miRNAs demonstrating acceptable standard curves, low variability in urine from control rats, and 2-fold increases in group mean CNIV were considered changed. Use of this strategy identified 11 miRNAs (miR-19b,-106b,-20a,-27a,-30a,-30d,-145 -192,-203, -218,-365) that were changed and correlated with renal injury on day 5, at least 7 of which have previously been identified in the literature related to renal injury. Use of the CNIV mitigates sample variability and clarifies meaningful changes among treatment groups; this method of normalization should be considered when examining urinary miRNAs.

ABSTRACT FINAL ID: 3753 Poster Board: P445
TITLE: Development of a Toxicogenomics Signature of Teratogenicity in H9 Human Embryonic Stem Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: N. Chepelev[1], A. Williams[2], R. Gagne[2], C. Yauk[2], L. Recio[1] .[1]*Integrated Laboratory Systems, Research Triangle Park, NC,*[2]*Health Canada, Ottawa, ON, Canada.*
KEYWORDS: Developmental/Teratology; Biomarkers; Predictive Toxicology

ABSTRACT BODY: *In vitro* gene expression biomarkers offer several advantages for toxicity testing, including reduced use of animals, increased throughput, and greater mechanistic understanding of toxicity pathways. The assessment of teratogenicity of chemical compounds in embryonic mouse cells has been approved by some regulatory authorities; however, animals respond to some teratogens differently than humans. Therefore, human embryonic stem cells (hESCs) may be more suitable for *in vitro* testing to predict teratogenicity in humans. In this work, H9 (WA09) hESCs were dosed with seven teratogens and 10 non-teratogens. The mRNA was sequenced on an Ion Proton Sequencer at 20 million reads/sample. The data were used to calculate counts per million in R, and data quality was assessed by boxplots, hierarchical clustering, and principal component analysis. The data were then standardized to the mean of control samples and quality assessment was repeated. Genes with no ensemble IDs or less than 10 counts were removed. The Kruskal-Wallis rank sum test (significance level of 0.001) was used to identify differences between teratogens and non-teratogens. The filtered data were used to estimate shrunken centroids using the pamr.R and the classifier was assessed using 7-fold cross validation. The resulting classifier contains 58 genes, including ERCC1 (Excision Repair Cross-Complementation Group 1) that is important for *in utero* development, and correctly assigned the 16 out of 17 compounds tested to either teratogens or non-teratogens with the 90% probability cut-off. Overall, this work produced gene expression signature for teratogenicity testing that potentially further expands on our mechanistic understanding of chemically-induced teratogenicity. This work was funded by NIEHS SBIR No. 2R44ES022114-02 and Health Canada funds.

ABSTRACT FINAL ID: 3754 Poster Board: P446
TITLE: Cytochrome P450-Mediated Metabolism of Triclosan Attenuates Its Cytotoxicity in Hepatic Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: Y. Wu, F.A. Beland, J.-L. Fang. *National Center for Toxicological Research, Jefferson, AR.*
KEYWORDS: Cytochrome P450; Cytotoxicity; Phase Ii Metabolism; Triclosan

ABSTRACT BODY: Triclosan is a widely used broad spectrum anti-bacterial agent. The objectives of this study were to identify which cytochrome P450 (CYP) isoforms metabolize triclosan and examine the effects of CYP-mediated metabolism on triclosan-induced cytotoxicity. A panel of HepG2-derived cell lines was established, each of which overexpressed a single CYP isoform including CYP1A1, 1A2, 1B1, 2A6, 2A7, 2A13, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, 3A7, 4A11, and 4B1. Seven isoforms were capable of metabolizing triclosan, with the order of activity being CYP1A2 > 2B6 ≈ 2C19 > 2D6 ≈ 1B1 > 2C18 ≈ 1A1. The remaining 11 isoforms (CYP2A6, 2A7, 2A13, 2C8, 2C9, 2E1, 3A4, 3A5, 3A7, 4A11, and 4B1) had little or no activity toward triclosan. Two metabolites were detected: 2,4-dichlorophenol and a currently unidentified product. Consistent with the *in vitro* screening data, triclosan was extensively metabolized in HepG2 cells overexpressing CYP1A2, 2B6, 2C19, 2D6, and 2C18, and these cells were much more resistant to triclosan-induced cytotoxicity compared to vector cells, suggesting that CYP-mediated metabolism of triclosan attenuated its cytotoxicity. This was further substantiated by the fact that 2,4-dichlorophenol was less toxic than triclosan to HepG2 cells. Triclosan-induced cytotoxicity was also attenuated in cells overexpressing CYP2A6, 2A13, 2C9, 3A4, and 4A11, which barely or minimally metabolized triclosan. In the culture medium of these cells, the triclosan concentration was decreased, accompanied by increased levels of triclosan glucuronide and triclosan sulfate, indicating that the glucuronidation and sulfation of triclosan are also detoxification metabolic pathways. Among the 18 CYP-overexpressing cell lines, an inverse correlation was observed between cell viability and triclosan concentration. In conclusion, we identified major CYP isoforms that metabolize triclosan, and found that CYP-mediated phase I metabolism and UGT- and SULT-mediated phase II metabolism of triclosan decreased its cytotoxicity in hepatic cells.

ABSTRACT FINAL ID: 3755 Poster Board: P447
TITLE: Human Liver Cytochrome P450 Activity, Abundance, and Expression Throughout Development

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: A.T. Wright, N.C. Sadler, P. Nandhikonda, J.N. Smith, R.A. Corley. *Pacific Northwest National Laboratory, Richland, WA.*
KEYWORDS: Cytochrome P450; Proteomics; Developmental Toxicity; Prenatal

ABSTRACT BODY: The expression and activity of drug metabolizing enzymes (DME), such as the cytochrome P450 superfamily, play a major role in the overall pharmacokinetic differences observed between adults, children, neonates, and fetuses. The expression and activity of P450 enzymes varies considerably throughout human development, and the deficit in our understanding of these dynamics limits our ability to predict environmental and pharmaceutical exposure effects. A comprehensive understanding of P450 expression and activity throughout human development is needed to enable predictive therapeutic dosing, and enable the avoidance of potentially adverse and harmful reactions during maturation from both therapeutic drugs and environmental toxins/agents. To develop a more comprehensive understanding of the ontogeny of P450 expression and activity we performed a multi-omic characterization of P450 transcript expression, protein abundance, and functional activity on liver tissues from fetus to late adulthood. We measured gene and protein expression by RT-PCR and global proteomics, respectively, and we employed activity-based protein profiling (ABPP) to interrogate P450 activity. We characterized a number of age-dependent features: (1) P450 1A2 was identified at very low levels by both qRT-PCR and global proteomics, but no measure of activity was made by ABPP in fetal samples. (2) P450 2D6 was observed with considerable gene expression but limited protein expression and enzyme activity. (3) Fetal activity in the 3A family was largely observed as isoform 3A7, but during postnatal development 3A7 is rapidly replaced by isoforms 3A4 and 3A5. (4) Family 1-3 P450 enzymes are generally not active in fetuses, but rapidly become active after birth. The data herein yield further evidence for classifying P450s to various life-stages based on expression and activity, but also serve to modify prior classifications and add data for a number of other P450s not previously studied. *Funded by NIEHS Grant No. P42 ES016465*.

ABSTRACT FINAL ID: 3756 Poster Board: P448
TITLE: Meperidine Metabolism: Roles of CYP2B6, CYP2C19, and CYP3A4 in Generation of the Neurotoxic Metabolite, Normeperidine

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J.L. Murray, S.L. Mercer, K.D. Jackson. *Pharmaceutical Sciences, Lipscomb University College of Pharmacy and Health Sciences, Nashville, TN.*
KEYWORDS: Cytochrome P450; Metabolism; Neurotoxicology

ABSTRACT BODY: Meperidine, or Demerol®, is an opioid analgesic for moderate to severe pain. When given in high doses or over prolonged periods, accumulation of the neurotoxic metabolite normeperidine can cause agitation, tremors, and seizures. Previous studies have shown that normeperidine is formed via N-demethylation by cytochromes P450 (CYP) 2B6, 2C19, and 3A4; however, the relative enzyme contributions are not well established. The goals of this project were to define the roles of CYP2B6, CYP2C19, and CYP3A4 to meperidine N-demethylation, and evaluate the effect of P450 polymorphisms on normeperidine formation. Reaction phenotyping studies were performed by incubating meperidine with individual recombinant P450s, and normeperidine levels were measured by LC-MS/MS. Recombinant CYP2B6 and CYP2C19 generated higher levels of normeperidine than CYP3A4 and CYP3A5 in the following order: CYP2B6 ≈ CYP2C19 > CYP3A4 > CYP3A5. To confirm the enzyme contribution, meperidine was incubated with pooled human liver microsomes (HLM) in the presence of P450-selective inhibitors. Ketoconazole, a CYP3A inhibitor, and ticlopidine, a CYP2B6 and CYP2C19 inhibitor, were added to reactions containing pooled HLM. Ticlopidine (5 µM) decreased normeperidine formation by 37% ($p = 0.006$), whereas ketoconazole (1 µM) decreased normeperidine formation by 23% ($p = 0.038$), compared to control. Normeperidine formation was also examined in a small set of individual genotyped HLM from CYP2C19*1/*1 (rapid metabolizer), *1/*2 (intermediate metabolizer), and *2/*2 (poor metabolizer) donors. Normeperidine levels were significantly higher in CYP2C19*1/*1 HLM compared to CYP2C19*2/*2 ($p = 0.0075$). In microsomes from two CYP2C19*1/*2 donors, normeperidine formation was higher from a younger (21 yrs) vs. older (62 yrs) donor ($p = 0.0225$). Moreover, Pearson correlation analysis revealed that normeperidine formation correlated with CYP2B6 activity ($r = 0.96$, $p = 0.0427$), and to a lesser extent with CYP2C19 activity ($r = 0.92$, $p = 0.0801$) and CYP3A4 activity ($r = 0.80$, $p = 0.201$). Collectively, these data suggest that CYP2C19 is involved in meperidine metabolism; however, CYP2B6 may play a more important role. Since CYP2B6 is highly polymorphic, future analysis of normeperidine formation with different CYP2B6 allelic variants should provide valuable insight into the pharmacogenetics of meperidine neurotoxicity.

ABSTRACT FINAL ID: 3757 Poster Board: P449
TITLE: Alginate Immobilization of Metabolic Enzymes (AIME) for High-Throughput Screening Assays

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: D.E. DeGroot, R. Thomas, S.O. Simmons. *National Center for Computational Toxicology, US Environmental Protection Agency, Research Triangle Park, NC*.
KEYWORDS: *In Vitro* and Alternatives; Metabolism

ABSTRACT BODY: The EPA's ToxCast program utilizes a wide variety of high-throughput screening (HTS) assays to assess chemical perturbations of molecular and cellular endpoints. A key criticism of using HTS assays for toxicity assessment is the lack of xenobiotic metabolism (XM) which precludes both metabolic detoxification as well as bioactivation of chemicals tested *in vitro* thereby mischaracterizing the potential risk posed by these chemicals. To address this deficiency, we have developed an extracellular platform to retrofit existing HTS assays with XM activity. This platform utilizes the S9 fraction of liver homogenate encapsulated in an alginate gel network which reduces the cytotoxicity caused by direct addition of S9 to cells in culture. Alginate microspheres containing encapsulated human liver S9 were cross-linked to solid supports extending from a 96-well plate lid and were assayed using a pro-luciferin substrate specific for CYP3A4 (IPA). We demonstrate that S9 was successfully encapsulated and remained enzymatically active post-encapsulation with 5-10X the CYP3A4 activity as compared to 1 µg solubilized human liver S9. Ketoconazole, a known inhibitor of human CYP3A4, inhibited CYP3A4 activity in a concentration-dependent manner (IC50: 0.27 µM) and inhibition was similar to that of solubilized S9 (IC50: 0.15 µM). Inhibition of CYP3A4 with ketoconazole and the passive diffusion of IPA and the resulting D-luciferin metabolite demonstrates small molecule permeability into and out of the microsphere which is a necessary requirement for testing ToxCast chemicals with this platform. Overall, these results demonstrate that HTS assays may be retrofitted with XM activity using immobilized alginate-S9 microspheres. This abstract does not necessarily reflect the policy of the US EPA.

ABSTRACT FINAL ID: 3758 Poster Board: P450
TITLE: Developmental Expression of the SULT1C Genes in Human Liver

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S. Dubaisi[1], T.A. Kocarek[2], M. Runge-Morris[2]. *[1]Pharmacology, Wayne State University, Detroit, MI; [2]Institute of Environmental Health Sciences, Wayne State University, Detroit, MI.*
KEYWORDS: Gene Expression/Regulation; Liver; Sulfation

ABSTRACT BODY: The cytosolic sulfotransferase (SULT) superfamily of conjugating enzymes includes 13 human genes that are classified into four families, SULT1, SULT2, SULT4, and SULT6. The human SULT1C subfamily includes three enzymes, SULT1C2, SULT1C3, and SULT1C4. SULT1C enzymes have been implicated as catalysts of drug, hormone, and neurotransmitter metabolism, as well as of pro-carcinogen bioactivation. Relatively little is known about the expression and regulation of the human SULT1C genes, and the limited information about hepatic expression suggests that the human SULT1Cs are mainly expressed during gestation. To study hepatic expression of the SULT1C genes during development, we measured the mRNA levels for these SULTs in 30 samples of human livers that were collected from prenatal (16-20 weeks of gestation), infant (1-12 months old), and adult (18-50 years old) life stages. SULT1C2 mRNA was readily detectable only in the prenatal and infant specimens. The median RNA level of SULT1C2 in the infant specimens was ~20-fold higher than it was in the prenatal specimens. SULT1C2 expression was relatively consistent among the prenatal specimens. However, the infant specimens demonstrated substantial inter-individual variability, which appeared as two clusters; the mean mRNA level in the higher expression cluster of specimens was ~50-fold higher than it was in the lower expression cluster. SULT1C4 was also readily detectable in the prenatal and infant specimens. The median prenatal SULT1C4 mRNA level was ~29- and ~100-fold higher than were the median infant and adult mRNA levels, respectively. SULT1C4 expression displayed substantial inter-individual variability among the prenatal specimens. SULT1C3 was minimally expressed in the liver samples that were examined (Ct values > 35). These findings suggest that hepatic SULT1C2 and SULT1C4 might play important roles in determining the susceptibility of individuals to chemical exposures during early stages of life. This work was supported by NIEHS grant R01ES022606 and Center Grant P30ES020957. Liver specimens were provided by the University of Maryland Brain and Tissue Bank (NIH Contract Number HHSN271201400045C).

ABSTRACT FINAL ID: 3759 Poster Board: P451
TITLE: A Novel Triterpenoid Inhibit Rhabdomyosarcoma by Reactive Oxygen Species-Mediated Downregulation Specificity Protein Transcription Factors

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: R. Kasiappan[1], I. Jutooru[2], S. Safe[1]. [1]*Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX;* [2]*Covance, Inc, Madison, WI.*
KEYWORDS: Gene Expression/Regulation; Carcinogenesis; Oncogene

ABSTRACT BODY: Rhabdomyosarcoma (RMS) is the most common malignancy during childhood and consists of two major types, namely, alveolar rhabdomyosarcoma (ARMS) and embryonal rhabdomyosarcoma (ERMS). The treatment strategies for RMS include, surgery, chemotherapy with cytotoxic agents, which increases patient survival but also has side effects that severely limit its clinical effectiveness and results in adverse health conditions later in life. Therefore, development of novel therapeutic approaches with minimal toxic side effects is needed for both single and combined therapies to diminish the adverse health effects of current chemotherapies. In the present study, we investigated the therapeutic potential of a novel synthetic triterpenoid, methyl 2-trifluoro-3, 11-dioxo-18β-olean-1, 12-dien-30-oate (CF3-DODA-Me) and its molecular mechanisms of action in rhabdomyosarcoma (RMS) cells. Here, we report that CF3-DODA-Me induces reactive oxygen species (ROS) and inhibits RMS cell proliferation, migration and induces apoptosis at 2.5 micro molar concentration in both RD (ERMS) and Rh30 (ARMS) cell lines. We also observed that CF3-DODA-Me downregulates specificity protein (Sp) transcription factors, including Sp1, Sp3 and Sp4 and this was accompanied by decreased expression of several oncogenic Sp-regulated genes, including survivin, VEGF and cyclin D. CF3-DODA-Me also decreased c-Myc-regulated miR-27a/miR-20a:miR-17, which in turn, results in the induction of the miR-regulated Sp repressors ZBTB10/ZBTB4 and ZBTB34. CF3-DODA-Me-induced ROS and the inhibitory effects of this drug on cell proliferation, survival and migration as well as inhibition of Myc-miR:ZBTB-Sp transcription factor axis were attenuated in RMS cells after cotreatment with antioxidant glutathione. Taken together, our results suggest that CF3-DODA-Me is a potential ROS-inducing anticancer agents that targets Sp transcription factors and represents a promising therapeutic agent for a future clinical applications for treating RMS.

ABSTRACT FINAL ID: 3760 Poster Board: P452
TITLE: Neonatal Exposure to PXR and CAR Activators Permanently Altered the Expression of Distinct Drug-Processing Genes in Adult Mouse Intestine

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S. Lee[1], C. Yanfei Li[2], J.Y. Cui[2]. [1]*University of Washington, Seattle, WA;* [2]*Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.*
KEYWORDS: Gastrointestinal; Receptor; Nuclear Hormone; Developmental Toxicity; Post-Natal

ABSTRACT BODY: Newborns and children are more vulnerable to xenobiotic insult, partly because little is known regarding the developmental regulation of drug-processing genes (DPGs) by drugs and environmental chemicals. The xenobiotic-sensing nuclear receptors, namely pregnane X receptor (PXR) and constitutive androstane receptor (CAR), are well known to up-regulate many DPGs in liver. However, relatively less is known regarding the developmental exposure to PXR and CAR activators on the expression of DPGs in intestine, where the biotransformation of orally-exposed xenobiotics takes place. The goal of this study was to determine both the acute and the potential long-term effects of neonatal exposure to PXR and CAR activators on the regulation of DPGs in newborn and adult intestine. Wild-type mice were intraperitoneally administered the PXR-ligand PCN, the CAR-ligand TCPOBOP, or vehicle, at 3-day-old neonatal age. Intestines were collected 24h post-dose (whole intestine) or at 60-day-old adult age (duodenum, jejunum, ileum, and colon). Neonatal exposure to TCPOBOP and PCN up-regulated many DPGs 24h postdose. For example, the mRNAs of Gstm1-m3 were up-regulated by both chemicals; Cyp2b10 mRNA uniquely by TCPOBOP; and Cyp3a11, Cyp4b1, Adh1, Aldh1a1, Ugt2b34, Ugt2b35, Gsta1, Gsta2, Mdrp1a, and Mrp2 uniquely by PCN in newborns. Interestingly, at 60-days adult age, neonatal exposure to TCPOBOP persistently up-regulated many DPGs such as Cyp2b10 preferably in small intestine, but down-regulated many DPGs such as Adh1 and Aldh2 preferably in colon. Neonatal exposure to PCN persistently up-regulated 6 DPGs but down-regulated 11 DPGs in specific sections of intestine. For example, neonatal PCN treatment persistent increased Cyp1a2 mRNA from jejunum to colon in a PXR-dependent manner. In conclusion, this study has demonstrated that neonatal exposure to xenobiotics that activate PXR and CAR not only produces acute induction effect on the DPG expression in newborns, but also leads to permanent alteration on the expression of certain DPGs in adult intestine, and this may potentially alter the pharmacokinetics of orally exposed chemicals in adults (supported by the Mary Gates Research Scholarship to SooWan Lee, as well as NIH grants ES019487, GM111381, and P30 ES007033).

ABSTRACT FINAL ID: 3761 Poster Board: P453
TITLE: BDE-99 Increases Lipid Development in Mouse and Human Pre-Adipocytes

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: L.E. Armstrong, M. MacArthur, S. Akinbo, A. Slitt. *University of Rhode Island, Kingston, RI.*
KEYWORDS: Exposure, Environmental; Cell Culture; Lipids; BDE-99

ABSTRACT BODY: Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants that were part of the largest marketed group of flame retardants in 2004 due to their effectiveness and low cost. PBDEs are found in electronics, textiles, and polyurethane foams, with the major routes of exposure being inhalation and ingestion through indoor air/dust, outdoor air, animal food, and aquatic biota world-wide. Due to the lipophilic and highly stable nature of PBDEs, concern grew over their bioaccumulation in the environment, and a remarkable decline in PBDE exposure has not been noted since initial bans of certain PBDE use. The persistence of PBDEs and their potential effects on the environment and human health has been brought to the forefront by the US EPA, who identified PBDEs a priority human health concern. PBDEs are detected in human adipose tissue and breast milk, with the most prevalent congeners described as BDE-47 and BDE-99. Our lab is currently researching BDE-99 for its adipogenic properties, as BDE-99 congener effects alone have not been well studied. BDE-99 treatment throughout the differentiation of 3T3-L1 pre-adipocytes and human pre-adipocytes induced greater lipid deposition. An increased in lipid development during differentiation may be time-dependent during exposure. There are no consistent changes in Pparγ expression during differentiation to contribute to increased lipids, but there is a significant induction of FAS (fatty-acid synthase) in both 3T3-L1 and non-induced human pre-adipocyte BDE-99 exposure models. FAS is induced 4-fold and 2-fold following 4 and 6 days of exposure in 3T3-L1 preadipocytes, and 2-fold in non-induced human pre-adipocytes following 7 days of exposure. Protein data in 3T3-L1 preadipocytes support the overall increase in Fas. This suggests that an increase in lipid development during adipocyte differentiation may occur not through hyperplasia, but through *de novo* lipogenesis resulting in hypertrophy. Lastly, Nqo1 gene expression is significantly down-regulated throughout differentiation in 3T3-L1 preadipocytes suggesting a role of Nrf2 regulation on lipid regulation in response to the environmental toxicant BDE-99.

ABSTRACT FINAL ID: 3762 Poster Board: P454
TITLE: Mutations in the Esa1 Histone Acetyltransferase Gene Alters Post-translation Modification

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: B. Thomas. *Microbiology, Southern University A&M College, Baton Rouge, LA.*
KEYWORDS: Gene Expression/Regulation

ABSTRACT BODY: The enzymatic activities of histone acetyltransferases and histone deacetylases often function in opposition to each other to regulate the balance of post-translational histone modification in chromatin. Histone acetyltransferases (HATs) have a significant role in regulating cell function through its post-translational modification of lysine residues on histones and other non-histone proteins. Esa1 is the only essential histone transferase in *Saccharomyces cerevisiae*. It has been implicated in diverse chromatin-mediated processes, transcriptional silencing, and DNA damage repair. Esa1 is a member of the MYST family of HATs, which is conserved in yeast and humans. It is the catalytic subunit of two enzyme complexes, NuA4 and Piccolo-NuA4 (picNuA4). These complexes function to maintain the global balance of histone H4 acetylation as well as numerous non-histone substrates. Genetic screens have identified esa1 mutants that are hypersensitive to DNA damaging agents, suggesting that Esa1 has several different biological activities. Based on genetic, biochemical and molecular data, we have proposed a model in which the essential function of Esa 1 is to function as a “molecular switch" that controls gene regulation. We hypothesize that selective mutations of Esa1 will result in a severe defect in its catalytic activity. To accomplish this, we generated bypass suppressor point mutants in *sds3*, *cti6* and *sap30*, then evaluated these mutants under galactose or glucose selection conditions. We found that the double-point mutations in all three acetyltransferases causes a switch in the bypass suppression from glucose to galactose in bacterial strains containing this mutation. We concluded that the essential function of Esa1 in catalyzing post-translation modification reactions requires residues within the catalytic pocket of Esa1.

ABSTRACT FINAL ID: 3763 Poster Board: P455
TITLE: Assessing DNA Damage through Direct Measurement of Double Strand Breaks

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: K.M. Dunnick, S. Rowley, S.M. Ross, M.M. Miller, C. Deisenroth, R.A. Clewell. *ScitoVation, Research Triangle Park, NC.*
KEYWORDS: *In Vitro* and Alternatives; Safety Evaluation; Risk Assessment

ABSTRACT BODY: The development of fit-for-purpose *in vitro* toxicity assays to better predict and define DNA damaging chemicals has become necessary to predict adverse outcomes via *in vitro* rather than *in vivo* methods. Current genotoxicity assays rely on indirect measurements of DNA damage through assessment of DNA repair foci or micronuclei formation. While these provide valuable information, they rely on high chemical concentrations and lack sufficient resolution at relevant chemical concentrations for human exposures. We adapted a published method (direct *in situ* labeling of double strand breaks (DSBs)) to a high-throughput compatible toxicological assay. Effectiveness of this method to detect DNA double strand breaks was tested against known prototype DNA damaging compounds. Initial studies were conducted utilizing aphidicolin, an inhibitor of DNA polymerase α and δ, as a test compound in HT-1080 fibrosarcoma cells. Preliminary data indicate that the DSB labeling method allows for detection of DSBs that closely models data from genotoxicity assays used in our laboratory (micronucleus and DNA repair centers). Studies were then conducted to assess assay sensitivity using prototype chemicals, etoposide (ETP) and methyl methanesulfonate (MMS). The direct DSB labeling method was approximately 10 times more sensitive than traditional micronucleus or DNA repair center assays (detected changes in DSBs compared to control cells at 0.001 µM vs. 0.02 and 0.01 µM ETP and 10 µM vs. 100 and 60 µM MMS, respectively). Based on this preliminary data, the direct DSB labeling method provides a novel tool for determining genotoxic potential at concentrations that are more relevant to human exposure levels than traditional *in vitro* models. Future studies are focused on determining accuracy of the assay using positive and negative controls for genotoxicity, and miniaturizing the assay for high-throughput screening.

ABSTRACT FINAL ID: 3764 Poster Board: P456
TITLE: Expert Review of Ames Data Impacts Genotoxic Impurity Classification

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: K. Dobo, W. Gunther, J. Cheung, M. Kenyon. *Pfizer Global R & D, Groton, CT.*
KEYWORDS: Genetic Toxicology; Risk Assessment; Computational Toxicology; Mutagenic Impurities

ABSTRACT BODY: The ICH M7 Guideline requires low level control of mutagenic impurities in pharmaceutical products to minimize cancer risk in patients. Bacterial mutagenicity (Ames) data is generally used to determine mutagenic and possible carcinogenic potential of compounds. Recently, a publication on experiences of using two *in silico* systems to identify potentially mutagenic impurities, highlighted the importance of performing a critical review of published Ames data utilized as part of a mutagenicity assessment of impurities (Greene et al, 2015). Four compounds (2-amino-5-hydroxybenzene, 2-amino-3-chlorobenzoic acid, methyl 2-amino-4-chlorobenzate and 4-morpholinopyridine) reported as mutagenic were identified in a two system *in silico* assessment as non-mutagenic and expert review of the structures (not including a review of the database containing the Ames result) agreed with classifying the structures as non-mutagenic. Likely reasons for mutagenicity of the test compounds were not identified and purity of the test compounds was proposed as a possible reason for the discrepancy between the *in silico* assessment and the published Ames results. The most pure sample that could be obtained of each of the four compounds has been tested in an OECD-compliant Ames test. The compounds were all found to be non-mutagenic. Possible reasons for the discrepancy between previously reported and current results are discussed. Additionally, guidelines are provided for minimum requirements for Ames data used in mutagenicity assessments particularly when the Ames outcome is discrepant with a two system *in silico* assessment.

ABSTRACT FINAL ID: 3765 Poster Board: P457
TITLE: Chemical Characterization and Genotoxicity of Emissions From Diesel and Gasoline Fuels

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KEYWORDS: DNA Adducts; Oxidative Injury; Polycyclic Aromatic Hydrocarbons; Biofuels

ABSTRACT BODY: Biofuels and their blends with standard fuels became a popular alternative to diesel and gasoline. We compared chemical composition and genotoxicity of organic extracts from extractable organic matter (EOM) obtained from emissions from various blends of biofuels with diesel and gasoline. We studied: 1. standard diesel fuel (B0), diesel fuel with 30% biodiesel (B30), biodiesel only (B100) and a new generation biodiesel (NEXBTL100); 2. Direct Injection Spark Ignition engine (DISI; operated using standard gasoline, gasoline with 15% ethanol (E15), 25% n-butanol and 25% iso-butanol) and Multi-Point Injection engine (MPI; operated using standard gasoline and E15). For the diesel fuels, the highest content of polycyclic aromatic hydrocarbons (PAHs), their oxy-, nitro- and dinitro- derivatives/mg EOM was found for B30 and B100; the lowest PAHs concentrations were observed for NEXBTL100. For gasoline-operated engines, the lowest PAH content was detected in EOMs from MPI/standard gasoline; MPI/E15 produced highest levels of both PAHs and oxy-PAHs. EOMs from DISI/standard gasoline were characterized by low PAH-derivatives content. Genotoxicity was tested in calf thymus DNA (CT-DNA) acellular system in the presence (+S9)/absence (-S9) of rat liver microsomal S9 fraction. Levels of bulky DNA adducts and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in CT-DNA after 24-h treatment with EOMs (50, 100, 250 µg/mL) were analyzed. In the +S9 system, all diesel emission EOMs induced similar DNA adduct levels; in the -S9 system, NEXTBTL was least genotoxic. B30 increased 8-oxodG levels. EOMs from emissions from all DISI fuels had 2-3-fold higher genotoxicity then EOMs from MPI emissions. All results were evaluated per mg of extract and do not take into the account changes in the total mass of the emitted particulate matter as a function of the fuel used. In summary, EOMs from the biofuels contained comparable or higher PAHs levels than standard fuel. The highest genotoxicity was observed for EOMs generated by the DISI engines regardless the fuel used. Support: Czech Science Foundation (13-01438S), EU LIFE+ (LIFE10 ENV/CZ/651).

ABSTRACT FINAL ID: 3766 Poster Board: P458
TITLE: Detection of γH2AX Induced by Formaldehyde and Acetaldehyde Using High Content Screening

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KEYWORDS: Genotoxicity; Cell Culture; Dose-Response; Aldehyde

ABSTRACT BODY: Formaldehyde (FA) and acetaldehyde (AA) are the ubiquitous environmental pollutants widely existing in the air, water and food. They have been listed as genotoxic carcinogen by WHO International Agency of Research on Cancer. Their genotoxicity are closely related to DNA lesions. Double-strand breaks (DSBs) are very deleterious DNA lesions as they can lead to chromosome aberration and/or cells apoptosis. As a sensitive and effective biomarker of DSBs, γH2AX is a widely used to assess the DNA lesions and genotoxicity of chemicals or radiation. However, the dose-effect relationship and expression kinetics of γH2AX induced by aldehydes have not been reported. So a novel γH2AX assay based on high content screening (HCS) has been applied in this study to evaluate inducing potential of DBSs for FA and AA in A549 cells. The results show that relative cell counts (RCC) decrease in a dose-dependent manner for FA and AA while the extent of H2AX phosphorylation increase in a dose-dependent manner in the cell nucleus. The FA and AA could produce significant increase in γH2AX frequency (1.5-fold) compared with control treatment at concentrations of 125 and 1000 µM, respectively. In addition, the exposure time has significant effects on the induction of γH2AX for FA and AA. The peak levels of γH2AX induced by 125, 250 and 500 µM of FA appears at 12, 4 and 24 h, respectively. The time for maximal γH2AX expression induced by 1000, 2000 and 4000 µM of AA was 12 h. RCCs of FA and AA were both decrease in time-dependent manner for each exposure concentration. In conclusion, exposure to the FA and AA can significantly induce γH2AX in A549 cells respectively, which displaying potential genotoxicity to A549 cells. And the exposure time and concentration have significant effects on the induction of γH2AX and DSBs. Keywords: Aldehyde;γH2AX;genotoxicity;high content screening (HCS)

ABSTRACT FINAL ID: 3767 Poster Board: P459
TITLE: Particulate Matter (PM) From Typical Household Biomass Fuel Combustion and Its Cytotoxic and Genotoxic Activity

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KEYWORDS: *In Vitro* and Alternatives; Particulates; Genotoxicity

ABSTRACT BODY: About 50% of all households use solid fuels as their main source of energy. Biomass combustion produces emissions of particulate matter (PM) containing minerals, heavy metals or polycyclic aromatic hydrocarbons (PAHs). Research on this PM shows increased risk of asthma, chronic bronchitis, heart disease and lung cancer. The aim of this study was to characterize PM from different biomass fuels used in residential fireplaces (pine and beech wood chips, Miscanthus straw), and to investigate their cytotoxic and genotoxic effects in human lung cells in comparison to coal-fly ash (CFA) and diesel exhaust particles (DEP). The materials mentioned above were burned in a 40 kW laboratory boiler. The particles were sampled by a cascade impactor and characterized by mineralogical and chemical techniques. They were investigated for their *in vitro* cytotoxic (WST-1 assay) and genotoxic effects (DNA alkaline unwinding assay) on human lung cell line A549 and the immortalized human bronchial epithelial cell line BEAS-2B using submerse 2D culture conditions. PM from biomass combustion contains numerous solid chemical compounds such as quartz, cristobalite, various carbonates, halides, sulfates and PAHs. Our study showed that compared with CFA and DEP the PM emitted from combustion of pine and beech wood chips displays genotoxicity at 30 µg/cm². CFA and DEP have significant DNA-damaging effects, even at very low concentrations (10 µg/cm²). PM from Miscanthus straw displayed no toxicity up to 100 µg/cm². We conclude that biomass fuel heating, especially with Miscanthus straw, may be a good alternative to heating with fossil fuels. To lessen biomass smoke-induced disease it is strongly recommended to reduce exposure to biomass smoke through “wise heating” or to use special filter techniques.

ABSTRACT FINAL ID: 3768 Poster Board: P460
TITLE: Electronic Cigarette Aerosols Induce Significant Oxidative DNA Damage

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: V. Ganapathy[1], D. McGuire[1], J. Manyanga[1], L.S. Brame[1], D. Rubenstein[2], I. Ramachandran[3], L. Queimado[1] . *[1]Otorhinolaryngology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK; [2]Biomedical Engineering, Stony Brook University, New York, NY; [3]Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, Tamil Nadu, India.*
KEYWORDS: Safety Evaluation; Genotoxicity; DNA Repair; Electronic Cigarette

ABSTRACT BODY: E-cigarettes (ECs) are battery-operated devices that deliver nicotine through inhaled aerosols. The health risks posed by exposure to EC aerosols are unknown. Nonetheless, the use of ECs has increased exponentially since 2003, with EC users reporting inhaling on average 200 puffs a day. EC aerosols contain unique constituents (e.g. tin and flavorants), as well as toxicants that are also present in tobacco smoke, including carcinogens (e.g. formaldehyde and nickel) and reactive oxygen species (ROS). Few toxicology studies have focused on EC aerosols. To determine the cytotoxicity and genotoxicity of diverse EC aerosol extracts. EC aerosols and tobacco smoke extracts were generated from distinct brands of ECs containing diverse nicotine concentrations and a reference combustible cigarette, in controlled conditions using a modified smoking apparatus. Human normal epithelial and oral cancer cell lines were exposed up to 2 weeks to diverse doses of EC aerosol extracts equivalent to 1 to 100 EC puffs. Overall DNA damage was quantified using q-PADDA. 8-oxo-7,8-dihydroguanine (8-oxoG) was quantified using a commercial ELISA kit. Cell viability was determined by MTT assay. Data were analyzed by Student’s t-test. No cytotoxicity was observed at the range of EC aerosol extract concentrations used in this study. Short- and long-term exposure to EC aerosol extracts induced significant DNA damage in normal and cancer cells. Moreover, a significant increase in 8-oxoG, one of the most common DNA lesions caused by ROS, was observed after exposure to EC aerosol extracts compared to the control. Short- and long-term exposure to EC aerosols can cause DNA lesions that are highly mutagenic and could contribute to cancer initiation and progression. Our study suggests that EC aerosols are harmful and emphasizes the urgent need to further evaluate their safety to ensure evidence-based public health policies and regulations.

ABSTRACT FINAL ID: 3769 Poster Board: P461
TITLE: Improving (Q)SAR Prediction of Alkyl Halide Mutagenicity Using Knowledge From Public, Regulatory, and Proprietary Databases

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KEYWORDS: Mutagenesis; QSAR; Predictive Toxicology

ABSTRACT BODY: The International Conference on Harmonisation (ICH) published the finalized ICH M7 guideline that outlines the steps to identify, categorize, qualify and control DNA reactive mutagenic impurities to ensure they pose a negligible risk of carcinogenicity. As part of hazard assessment for an impurity, the ICH M7 guideline permits the use of (Q)SAR models for the prediction of bacterial mutagenicity when no or inadequate experimental data are available. The list of impurities being assessed includes residual reagents and synthetic intermediates, such as alkyl halides. As a class, alkyl halides are also a structural alert for mutagenicity. The current (Q)SAR methods poorly predict alkyl halide mutagenicity. This poster describes an approach for incorporating proprietary knowledge into regulatory-acceptable public (Q)SAR models using a Structure Activity Relationship or SAR fingerprint. 3,312 alkyl halide substructures define the SAR fingerprint including different types and combinations of halide atoms, different substitution patterns on the adjacent atom, and different functional groups and ring systems proximal to the halide group. By running this fingerprint over multiple public and proprietary databases, it is possible to extract knowledge from thousands of alkyl halide compounds without the necessity to release the individual chemical structures or their associated data from proprietary databases. Classes of alkyl halides that activate or deactivate mutagenicity were identified and used to improve the predictive performance of the Leadscope alkyl halide structural alerts. The performance of the modified structural alerts was compared against alkyl halide alerts derived from different publications using a test set of 949 compounds containing alkyl halides. This resulted in an increase of 14.5% in the accuracy of the alert, including a 25% increase in the sensitivity.

ABSTRACT FINAL ID: 3770 Poster Board: P462
TITLE: Transcriptional Profiling in Testes of Rats Reveals Acrylamide Effects on Actin Filaments, Calcium Signaling, and Cell Proliferation

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KEYWORDS: Carcinogenesis; Genomics; Non-Genotoxic; Acrylamide

ABSTRACT BODY: Acrylamide exposure (5 mg/kg x 10 weeks) induces dominant lethal mutations in rats and chronic acrylamide exposure increases the incidence of benign spontaneous mesotheliomas of the tunica vaginalis testis. The mechanisms leading to these testes-specific effects of acrylamide are uncertain. We used next generation RNA-sequencing (RNASeq) to identify differentially expressed genes (DEG) and explore the pathway perturbations that could contribute to acrylamide-induced testicular toxicity. Male F344/DuCrl rats were administered 0.0, 0.5, 1.5, 3.0, 6.0, or 12.0 mg acrylamide/kg bw/day in drinking water for 4, 14, or 30 days. This design spanned and exceeded the doses used in the dominant lethal and cancer bioassays using the same route of administration. Testes were harvested, RNA was extracted, and mRNA was sequenced at 10 million single reads per sample by poly-A RNASeq on an Illumina NextSeq500. DAVID was used for pathway analysis and to explore the molecular perturbations induced by acrylamide. At 12.0 mg/kg there were 38, 33 and 65 DEG (p-value < 0.005; fold-change > 1.5) in the testes after 4, 14, or 30 days of exposure, respectively. At 30 days, there was a dose-dependent increase in the number of DEG and at 12.0 mg/kg day the top three functional clusters affected by acrylamide exposure were actin filament organization, response to calcium ion, and regulation of cell proliferation. Notably, at this same time point Cyp2a1 (testosterone 7 alpha hydroxylase) was significantly induced at 6.0 (1.5-fold) and 12.0 mg/kg day (2.0 fold). These results are consistent with earlier reports of the effects of acrylamide on cytoskeletal actin filaments and cell proliferation, suggesting that acrylamide causes rat dominant lethal mutations and could lead to benign tumors by impairing chromosome segregation during cell division but not by direct DNA damage.

ABSTRACT FINAL ID: 3771 Poster Board: P463
TITLE: Effect of Arsenic on Type 2 Diabetes

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KEYWORDS: Environmental Toxicology

ABSTRACT BODY: Type 2 diabetes (T2D) affects approximately 364 million individuals worldwide including ~8% of the US population, with this number expected to rise. Many studies demonstrate that obesity contributes to the development of T2D, and chronic inflammation in adipose tissue is a complication associated with obesity. While the link between arsenic exposure and T2D in the US is not well established a clear association has been found in Bangladesh and Taiwan where exposure levels are as high as 1000µg/L. While these studies have established a correlative affect between arsenic exposure and T2D, a direct mechanism linking the two is still elusive. Male mice (Leptin receptor deficient - db/db, heterozygous - db/+, and wild-type) were exposed to low dose arsenic trichloride (50ug/kg) every other day by oral gavage, from 4-12 weeks of age. Every 4 weeks we evaluated glucose tolerance (oral glucose tolerance test, OGTT) and body composition (DEXA scan). Mice were euthanized at 12 weeks. At this time we do not have sufficient numbers of db/db mice to include in the study. We found that arsenic exposure increased measures of metabolic disorders and diabetes in heterozygous but not wild-type mice. Insulin resistance was increased in those potentially predisposed to disease. And heterozygous animals increased bodyweight over time while wild-type did not. Interestingly this increase in body weight was not the result of increased adiposity. In this project we have established that the heterozygous db/+ mouse is susceptible to metabolic disorder and diabetes with exposure to arsenic, suggesting that this genotype may not be the best control for studies in the db/db model. Future studies in this model will examine the effects of arsenic on the immune system in a diabetic mouse model to determine whether arsenic-induced changes in inflammatory markers could induce a shift towards a diabetic state.

ABSTRACT FINAL ID: 3772 Poster Board: P464
TITLE: Evaluation of Immune Modulatory Effects of PD-1 Blockade in a Murine Primary and *Reca* ll Antibody Response Model

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: N. Li[1], F. Poulet[1], S. Born[2], L. Plitnick[2], J. Wolf[2], S. Kulkarni[1], B. Bhatt[1], M. Viola[2], D.T. Thudium[2], S.A. Dubey[3], S. Zhang[4], J. Lebron[2], R.P. Amin[1], D. Herzyk[2]. *[1]Safety Assessment & Laboratory Animal Resources, Merck Research Laboratories, Kenilworth, NJ; [2]Safety Assessment & Laboratory Animal Resources, Merck Research Laboratories, West Point, PA; [3]Biology-Discovery, Cellular Assays & Critical Ab Reagents, Merck Research Laboratories, West Point, PA; [4]Assay Development, Drug / Immunogenicity Assay Group, Merck Research Laboratories, Palo Alto, CA*.
KEYWORDS: Immunotoxicity; Biomarkers; Cytokines

ABSTRACT BODY: Programmed cell death protein-1 (PD-1) is a negative regulatory protein expressed on T cells to ensure an effective but appropriate immune response. While monoclonal antibodies blocking PD-1 produce robust anti-tumor responses with manageable side-effects in the clinic, a concern that blocking this critical checkpoint may result in immune-mediated toxicity following vaccination and recall response has been raised. A murine model of primary and recall antibody responses to Hepatitis B Vaccine (Hepatitis B Surface Antigen, HBsAg, with an aluminum-based adjuvant) was developed. CD1 mice were administered either phosphate buffered saline (PBS) or a murine anti-PD-1 monoclonal antibody (muDX400) as a single dose or as once-weekly doses. All animals also received primary and secondary immunizations of Hepatitis B Vaccine. Hepatitis B vaccine was immunogenic and anti-HBsAg antibody titers followed typical primary and recall antibody response kinetics. The magnitude and kinetics of both the primary and recall antibody responses in muDX400-treated groups were similar to those that occurred in PBS-treated animals. A slight increase (<30%) in absolute counts of splenic lymphocytes (B and T cell subsets) was observed in muDX400-treated groups, which were consistent with findings in PD-1 knockout mice and were considered to be of minimal toxicological significance. There were no muDX400-related hematology or serum cytokine changes observed in vaccinated animals. The murine model of primary and recall antibody responses to a vaccine antigen is useful to assess the potential immune-modulatory effects by immune-checkpoint inhibitors. Our results indicate that PD-1 inhibition does not alter the immune response to vaccination. More importantly, PD-1 blockade did not induce adverse immune-related events in mice receiving primary and secondary doses of Hepatitis B Vaccine.

ABSTRACT FINAL ID: 3773 Poster Board: P465
TITLE: Global Effects of Methylsiothiocyanate by Inhalation on Innate Immunity in a Mouse Model

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KEYWORDS: Pesticides; Immunotoxicity; Inflammation; Microarray; Methylsothiocyanate

ABSTRACT BODY: Sodium methyldithiocarbamate (SMD) is the third most abundantly used conventional pesticide in the United States. It is primarily used as a soil fumigant, and both in soil and in exposed mammals it undergoes a spontaneous hydrolysis reaction yielding methyl isothiocyanate (MITC) and hydrogen sulfide. Oral exposure of mice to SMD significantly decreases resistance to infection in response to intravenous lipopolysaccharide (LPS). The effects of MITC via a more relevant route of exposure (intranasal) on innate immune responses have not previously been reported. This was addressed in the present study. It caused a dose-dependent suppression of cumulative production of IL-6 and IL-12 and an increase in cumulative production of IL-10 induced by LPS. However, MITC-induced changes in cytokine and chemokine production measured at a single time after LPS administration was less pronounced that previously reported for SMD. Thus, it was not surprising that intranasal administration of MITC at 25 mg/kg did not significantly decrease survival in response to E. coli - induced sepsis. Microarray analysis of changes in gene expression in peritoneal cells revealed suppression of pro-inflammatory responses to LPS in MITC-treated mice (e.g., expression of mRNA for CXCL9, CXCL16, TNF-alpha, Type I interferon receptor, and JAK kinase). However, some changes in gene expression suggested compensatory mechanisms that could enhance survival in sepsis (e.g., decreased expression of proteasome components and increased expression of I-kappaB alpha, p53, septin 7, stefin A3, vacuolar protein sorting 3A, exosome component 8, and syntaxin binding protein 3A). Many of these genes are involved in phagocytosis, and enhanced expression may increase phagocytic activity. We conclude that exposure to MITC by inhalation (the most common route in humans) does not substantially affect innate immunity. This work was supported by R01ES01378 from the National Institute for Environmental Health Sciences, and S.B.P. is supported by Center for Biomedical Research Excellence P20GM103646 from the National Institute for General Medical Sciences.

ABSTRACT FINAL ID: 3774 Poster Board: P466
TITLE: Determination of Immunotoxic Potential of Fenbutatin Oxide in Hsd:ICR (CD-1®) Mice

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KEYWORDS: Immunotoxicity; Immunosuppressive Effect; Fenbutatin Oxide Technical

ABSTRACT BODY: This study was conducted to determine the immunosuppressive effect of Fenbutatin Oxide (FBTO) by repeated daily oral administration via diet for 28 days in mice following guidelines of EPA OPPTS 870.7800. Fifty mice of each sex were randomly divided into five groups (G1 to G5), 10 mice/sex/group. FBTO was given at 0 (G1, control; basal diet), 50 (G2), 150 (G3) and 450 (G4) ppm in diet. Mice from positive control group (G5) were treated with cyclophosphamide (CYM) at 50 mg/kg b. wt./day for a period of 4 consecutive days (Day 25-28) via intraperitoneal injection. All mice (G1-G5) were treated with sheep red blood cells (SRBC) in normal saline at fixed dose volume of 0.2 mL/mouse (7.8 x 107 RBCs) by intravenous injection on day 25. Mice were observed twice for clinical signs, morbidity and mortality. Body weight and food consumption of individual mouse were determined twice weekly throughout the study. An ELISA was performed using Mouse Anti-SRBC IgM ELISA kit to determine the effects on serum anti-SRBC IgM levels. Animals were subjected to gross pathology and organ weight was determined for spleen and thymus. All mice were alive without showing any clinical sign during experiment. Marked reductions in body weight, food consumption and anti-SRBCs IgM level was observed for males and females at 450 ppm of FBOT. Statistically, significant reduction in absolute weights of spleen and thymus were observed in the group treated with 450 ppm FBOT which was similar to that of CYM treated group. External and internal examination of terminally sacrificed animals did not reveal any pathological lesions. Based on the results, it was concluded that, FBOT produced immunosuppressive effects at 450 ppm dose level when administered orally through diet for 28 consecutive days in male and female mice of Hsd:ICR (CD-1®) strain. FBOT induced immunosuppression was comparable in both sexes.

ABSTRACT FINAL ID: 3775 Poster Board: P467
TITLE: The Use of 1-Methyl-D-Tryptophan in a Mouse Model of Amodiaquine-Induced Liver Injury to Inhibit Immune Tolerance

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: T.E. Cho, J. Uetrecht. *Pharmaceutical Sciences, University of Toronto, Toronto, ON, Canada.*
KEYWORDS: Immunotoxicology; Liver; Amodiaquine

ABSTRACT BODY: Idiosyncratic drug reactions are unpredictable adverse reactions. In particular, the mechanism of idiosyncratic drug-induced liver injury (IDILI) remains elusive. Most cases of IDILI appear to be immune-mediated and likely caused by reactive metabolites. Recently, we have developed a mouse model of amodiaquine (AQ)-induced liver injury that reflect the clinical characteristics of idiosyncratic liver injury in humans. This was accomplished by impairing immune tolerance with the use of PD-1-/- mice and an antibody against CTLA-4. These immune checkpoint proteins are known negative regulators of lymphocyte activation that promote immune tolerance. Another agent that is being developed to block immune tolerance for cancer chemotherapy is 1-methyl-D-tryptophan (1-D-MT), which inhibits indoleamine 2,3-dioxygenase (IDO). We hypothesize that the use of 1-D-MT may increase the liver injury in our current model. Antibodies were administered at 300 μg/mouse for PD-1 and CTLA-4 blockade via the intraperitoneal route. 1-D-MT was prepared as a 2 mg/mL solution in drinking water for oral delivery. Weekly ALT measurements were conducted to quantitatively assess liver injury. These treatments led to a moderate increase in serum ALT in all AQ-treated groups; however, there was no greater liver injury in the 1-D-MT co-treated animals. These results suggest that the IDO pathway does not play an important role in the immune tolerance to amodiaquine that appears to limit the liver injury associated with this drug. In addition, the anti-PD-1 antibody was not as effective as the genetic deficiency in PD-1. Future studies will continue to explore methods to modulate immune tolerance and liver injury in this model. Such studies should provide mechanistic clues and possibly methods to predict and prevent these currently unpredictable adverse reactions.
This research was supported by grants from the Canadian Institutes of Health Research.

ABSTRACT FINAL ID: 3776 Poster Board: P468
TITLE: Significant Differences Between Cynomolgus Macaque and Human $CD4^+$ Immune Cell Subsets Using Single Platform Polychromatic Flow Cytometry

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KEYWORDS: Immunotoxicology; Flow Cytometry

ABSTRACT BODY: T cell biology function and analysis is a fast growing area in many therapeutic areas. The detection of T cells that can differentiate into multiple cell types allowing biomarker generation has unique applications in toxicology, safety assessment, oncology, immunology, and translational medicine. To our knowledge, there is no available centralized assay to bridge the preclinical to clinical transition. Our goal in this study is to provide a single platform flow cytometry assay that can be used in cross-species and cross-therapeutic platform studies with the flexibility to simultaneously evaluate proprietary biomarkers to assimilate compound effects. Ten fresh peripheral whole blood samples from both cynomolgus macaque and human donors were analyzed using a single polychromatic panel containing cross-reactive antibodies to both species. The backbone of the panel characterized the following subsets: T cells, Cytotoxic T cells, Helper T cells, Regulatory T cells, as well as the following on both Cytotoxic T and Helper T cells: $PD\text{-}1^+$, Effector Memory RA, Naïve, Central Memory and Effector Memory. In addition, the panel has deliberate flexibility to allow for the inclusion of lab-critical markers in the most common fluorophore channels: PE, APC and PerCP. Both the relative percentages and absolute counts of each subset were analyzed. Assessment of variability was performed using mean, standard deviation, a two-tailed homoscedastic t-test ($p < 0.05$), followed by Bonferroni's correction for multiple hypothesis testing to compare individual population differences between species. Results demonstrate that for both percentage and absolute count there were only significant differences between species in three out of fourteen cell subsets. Absolute counts of human regulatory T cells were 2.1-fold higher and human Central Memory of the $CD4^+$ subset were 2.8-fold higher, both relative to cynomolgus macaque. Additionally, human Effector Memory of the $CD4^+$ subset were 3.5-fold lower than cynomolgus macaque. In conclusion, our data emphasizes that in critically important T helper subset populations, there are significant species differences, and results from this specific cross-species testing has utility for translation from preclinical studies to the clinical environment.

ABSTRACT FINAL ID: 3777 Poster Board: P469
TITLE: An Evaluation of the Cytotoxic and Pro-Inflammatory Effects of Various E-Cigarette Flavors on THP-1 Monocytes

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KEYWORDS: Immunotoxicity; Macrophage; Inflammation

ABSTRACT BODY: The use of electronic cigarettes has been increasing rapidly since their introduction to the US market in 2006. Although the popularity of these products is increasing, they are not currently regulated by the Food and Drug Administration and long-term health effects remain unclear. The objective of this study was to identify the potential cytotoxic and inflammatory effects of various flavors of E-cigarette liquids in THP-1 monocytes. Our hypothesis was that E-cigarette liquids would induce a pro-inflammatory response in THP-1 cells, induce cytotoxicity, and impair phagocytic function. THP-1 cells were used as experimental models to show the effect of these substances on respiratory innate immune defense. Macrophages are the most predominant immune cell in the healthy human lung and are critical in orchestrating the immune response in this tissue through cytokine signaling. THP-1 cells were challenged with seven different flavors of e-cigarette liquids. The cells were harvested 24 hours post-challenge and the Enzyme Linked Immunosorbent Assay (ELISA) was used to detect and quantify the IL-6 and IL-8 that had been secreted, hallmark cytokines of an inflammatory immune response. Results showed that various flavors of e-liquids differentially induce both IL-6 and IL-8 in THP-1 monocytes. We also calculated cell viability following challenge, using trypan blue exclusion. Our data suggests that of the seven E-cigarette liquids tested, two induced significant cytotoxicity in THP-1 monocytes, and these flavors, which contain varying levels of the flavoring cinnamaldehyde, also suppressed phagocytic function. These data contradict the prevailing narrative of E-cigarettes being a healthy alternative to traditional combustible tobacco products. Disruption of innate immune function by flavoring components in E-cigarette liquids may leave users vulnerable to pathogenic and inflammatory respiratory conditions.

ABSTRACT FINAL ID: 3778 Poster Board: P470
TITLE: Long-Term Effects of Early-life Arsenic Exposure on Innate Immunity

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KEYWORDS: Immunotoxicity; Macrophage; Metabolomics; Early-Life Exposure; Arsenic

ABSTRACT BODY: In a unique study area in Chile, our research group found that relative risks of adult mortality from cancer, bronchiectasis and tuberculosis (TB) are much greater when arsenic exposure occurred only *in utero*/early-life, rather than later in life. Notably, these risks remain high up to 40 years after the exposures ended. This provides rare human evidence in support of the "Developmental Origins of Health and Disease" hypothesis. However, the mechanisms behind this phenomenon remain unclear. We hypothesize that early-life arsenic exposures permanently impact immune development and increase the risks of immune-related diseases later in life. Here we focus on macrophages, innate immune cells known to influence tumor progression and TB pathogenesis. We assessed the effects of different *in vitro* doses of inorganic arsenic and arsenic metabolites on mouse bone-marrow-derived macrophages (BMDMs) and human monocyte-derived macrophages (MDMs). Multiplexed cytokine/chemokine profile analysis on homeostatic and activated BMDMs revealed significant dose- and time-dependent expression changes. Several of these cytokines/chemokines are involved in pattern recognition receptor pathways that are critical for the innate immune response against TB. Metabolomics analysis of these BMDMs showed that arsenic also led to changes in pro-inflammatory and tumor-promoting signaling lipids. As for BMDMs, we found significant differences in immunogenic and metabolic profiles when arsenic treatment occurred during differentiation vs. after complete maturation of human MDMs. Correspondingly, we observed differential effects of arsenic on *Mycobacterium tuberculosis* infections. Our preliminary analysis of plasma samples from early-life arsenic exposed adults suggests the involvement of macrophages in the observed changes in immune signaling profiles. We are currently validating our findings in human early-life exposed macrophages and are investigating how arsenic-induced immunogenic and metabolic alterations in macrophages influence TB and tumor cell pathogenicity *in vivo*. Supported by NIEHS Superfund Research Program P42ES004705 (M.S.) and NIEHS K99ES024808 (F.S.)

ABSTRACT FINAL ID: 3779 Poster Board: P471
TITLE: Elucidating the Physiological and Toxicological Role of the AhR in Human Ig Expression Using CRISPR/CAS 9 Gene Editing

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KEYWORDS: Dioxin; Receptor; Aryl Hydrocarbon; CRISPR/CAS 9

ABSTRACT BODY: In various animal models, the environmental toxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits the expression of immunoglobulin (Ig) and the differentiation of B cells into antibody secreting cells. Within the Ig heavy chain locus (Igh) is a 3' regulatory region (3'RR), which in mouse controls Igh expression and is a sensitive target of TCDD. TCDD inhibits mouse Ig expression and 3'RR activity in an aryl hydrocarbon receptor (AhR)-dependent manner. In humans, the role of the human 3'RR and the effect of TCDD and the AhR on Ig expression are unclear. Our previous studies using a human B-cell line (CL-01) and AhR inhibition by shRNA or chemical antagonism demonstrated a discordant effect of TCDD on human IgG secretion vs. hs1.2 enhancer activity, which appears to be AhR-dependent. The hs1.2 enhancer is one of three enhancers within the human 3'RR and it has the greatest transcriptional activity. Additionally, polymorphisms within the hs1.2 enhancer have been associated with several autoimmune diseases. Because of limitations in stability of our clones expressing shRNA against the AhR, the goal of the current study was to utilize CRISPR/Cas 9 gene editing to disrupt endogenous AhR expression in the CL-01 cell line. Several clones were isolated and analyzed for AhR expression and function by Western blot analysis and TCDD-induced CYP1A1 reporter activity, respectively. Additionally, sequence analysis of the CL-01 cells identified heterozygous SNPs associated with a dominant transcriptionally inactive AhR, which was confirmed by a lack of TCDD-induced CYP1A1 reporter activity in the wildtype cells. Analysis of our CRISPR/Cas9 clones suggest monoallelic gene editing with some clones expressing only the functional AhR allele and others the nonfunctional allele. Studies are underway to characterize Ig expression in these cells. These clones should provide a comprehensive model to elucidate both the physiological and toxicological role of the AhR in human Ig expression.

ABSTRACT FINAL ID: 3780 Poster Board: P472
TITLE: Increased Inflammatory Cytokine Output by Manganese-Mycobacterium Tuberculosis (Mtb) Co-Exposure *In Vitro*: Relevance to Occupational Manganese Toxicity

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: N.M. Filipov[1], L. Sellers[2], K. Sakamoto[2]. *[1]Physiology and Pharmacology, University of Georgia, Athens, GA; [2]Pathology, University of Georgia, Athens, GA.*
KEYWORDS: Inflammation; Mycobacterium Tuberculosis; Manganese

ABSTRACT BODY: Overexposure to manganese (Mn) results in manganism, a basal ganglia disorder, which, in its late stages, resembles Parkinson's disease (PD) and is of neuroinflammatory nature. Occupationally, welders and, especially, miners are chronically exposed to excessive Mn, but mining-relevant Mn research is scarce. Miners in South Africa where most of todays' Mn is mined also have extremely high incidence of Mycobacterium tuberculosis (Mtb) infection and tuberculosis (TB), with Mn miners having the highest prevalence of active pulmonary TB amongst all mining groups. Thus, the likelihood of co-exposure to Mn and Mtb is distinct, but the nature of their interaction within the context of Mn-induced neuropathology is unknown. We and others have already shown that Mn enhances inflammatory mediator output by activated microglia; in this study, using macrophages, we sought to determine whether co-exposure to Mn and Mtb will affect inflammatory cytokine output and whether such an effect will be associated with increased Mtb infectivity. Mouse RAW264 macrophages (0.5 X 106 cells per well) were infected with Mtb (H37Rv strain); at time 0, vehicle or Mn was added to the inoculation medium and the cells were incubated for 48 or 72 h. At both time points, supernatant cytokine (TNF-alpha and IL-6) levels and the number of intracellular Mtb were determined by ELISA and counting the colony-forming units (CFUs), respectively. Exposure of macrophages to Mtb and up to 500 uM Mn (48 or 72 h) did not result in increased numbers of intracellular Mtb. However, while Mtb on its own increased TNF-alpha, but not IL-6, media concentration of both cytokines was dose-dependently increased by the presence of Mn in the culture medium at both time points. Thus, much like other inflammagens, Mtb infection interacts with Mn exposure to increase inflammatory cytokine output. Such Mn/Mtb interactions *in vivo* would result in peripheral inflammation, increased neuroinflammation, and increased neuronal susceptibility to the effects of Mn, providing a potential explanation of differential susceptibility to similar Mn exposure levels among the Mn mining population.

ABSTRACT FINAL ID: 3781 Poster Board: P473
TITLE: Tetrachlorobenzoquinone Exerts Neurological Pro-inflammatory Activity by Promoting HMGB1-TLR4 Signaling

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KEYWORDS: Inflammation; Receptor; Neurotoxicology; HMGB1; TLR4; Tetrachloro-p-benzoquinone (TCBQ)

ABSTRACT BODY: Pentachlorophenol (PCP) has been extensively used as herbicide and insecticide in the agriculture and industry due to its broad applicability, however, PCP has been listed as a group 2B (possibly carcinogenic to humans) carcinogen by the International Agency for Research on Cancer (IARC) and a group B2 carcinogen (probable human carcinogen) by Environmental Protection Agency (EPA) . Tetrachloro-p-benzoquinone (TCBQ) is an oxidative metabolite of pentachlorophenol. TCBQ is a widely used fungicide as well. Thus, TCBQ may pose a considerable humans and environmental health risk. The Toll-like receptors (TLRs) play a crucial role in the molecular mechanisms of inflammatory processe. Upon stimulation, MyD88 recruits IL-1 receptor-associated kinase (IRAK) to TLRs, which in turn leads to the activation of NF-κB , the mitogen-activated protein kinase (MAPK) transduction cascades. High mobility group protein box 1 (HMGB1) is a potent agonist at multiple TLR receptors (TLR2, TLR4 and TLR9) and the receptor for advanced glycation end-products (RAGE) , which is capable of conveying danger or damage-related signaling after release from immune, activated or neurotic cells[3]. HMGB1 has been shown to be a mediator of inflammation in various models of chronic inflammation. Currently, the information of TCBQ-induced neurotoxicity is not available. Here, we selected PC12 cell line, a well-established *in vitro* model for this assessment. In our study,TCBQ elevated the level of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) expressions (Fig.1). TCBQ incresed protein expressions of TLR4, MyD88 and Melatonin exhibited a statistically inhibitory effect (Fig.2). More over TCBQ markedly induced the phosphorylation of p38, JNK and ERK1/2 MAPKs.However, these increases were attenuated by melatonin treatment (Fig.3). our results clearly exhibited the decreased IκBα level and increased p-IκBα levels in TCBQ-treated PC12 cell. Furthermore, our results showed that the p-p65, p65 and was up-regulated by TCBQ treatment (Fig.4). By an immunofluorescent staining assay, the translocation of endogenous HMGB1 was observed after TCBQ treatment (Fig.5). our data indicate that TCBQ exerts pro-inflammatory activity by promoting HMGB1 release, which induces TLR4 signaling in PC12 cells.

ABSTRACT FINAL ID: 3782 Poster Board: P474
TITLE: Design, Synthesis and Anticancer Activity of Some Novel Thioureido-benzenesulfonamides Incorporated Biologically Active Moieties

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KEYWORDS: Cell Culture; Apoptosis; Cell Proliferation; Early/Late Apoptosis, Cell Cycle Effects, Reactive Oxygen Generations; Sulfonamides, Thioureido, Anticancer Activities

ABSTRACT BODY: Novel series of thioureidobenzenesulfonamides incorporating miscellaneous biologically active moieties 3-17 were designed and synthesized utilizing 4-isothiocyanatobenzenesulfonamide 2 as strategic starting material. The structures of the newly synthesized compounds were established on the basis of elemental analyses, IR, 1H-NMR, 13C-NMR and mass spectral data. All the newly synthesized compounds were evaluated for their *in vitro* anticancer activity against various cancer cell lines. Most of the synthesized compounds showed good activity especially compounds 3, 6, 8, 9, 10, 15 and 16 which exhibited good activity higher than to the reference drugs, DCF and Doxorubicin, except for the colorectal cancer cell line and breast cancer line. As a trial to suggest the mechanism of action of the active compounds, molecular docking on the active site of mitogen kinase enzyme (MK-2), early/late apoptosis, cell cycle effects and reactive oxygen generations were performed and promising results were obtained especially for compound 3. More importantly, compound 3 had *in vivo* antitumor activity in a murine model without causing systematic toxicity in mice. Our findings indicated that compound 3 has significant potential for further drug development.

ABSTRACT FINAL ID: 3783 Poster Board: P475
TITLE: ER Stress and ERK1/2 Signaling Pathway Underlies the Toxicity of Nefazodone

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KEYWORDS: Hepatocytes; Apoptosis; Cytotoxicity; ER Stress, ERK1/2; Nefazodone

ABSTRACT BODY: Nefazodone is a phenylpiperazine antidepressant drug and is withdrawn from market due to its severe potential in inducing hepatic failure. The detailed mechanism and pathways underlying its effect, however, is not clear yet. In current study, we used human HapG2 cells to investigate the toxicity of Nefazodone. Nefazodone at the concentration of 20 - 100 μM decreased cell viability and induced apoptosis in a dose- and time- dependent manner. Nefazodone induced ER stress as ER stress markers including CHOP, ATF4, PDI and phosphor-eIF2α were upregulated upon Nefazodone treatment. This up-regulation was abolished by pre-treatment by 4-PBA, an ER stress inhibitor, at the concentration of 2 mM. In addition, components of MAPK family, including p38 and ERK1/2, were activated by Nefazodone. Further experiments suggested that ERK1/2 was involved in the ER stress and cytotoxicity, because inhibition of ERK1/2 by PD184352 at 2.5 μM increased cell viability, and attenuated the boost of ER stress related proteins. Together, these results shed light on the mechanism of Nefazodone induced hepatic failure, and can further our understanding on Nefazodone-induced liver injury.

ABSTRACT FINAL ID: 3784 Poster Board: P476
TITLE: Death Receptor 5 Regulate Tetrachlorobenzoquinone-Induced Apoptosis by Enhancing Formation of DISC in PC12 Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: Z. Liu, Y. Song. *College of Pharmaceutical Sciences, Southwest University, Chong Qing, China.*
KEYWORDS: Environmental Toxicology; Apoptosis; Neurotoxicology; DR5, Endoplasmic Reticulum Stress, DISC; TCBQ

ABSTRACT BODY: Tetrachlorobenzoquinone (TCBQ) was generated by pentachlorophenol (PCP) metabolism. Although there are few studies suggested that PCP exposure induced developmental and behavioral disorders, however, the mechanism(s) responsible for the neurotoxicity of PCP have not yet to be fully established. Studies have shown that TCBQ is significantly toxic to neuronal cells. Whether this is the result of endoplasmic reticulum (ER) stress-mediated the programmed cell death remains unknown yet. In this study, we explore the mechanism of TCBQ induced apoptosis by ER stress and the role of Death Receptor 5 (DR5) as a regulator of apoptosis in pheochromocytoma (PC12) cells. In order to determine whether TCBQ induces apoptosis in PC12 cells, three assays were used. Our result indicated that TCBQ-induced cytotoxicity in a time-dependent behavior (Fig.1). DR5 is an important mediator of the extrinsic apoptotic signalling pathway. In our study, we found DR5 protein levels increased in a time-dependent manner. DR5 siRNA transfection not only decreased both the basal level of DR5 expression and the level of TCBQ-increased DR5 expression but also decreased the TCBQ-increased levels of cleaved caspase 8, caspase 3 and PARP (Fig.2). The endoplasmic reticulum (ER) is the multifunctional organelle of protein synthesis and folding. Our study showed that TCBQ induced an ER stress response in PC12 Cells. TCBQ activated PERK/eIF2a branch of the UPR and induced gene transcriptional program of ATF4/ATF3/CHOP pathway (Fig.3). The induction of DR5 expression by TCBQ occured at transcriptional level, including induction of the transcriptional factors such as ATF3 and CHOP. Consistent with this, ATF4/ATF3/CHOP pathway played a significant role in the TCBQ-induced expression of DR5 (Fig.4). This resulted in an increased assemble of DR5 on the cell surface and caspase 8 was recruitment by DR5 through FADD binding of the death inducing signaling complex (DISC) (Fig.5). DISC induced the activation of caspase 3 and the cleavage of PARP. These results confirmed that DR5 play a central role in TCBQ-induced neurotoxicity. In summary, our present work demonstrated that TCBQ caused the ER stress and increased the expression of DR5, then induced PC12 cells to apoptosis. Particularly, DR5 enhance DISC formation by recruitment of caspase 8. Understanding the mechanisms of TCBQ-induced neurotoxicity will provide insight into limiting its effect on the CNS.

ABSTRACT FINAL ID: 3785 Poster Board: P477
TITLE: Assessment of *In Vitro* Acaricidal Potential and Cytotoxicity of Crude Extract of Ptaeroxylon Obliquum

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KEYWORDS: Cytotoxicity; Natural Products; Pesticides; Ptaeroxylon Obliquum

ABSTRACT BODY: Infections of domestic and wild animals that are transmitted by ticks to humans are major causes of morbidity and mortality. To date, chemical treatment of host animals with acaricides remains the method of choice to reduce the influence of the parasites on animal and human health. The environmental contamination posed by these acaricides, development of resistance by ticks to these chemicals leading to recurrent ectoparasitism, danger of misuse and presence of toxic residues in food, water and animal by-products has led to the search for safer and more environmentally-friendly alternatives, one of which is the use of medicinal plants. The aim of this study was to determine the acaricidal potential as well as the cytotoxicity of the crude acetone extract of *Ptaeroxylon obliquum* leaves. The acaricidal activity was determined using an *in vitro* toxicity bioassay against *Rhipicephalus sanguineus* ticks, while cytotoxicity was determined by evaluating the viability of Vero African green monkey kidney cells and bovine dermis cells in the presence of the extract using the tetrazolium-based colorimetric assay. There was a dose-dependent increase in mortality of *R. sanguineus* ticks with more than 60% mortality at 20% concentration (0.2 mg/mL) after 24 hours incubation. In comparison with the positive control, cypermethrin at 0.5%, this extract was less effective at the tested concentrations. The extract was moderately toxic with LC_{50} of 148.92 ± 49.45 mg/mL and 185.10 ± 34.79 mg/mL respectively on Vero cells and bovine dermis cells. The crude extract of *Ptaeroxylon obliquum* leaves showed moderate acaricidal activity and cytotoxicity. Further work on fractionating and isolating active compounds with potentially higher activity and lower cytotoxicity may prove the usefulness of this plant and the fractions, or whether pure compounds from these plants may be of pharmacological value.

ABSTRACT FINAL ID: 3786 Poster Board: P478
TITLE: Evaluation of Schisandra Extract Using Sandwich-Cultured Human Hepatocytes and B-CLEAR® Technology for the Prediction of Clinically Relevant Clearance Interactions

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J.P. Jackson[1], K. Freeman[1], W. Friley[1], K.R. Brouwer[1], A.L. Roe[2]. *[1]Qualyst Transporter Solutions, Durham, NC,[2]Procter & Gamble, Cincinnati, OH.*
KEYWORDS: Natural Products; Pharmacokinetics; Biliary Excretion; Herb-Drug Interactions; Schisandra Extract

ABSTRACT BODY: The Schisandraceae family of plants is reported to have a wide range of pharmacological activities, including antioxidant, anti-inflammatory, and hepatoprotective effects. As with all herbal preparations, Schisandra extracts (SE) are complex mixtures composed of >50 lignans including schizandrins and schizandrols. Current *in vitro* drug-drug interaction (DDI) assessment strategies such as FDA and EMA DDI Guidances require each constituent to be interrogated separately for their interaction potential. However, this approach fails to account for synergistic effects among constituents. Therefore; a more integrative approach is required to evaluate complex mixtures. We assessed the herb-drug interaction (HDI) potential of SE utilizing a fully integrated hepatic cell system which generates physiologic intracellular concentrations of xenobiotics by maintaining key regulatory pathways (CAR/PXR) and drug clearance pathways (drug metabolism and transport). Following 72 hours of SE treatment, the clearance of midazolam, tacrolimus, and digoxin, and the hepatotoxicity of SE were assessed. No marked changes were observed in hepatocyte morphology or ATP content following 72 hours exposure to S. chinensis extract (SCE) or S. sphenanthera extract (SSE). Gene expression analysis of SCHH treated with SCE and SSE showed significant induction of CYP2B6 and CYP3A4 mRNA content. These results suggested that midazolam clearance in SCHH following treatment would likely increase. However, clinical pharmacokinetic (PK) interactions studies of SSE demonstrated a 56% and 63% decrease in the clearance of midazolam or tacrolimus, respectively. Using Transporter Certified™ hepatocytes and B-CLEAR® technology we observed a significant reduction in the clearance of midazolam, tacrolimus, and digoxin in SCHH treated with either SE which was consistent with the clinical observations and supports the use of this *in vitro* model to predict *in vivo* HDI.

ABSTRACT FINAL ID: 3787 Poster Board: P479
TITLE: Modulatory Effects of Guava (*Psidium guajava*) Extract on Adriamycin Induced Toxicities in Wistar Rats

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KEYWORDS: Systems and Integrative Toxicology; Natural Products; Clastogen; Guava Extract; Adriamycin

ABSTRACT BODY: Adriamycin (ADR), though, a drug of choice in cancer therapy, its use is associated with acute and chronic complications, which are capable of exacerbating the conditions of the patients. Thus, efforts are being directed at evaluating several compounds, antioxidants and natural products that are capable of ameliorating these complications whilst still retaining the potency and efficacy of Adriamycin as an anti cancer agent. The modulatory and ameliorative effects of methanol guava leaf extract (MGLE) (*Psidium guajava* Linn) against Adriamycin/Doxorubicin toxicity was evaluated in Wistar rats. Thirty Male rats were divided into six groups A - F of five rats each, Group A fed normal saline, B- single acute dose of ADR, C- 500mg/kg guava extract alone, D to F -ADR in combination with 125, 250 and 500mg/kg body weight of guava leaf extracts,(MGLE) respectively for seven days after which blood samples were collected from all the animals. Liver and kidney damage markers were assessed in serum, liver histopathology, and micronucleated polychromatic erythrocytes (mPCEs) from bone marrow were assessed, and data represented as mean ± SE. Adriamycin resulted in considerable liver and kidney damage as seen in the form of elevated liver enzymes, total protein as well as urea and creatinine levels. It also resulted in significant (P<0.05) increases in the total triglyceride and cholesterol levels. The use of the extract alone on the other hand showed hepatoprotective and nephroprotective properties of guava leaf extract in that the above parameters were significantly lower than those of the untreated control. Hepatic histopathology indicated no visible lesion in the control group, necrotic hepatocytes observed in ADR, no visible lesion seen in the MGLE treated group and mild mononuclear cellular infiltration in ADR +MGLE treated groups. ADR induced mPCEs formation in rat bone marrow (P < 0.05), a dose dependent decrease (P < 0.05) in mPCEs in ADR + MGLE rats compared with ADR was observed. The MGLE exhibited hepatoprotective and anticlastogenic potentials. Our findings suggest that MGLE may mitigate the effect of ADR-induced toxicities.

ABSTRACT FINAL ID: 3788 Poster Board: P480
TITLE: Benchmark Dose Modeling for 5-Hydroxymethylfurfural: A Comparison of Models for Superior Fit

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KEYWORDS: Food Safety/Nutrition; Risk Assessment; 5-HMF

ABSTRACT BODY: Hydroxymethylfurfural (5-HMF) can be found in foods either through addition as a flavoring or as a heat formed constituent of carbohydrate containing ingredients and foods. The European Food Safety Authority (EFSA, 2011) concluded that the use of 5-HMF as a flavoring does not raise safety concerns. Because 5-HMF can be found in numerous foods and beverages as a consequence of heating and cooking, it is important to develop the most scientifically appropriate risk assessment for 5-HMF. EFSA identified cytoplasmic effects in the renal proximal tubule epithelium of B6C3F1 male mice from a 13-week National Toxicology Program study (NTP, 2010) as the critical effect for use in risk assessment. For its benchmark dose (BMD) modeling, EFSA (2011) used the PROAST (v. 27.4) statistical package plus exponential and Hill models and reported results for all models. EFSA selected the lowest BMD Lower Bound (BMDL) of 20.2 mg/kg-day for 5-day per week dosing as generated by the One-stage model even though it misses the low dose data points. The data for the same study with male renal tubule epithelium cytoplasmic alterations as the critical effect data were re-analyzed using the US Environmental Protection Agency (US EPA) BMD modeling software (BMDS v. 5.0). The results were assessed for model fit using the criteria described in the US EPA BMDS guidance document, and several of the models provided an acceptable fit to the data according to these BMDS guidelines (US EPA, 2012). In general, the various BMDs and BMDLs developed were similar to those determined by EFSA (2011), given the same models. However, the number of criteria used for model choice differs between USEPA (2012) and EFSA (2011). EFSA (2011) included 0.05 as the P-value for goodness of fit and BMDL/BMDU < 10, both of which are also used with modifications by US EPA (2012). However, US EPA (2012) includes additional criteria of visual fit, scaled residuals, and Aikaike's Information Criterion (AIC) to enhance the evaluation of these data sets. The re-evaluation of the data (NTP, 2010) determined that the LogProbit model provided a superior fit to the data with a BMD and BMDL of 96 and 57 mg/kg-day, respectively, as compared to 20.2 mg/kg-day for the 5-day per week dosing determined by EFSA (2011).

ABSTRACT FINAL ID: 3789 Poster Board: P481
TITLE: Steviol Glycosides in Purified Stevia Leaf Extract Sharing the Same Metabolic Fate

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S. Purkayastha[1], A. Markosyan[1], I. Prakash[2], S. Bhusari[2], G. Pugh[2], B. Lynch[3], A. Roberts[3]. *[1]PureCircle, Oak Brook, IL,[2]The Coca-Cola Company, Atlanta, GA,[3]Intertek Scientific & Regulatory Consultancy, Mississauga, ON, Canada.*
KEYWORDS: Food Safety/Nutrition; Metabolism; Stevia, Steviol Glycosides, Rebaudiosides

ABSTRACT BODY: The stevia plant leaves contain more than 40 different sweet tasting components called steviol glycosides (SGs) present in the stevia leaf extract. Each of these SGs has its own unique taste profile and sweetness intensity, but all share a similar molecular structure where different sugar moieties like glucose, xylose and rhamnose are attached to aglycone steviol. The current safety database is generated based on the toxicology studies of SGs stevioside and rebaudioside (Reb) A. While, recent research have focused on the different aspects (e.g. chemical, metabolism and nutrition) of *Stevia rebaudiana* and few SGs, the metabolic fate of various SGs with different attached sugars, has not been reported. To address this greater detail, *in vitro* incubation assays with human fecal isolates, using Rebaudiosides A, B, C, D, E, F and M, as well as steviolbioside and dulcoside A, at two concentrations over 24-48 hours, were conducted to assess the metabolic fate of various steviol glycoside classes and to demonstrate that likely all steviol glycosides are metabolized to common end product, steviol. The data show that glycosidic side chains containing glucose, rhamnose, xylose, fructose and deoxy-glucose, including combinations of α(1-2), β-1, β(1-2), β(1-3), and β(1-6) linkages, were degraded to steviol mostly within 24 hours. Given similar structure and a shared metabolic fate, safety data available for individual steviol glycosides can be used to support safety of purified steviol glycosides in general. Therefore, steviol glycosides specifications adopted by the regulatory authorities should include all steviol glycosides belonging to the five groups of steviol glycosides and a group acceptable daily intake established.

ABSTRACT FINAL ID: 3790 Poster Board: P482
TITLE: In-House Standardization and Validation of Radio-Receptor Assays for Chloramphenicol Residues in Bovine Milk and Meat

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KEYWORDS: Food Safety/Nutrition; In-House Validation, Radio Receptor Assay, Chloramphenicol, Residues, Meat, Milk

ABSTRACT BODY: Radio receptor assay techniques were standardized and validated in-house for the detection of Chloramphenicol residues in bovine meat and milk matrices following CHARM Sciences Inc procedures. A Beckman Coulter LS 6500 scintillation counter was used to measure the β-emissions from Tritium (1H3) radionuclide tracers. The determined critical control point corresponding to the decision limit (CCα) was equivalent to 5739 and 2224 disintegrations per minute (dpm) in meat and milk samples, respectively. In both matrices, 20 blank and 20 spiked samples were analysed on different days to determine the method repeatability, accuracy and precision. Samples were spiked at 0.3 ng/mL, the Minimum Required Performance Limit (MRPL) for Chloramphenicol. The method detection capability (CCβ) was estimated at ≤ 0.3 ng/MRPL. The coefficient of variation (CV, %) for spiked samples, was 7.5 and 9.2 in meat and milk, respectively. The test methods were suitable for screening field meat and milk samples for Chloramphenicol residues in Faisalabad, Pakistan. Samples were found to be negative.

ABSTRACT FINAL ID: 3791 Poster Board: P483
TITLE: (2R,4R)-Monatin-Induced QT Prolongation Detected in Human Clinical Studies Not Previously Identified in Animal Safety Studies or in Ion Channel Evaluations

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KEYWORDS: Food Safety/Nutrition; Cardiovascular System; Regulatory/Policy; QT Prolongation; Monatin

ABSTRACT BODY: (2R,4R)-Monatin salt (herein referred to as "R,R-monatin") is a sweet isomer of a component first identified in the root bark of the Schlerochitin ilicifolious plant. Due to its intense sweet taste (up to 3000x of sucrose), enzymatically-synthesized R,R-monatin has been evaluated for potential use in foods. Comprehensive safety evaluation in rodent studies have shown that R,R-monatin is well tolerated at high dietary concentrations, is not overtly toxic or carcinogenic, and is not associated with any developmental or reproductive effects. *In vitro* studies have shown that R,R-monatin is not genotoxic/mutagenic, and does not cause significant inhibition of common cardiac ion channel activities. *In vivo* oral studies of R,R-monatin in dogs also showed no clear, consistent or dose-related effects on heart rate or electrocardiogram (ECG) parameters. In a pharmacokinetic and metabolism study involving 12 healthy male volunteers, consumption of a single oral dose of 2 mg/kg of R,R-monatin resulted in a small reduction of heart rate and prolongation of the QTcF interval. These findings were further evaluated in a randomized cross-over thorough Qt/QTc (tQT) study in 56 healthy male subjects using a single oral dose of 2 mg/kg of R,R-monatin, placebo (microcrystalline cellulose), and 400 mg moxifloxacin (positive control). Following consumption of R,R-monatin, a small reduction of heart rate was observed, but also a prolongation of the QTcF interval of 20-24 ms, with a peak effect corresponding to the maximum R,R-monatin plasma concentration at 3.1 hours. A clear correlation was observed between R,R-monatin plasma levels and the extent of the QTc prolongation, the peak of which was nearly 2.5 times higher than the positive control. These findings collectively demonstrate that R,R-monatin causes QTc prolongation in humans, which was not clearly detected in *in vivo* animal studies, nor *in vitro* ion channel studies specifically designed to screen for potential adverse cardiac effects.

ABSTRACT FINAL ID: 3792 Poster Board: P484
TITLE: Surveillance in Trends of Foodborne Illnesses Based on Assessment of Food Safety Lawsuits

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: C. Trusty[1], M. Ierardi[2], B. Tvermoes[3]. *[1]Cardno ChemRisk, Chicago, IL; [2]Cardno ChemRisk, Brooklyn, NY; [3]Cardno ChemRisk, Boulder, CO.*
KEYWORDS: Food Safety/Nutrition; Food Safety Surveillance

ABSTRACT BODY: Food is considered a major source of potential exposure to many different types of microbial or chemical contaminants. In fact, according to the most recent data provided by the Centers for Disease Control and Prevention (CDC), foodborne illness strikes 1 in 6 Americans each year. Hence, awareness of common sources and types of food contamination is necessary to address and manage food safety. To this end, knowledge regarding trends in foods implicated in foodborne diseases is important. While there are a number of ways to track foodborne illnesses and assess associated disease burden in the United States, only a fraction of the foodborne illnesses that occur each year in the US are captured and not all causes are well monitored. One way to improve surveillance of trends in foodborne illnesses is to utilize nontraditional approaches of disease surveillance. The aim of this study was to assess whether evaluation of food safety lawsuits could be used as a supporting surveillance tool for assessing trends in foodborne illnesses in the US. Using the LEXIS-NEXIS database, we examined the awards and damages for US food safety cases filed between 2003 and 2013 and compared the most commonly litigated foods and food contaminants to the most common causes of foodborne illness reported by the CDC. Our search parameters included food poisoning, adulterants, microbial contamination, imports, and contaminants. Of the 305 identified food safety cases, only 184 cases (39.6%) mentioned a contaminant by name. Among those cases that described a specific contaminant by name, bacterial etiologies were most commonly (n = 94) associated with food safety cases. E. coli was the most common bacteria associated with a food safety case and accounted for 36 cases (11.8%), followed by Salmonella (25 cases; 8.2%), and Listeria (6 cases; 1.9%). Only 161 food safety cases (52.8%) implicated a particular food and the food could be classified into 1 of 21 categories; the categories most commonly implicated were beef (28%) and chicken (8.1%). While there was overlap with the most common food pathogens reported by the CDC, we also identified additional food contaminants and food categories not captured by the CDC suggesting that assessment of food safety lawsuits may also provide insight into trends of foodborne illnesses.

ABSTRACT FINAL ID: 3793 Poster Board: P485
TITLE: Effects of Repeated Coffee Consumption on DNA Integrity in White Blood Cells (WBC): A One-Day Human Intervention Study

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: G. Eisenbrand, T. Bakuradze, E. Richling. *University of Kaiserslautern, Kaiserslautern, Germany.*
KEYWORDS: Genotoxicity; Toxicokinetics; Food Safety/Nutrition; Coffee

ABSTRACT BODY: Recent studies suggest regular, long-term coffee consumption to be associated with decreased background DNA damage in WBC of healthy humans [1, 2]. To gain information about the time dependence of these DNA protective effects, we monitored DNA integrity by the Comet Assay in a one-day intervention study with repeated coffee consumption. Healthy male volunteers (n=13, age 23 ± 2.4, BMI 23.8 ± 1.6 kg/m2) after a 72 h wash out period consumed every 2 hours 200 ml of freshly prepared coffee brew up to a total of 4 intakes (4 x 200 ml). Blood sampling was performed immediately before the first coffee intake (baseline), and subsequently every two hours. The results showed a significant ($p<0.05$) reduction of background DNA strand breaks in comparison to baseline. This DNA protective effect was detected already two hours after the first coffee consumption. Extended coffee consumption, up to a total of 800 mL, revealed a significant further incremental reduction of DNA damage ($p < 0.01$ and $p < 0.001$, respectively) in relation to the baseline [3]. The observed rapid DNA protective response may be ascribed to radical scavenging by coffee constituents such as chlorogenic acids or melanoidins. In addition, longer term response may be attributed to the activation of the Nrf2/ARE signalling pathway, resulting in activation of various phase II detoxification pathways as has been shown earlier [4]. Thus, this study indicates a very early onset of DNA protective efficacy in humans, augmented during further coffee intake during the day, eventually becoming sustained during long-term coffee ingestion.
References: 1. Bakuradze, T. et al., Consumption of a dark roast coffee decreases the level of spontaneous DNA strand breaks: a randomized controlled trial. Eur J Nutr, 2015, 54, 149-156. 2. Bakuradze, T. et al., Antioxidant-rich coffee reduces DNA damage, elevates glutathione status and contributes to weight control: results from an intervention study. Mol Nutr Food Res, 2011, 55, 793-797. 3. Bakuradze, T. et al., Coffee consumption rapidly reduces background DNA strand breaks in healthy humans: Results of a short term repeated uptake intervention study. Mol Nutr Food Res. 2015 Dec 3. doi: 10.1002/mnfr.201500668. [Epub ahead of print] 4. Boettler, U. et al., Coffees rich in chlorogenic acid or N-methylpyridinium induce chemopreventive phase II-enzymes via the Nrf2/ARE pathway *in vitro* and *in vivo*. Mol Nutr Food Res. 2011;55,798-802.

ABSTRACT FINAL ID: 3794 Poster Board: P486
TITLE: Chronic Aflatoxin Exposure, Growth, and Breastfeeding in Children Living in Bhaktapur, Nepal: Extension of the Mal-ed Study

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KEYWORDS: Food Safety/Nutrition; Aflatoxin

ABSTRACT BODY: Exposure to aflatoxin, a mycotoxin common in maize and groundnuts, has been associated with childhood stunting in sub-Saharan Africa. In an effort to further our understanding of growth impairment in relation to mycotoxins and other risk factors, biospecimens from a cohort of children enrolled in the Bhaktapur, Nepal MAL-ED study were assessed for aflatoxin exposure at 15, 24, and 36 months of age. Exposure was assessed through a well-established serum biomarker, the AFB1-lysine adduct. In this manuscript, the levels of aflatoxin exposure in the Nepal cohort were compared to z-scores, growth trajectories, age, and breastfeeding status. Results from this preliminary analysis demonstrated chronic aflatoxin exposure in children residing in Bhaktapur with a geometric mean of 3.62 pg AFB1-lysine/mg albumin. However, the chronic exposure did not show any significant associations with growth variables, age, or feeding status. Birth weight was the strongest predictor of length-for-age (LAZ), weight-for-age (WAZ), and weight-for-length (WLZ) z-scores. Future work needs to be conducted to observed mother exposure to aflatoxin during pregnancy and resultant birth weights and z-scores.

ABSTRACT FINAL ID: 3795 Poster Board: P487
TITLE: Smectite Presence in Mycotoxin Clay Based Adsorbents

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KEYWORDS: Food Safety/Nutrition; Clay

ABSTRACT BODY: Aflatoxin B1 (AFB1) is a group 1 carcinogen mainly produced by Aspergillus fungi. It is a common contaminant in animal feed and strategies to reduce its exposure are highly desirable. Some clay minerals have been widely uses as anticaking agent additives and some are also approved as aflatoxin binders in several countries. In this study, the mineral characteristics and sorption capacity for aflatoxin, zearalenone and fumonisin of commercial clays intended for animal nutrition were investigated. Clays were obtained from NUTRECO (The Netherlands). Mineral ingredients of clays were revealed using X-Ray diffraction analyses (XRD). Aflatoxin adsorption analyses were done in borosilicate glass tubes with 11 dilutions of AFB1 AFB1 (0.4, 0.8, 1.2, 2.4, 3.2, 4, 4.8, 5.6, 6.4, 7.2, and 8 µg/mL) in a total volume of 5 mL. Tubes with water, water + clay and AFB1 (8 µg/mL) were used as controls. An amount of 100 µg of clay was added to each dilution tube and then tubes were placed in agitation at 1000 rpm for 2 hours at a temperature of 25°C. After this, tubes were centrifuged at 2000 rpm and supernatant was read at 364 nm wavelength in a UV-VIS spectrophotometer (Shimadzu). Results showed that most effective clays contained either Na or Ca saturated montmorillonite. In the adsorption analyses, the most effective clay showed an L-2 (Langmuir) pattern of adsorption and a maximum adsorption capacity of 0.38 mol AFB1/kg clay while others ranged from 0.04-0.25 mol/kg. Funding NUTRECO-UANL 2013-2015.

ABSTRACT FINAL ID: 3796 Poster Board: P488
TITLE: Safety Evaluation of AB-LIFE(R) (*Lactobacillus plantarum* CECT 7527, 7528, and 7529): Antibiotic Resistance and 90-Day Repeated-Dose Study in Rats

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J.M. Roper[1], P. Mukerji[1], B. Stahl[2], A.B. Smith[2], F.R. Burns[1], J. M. Caverly Rae[1], N. Yeung[3], A. Lyra[3], L. Svard[3], M.T. Saarinen[3], E. Alhoniemi[4], A. Ibarra[3], A. Ouwehand[3] .*[1]Science and Innovation, DuPont, Newark, DE; [2]Nutrition and Health, DuPont, Madison, WI; [3]Nutrition and Health, DuPont, Kantvik, Finland,[4]Avoltus Oy, Turku Area, Finland.*
KEYWORDS: Food Safety/Nutrition; Biotech Products; Gastrointestinal; *Lactobacillus Plantarum*; Probiotic

ABSTRACT BODY: AB-LIFE® is a probiotic product consisting of equal parts of three strains of *Lactobacillus plantarum* (CECT 7527, 7528, and 7529). Whole genome sequencing was performed on each of the three strains. Antibiotic resistance was evaluated by genomic mining for resistance genes, followed by assessment for transferability. There was no risk of transfer potential identified for any antibiotic resistance genes in the three strains. AB-LIFE® was evaluated for potential subchronic toxicity in rats, with dosages of 300 and 1000 mg/kg/day (equivalent to 5.55 x 10^10 and 1.85 x 10^11 CFU/kg/day) administered by oral gavage for 90 days. The survival of the three test strains through the gastrointestinal tract was confirmed in the feces by strain-specific qPCR, in proportion to the administered dosage. There was no evidence of systemic translocation of the test strains beyond the extra-intestinal sites (liver and mesenteric lymph nodes), based on enumeration of the test strains. There were no adverse effects identified with respect to in-life parameters, clinical pathology, or anatomic pathology. Fecal chemical analyses (bile acids, neutral sterols, short or branched chain fatty acids, and lactic acid, using gas chromatography) did not reveal any adverse effects on metabolic activity of the intestinal microbiota. Therefore, the no-observed-adverse-effect level (NOAEL) for AB-LIFE® in male and female rats was 1.85 x 10^11 CFU of AB-LIFE®/kg/day, which was the highest dose level evaluated, representing an approximately 1,000-fold margin of safety. These results, in conjunction with a previous acute toxicity study in rats, support the conclusion that AB-LIFE® is safe for human consumption.

ABSTRACT FINAL ID: 3797 Poster Board: P489
TITLE: LC-MS/MS Analysis of Diarrhetic Shellfish Poisoning and Azaspiracid Shellfish Poisoning Biotoxins

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: L. Yang, R. Self, J. Stuart, T. Lapainis, D. Rice, W.-H. Wu, J. Hungerford. *Applied Technology Center, PRL-NW, US Food and Drug Administration, Bothell, WA.*
KEYWORDS: Food Safety/Nutrition; Methods/Mechanism; HPLC-MS/MS; Marine Biotoxins

ABSTRACT BODY: Marine biotoxins, produced by microalgae, are small molecules accumulated in filter-feeding fish or shellfish. Consumption of seafood contaminated with marine biotoxins by humans can lead to symptoms such as diarrhea, vomiting, paralysis and even death. These biotoxins therefore impact public health in multiple aspects including regulatory agencies, aquaculture, fisheries and international trade. Diarrhetic shellfish poisoning (DSP) and azaspiracid shellfish poisoning (AZP) are emerging issues in the US following recent DSP outbreaks in US shellfish harvesting areas and an AZP outbreak from imported seafood. To reach the goal of continually monitoring for DSTs and AXTs in the US seafood supply, in this study, a LC-MS/MS method is developed to detect both DSP and AZP marine biotoxins. The method was modified from an official European Union-approved protocol designed for the detection of several classes of EU-regulated lipophilic toxins.A liquid chromatography triple quadrupole mass spectrometry method for the separation and detection of prominent DSP and AZP marine biotoxin groups is described in this abstract. Separation was achieved by a C18 Hypersil gold column (50mm × 2mm, 1.9µm particle size) with a single run of 7.5 minutes. The mobile phase is comprised of a gradient acetonitrile/water containing ammonium formate and formic acid. Both positive and negative modes have been used for multiple reaction monitoring with a Thermo triple quadrupole (TSQ Quantum Ultra) mass spectrometer. The limit of quantification of okadaic acid, dinophysistoxin2, Azaspiracid (AZA) 1, 2 and 3 are 1.63, 0.81, 0.81, 0.81, and 0.81 pg/µL respectively. The method shows sufficient sensitivity, linearity and reproducibility. We conclude that this LC/MS-MS method could provide rapid testing for DSP and AZA biotoxins and is a viable strategy in monitoring selected types of marine toxins in the seafood supply.

ABSTRACT FINAL ID: 3798 Poster Board: P490
TITLE: Larvicidal Activity of *Clostridium bifermentans* Toxins on Aedes Mosquitos

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S.M. Andrews, S. Chawla, S.S. Gill. *Environmental Toxicology, University of California, Riverside, CA.*
KEYWORDS: Pesticides; Natural Products; Bacterial Toxins

ABSTRACT BODY: Mosquitoes are important vectors of human diseases, including malaria, dengue fever, and West Nile fever, which are vectored by Anopheles, Aedes and Culex species, respectively. Currently two bacteria serve as the major biological control methods against these vector mosquitoes. They are *Bacillus thuringiensis israelensis* (Bti) and *Lysinibacillus sphaericus* (Ls) that target Aedes and Culex mosquitoes, respectively. Their toxicity to Anopheles mosquitoes is, however, lower. But a bacterial strain of Clostridium has higher toxicity to anopheline mosquitoes. This strain *C. bifermentans* serovar malaysia, a gram positive, anaerobic bacteria, was isolated from mangrove swamp soil in Malaysia, and the strain has larvicidal activity against Anopheles and Aedes mosquitoes, both of which serve as disease vectors. In this clostridial strain there are two loci that are toxic to mosquitoes, the Cry loci and the *Clostridium mosquitocidal* protein (CMP) loci. The Cry locus, which contains four toxin proteins, was shown to have toxicity against Aedes mosquitoes, while CMP locus, which consists of seven proteins, showed toxicity against Anopheles. Further study of the Cry operon has shown that all four proteins are necessary for toxicity, and that these four proteins do in fact form a complex.

ABSTRACT FINAL ID: 3799 Poster Board: P491
TITLE: Pendimethalin Induced Oxidative Damage in Rat

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KEYWORDS: Pesticides; Oxidative Injury; Biomarkers; Herbicide, Oxidative Stress, Rat, Biomarkers; Pendimethalin

ABSTRACT BODY: Pendimethalin is a dinitroaniline pre-emergence herbicide used worldwide in agriculture for control of most annual grasses and many annual broad-leaved weeds. The objective of this study was to investigate the propensity of pendimethalin to induce oxidative stress, changes in biochemical parameters and enzyme activities in serum, kidney, liver and brain of female rats following four oral doses of 109.4 mg/kg b.wt every other day. Tissue malondialdehyde (MDA), antioxidant enzymes such as catalase (CAT), lactic dehydrogenase (LDH) and alkaline phosphatase activities were evaluated. Exposure of rats to pendimethalin caused a significant increase in tissue MDA, LDH and ALP levels (p<0.05) as compared to controls. The activities of CAT in serum, liver and kidney were significantly increased (p<0.05), while brain CAT activity was significantly decreased (p<0.05) due to pendimethalin treatment. It can be concluded that pendimethalin exposure had profound influence on the oxidative stress markers and enzyme activities of the exposed female rat and these enzymes can be used as biomarkers in determining pendimethalin toxicity.

ABSTRACT FINAL ID: 3800 Poster Board: P492
TITLE: Diaminochlorotriazine Induced Toxicity of N2α Mouse Neurons and BV2 Mouse Microglia

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KEYWORDS: Pesticides; Cell Culture; Neurotoxicity; Pesticides; Diaminochlorotriazine

ABSTRACT BODY: Atrazine (2-chloro-4-ethytlamino-6-isopropylamine-1,3,5-triazine) is a widely used herbicide that has been shown to effect hormone regulation, modulate catecholamine synthesis and induce dopaminergic apoptosis. Contemporary research investigating the effects of atrazine's neurotoxicity and neurodegeneration is budding. The primary investigative species for atrazine-caused neurodegeneration has been *in vivo* and *in vitro* rat models. In this study we investigated the neurotoxic and, secondarily, neurodegenerative effects of atrazine and its primary active metabolite, diaminochlorotriazine, (DACT) in mouse *in vitro* models. Exposure of DACT on mouse N2α neurons and BV2 microglia elicited dosimetric and neurotoxic results as indicated by the MTT assay. DACT exposure to BV2 and N2α cells resulted in greater cellular toxicity with increasing concentration. The results also illustrated a good correlation between polypeptide aggregation and neurodegeneration. The present experiments demonstrated that mouse *in vitro* assays could be used as an investigatory model into neurodegenerative disease.

ABSTRACT FINAL ID: 3801 Poster Board: P493
TITLE: Predicting MTDs for a 2-Year Rat Study Using Pharmacokinetics and Toxicity Differences Between Genders

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KEYWORDS: Pesticides; Pharmacokinetics; Carcinogenesis; MTD

ABSTRACT BODY: Pesticides are required to be tested up to the maximum tolerated dose (MTD) in chronic/oncogenicity studies. It is difficult to use the same high dose to achieve MTDs without overdosing the more sensitive gender or insufficiently challenging the less sensitive gender for chemicals with significant gender differences. When an inappropriate MTD is chosen, the assessment of the oncogenicity can be compromised. For a new pesticide currently under development, sub-chronic studies were conducted in rats. In the 28-day dietary study, a 3-4 fold higher systemic exposure was noted in females when compared to males as measured by areas under the curve (AUCs) and Cmax at dose levels ranging from 300 to 4000 ppm. In the 90-day dietary study with a 28-day recovery phase (0, 100, 450, 2000/6000 ppm females/males), 8% decrease in body weight gain (BWG), 15% increase in relative liver weights, 33% increase in relative thyroid weights, 60% increase in serum triglyceride, and hepatocellular hypertrophy were observed in the 6000 ppm males. Comparatively, in females at 2000 ppm the following changes were observed: 17% decrease in BWG, 15% increase in relative liver weights, 24% increase in relative thyroid weights, and hepatocellular hypertrophy. At the end of the 28-day recovery, males demonstrated full recovery while females showed only partial recovery. Thus, a large gender differences in PK and toxicity were observed in all sub-chronic dietary studies. These data demonstrated that it would not be possible to use the same high dose to reach MTD for both genders in the 2-year study. Using this PK and toxicity data, high doses of 1600 and 4800 ppm were selected for females and males, respectively. In the 2-year study, 14%, 13% and 13% reduction in BWG of males and 10%, 12% and 19% reduction in BWG of females were observed at weeks 13, 26 and 52, respectively in the high doses. In addition, food consumption was reduced by 8%, 4% and 8% in the 4800 ppm males and 7%, 7% and 6% in the 1600 ppm females at weeks 0-13, 0-26 and 0-52, respectively. Based on the BWG decrement data it is clear that MTDs were achieved in both genders with different high dose levels. In conclusion, as demonstrated by this example for a pesticide with large gender differences, it is scientifically sound and practical to use PK and toxicity data to predict different high dose levels to achieve MTDs to improve the quality and regulatory acceptability of chronic/oncogenicity studies.

ABSTRACT FINAL ID: 3802 Poster Board: P494
TITLE: Ameliorative Effect of Vitamin C on Amitraz-Induced Toxicity in Albino Wister Rats

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S.M. Anika, V. Omoja, U. I. Asuzu. *Veterinary Physiology and Pharmacology, University of Nigeria Nsukka, Nsukka, Nigeria.*
KEYWORDS: Pesticides; Epididymis; Exposure, Environmental; Vitamin C; Amitraz

ABSTRACT BODY: There is the need to investigate whether vitamin C could ameliorate amitraz-induced toxicity since amitraz poisoning has no specific antidote. Thirty adult male rats 180 ± 10 g assigned into five groups A to E using simple random sampling technique were used for this study. The groups were treated as follows: A (50 mg/kg bw amitraz only), B (50 mg/kg bw amitraz plus 30 mg/kg bw vitamin C), C (50 mg/kg bw amitraz plus 150 mg/kg bw vitamin C), D (50 mg/kg bw amitraz plus 750 mg/kg bw vitamin C) and E equivalent volume of distil water. The treatment was done daily using the oral route for 28 days. Haematological parameters and serum biochemical markers were taken and analyzed on day zero for base line data and at the end of the experiment. Testicular sperm reserve (TSR), body weight gain/loss index (BWI), epididymal sperm reserve (ESR), plasma antioxidant markers and histology of the liver and kidney were taken once at the end of the experiment and analyzed. The mean ESR and TSR of rats in groups D and E were significantly higher ($p \leq 0.05$) than those of rats in groups C, B and A. Sections of the testes from group A showed a diffuse degeneration of the seminiferous tubules while sections of the testis from rats in groups C, D and E showed no deviation from the normal testicular histo-architecture. The mean alanine aminotransferase of rats in groups A and B were significantly higher ($p \leq 0.05$) than those of groups C, D and E on day 28 of the experiment. There was dose-dependent significant reduction ($p \leq 0.05$) among the mean packed cell volume and haemoglobin concentration as the vitamin C in-take decreased in groups C, B and A on day 28 of the experiment, with group A having the least values. It was concluded that vitamin C at 750 mg/kg could ameliorate amitraz-induced toxicity.

ABSTRACT FINAL ID: 3803 Poster Board: P495
TITLE: Hemp Seed Formulations Deter and Inhibit Feeding by Disrupting the Nervous System of Beetles

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J. Robinson, C. Baldwin, R. Johnson, S.L. Chao. *Fayetteville State University, Fayetteville, NC.*
KEYWORDS: Pesticides; Food Safety/Nutrition; Neurotoxicity; Pesticides

ABSTRACT BODY: According to the United States Department of Agriculture, postharvest losses from insect infestation on corn and wheat in the United States are estimated at about $1.25 to $2.5 billion. One of the culprits is the Tenebrionidae family of beetles, specifically the *Tribolium* and *Tenebrio* species. Insecticides have been used to control these pests but not many people are comfortable with toxic chemicals being sprayed on and around their food. The objective of the current study was to determine the efficacy of a patent-pending formulation composed of hemp seeds of the *Cannabis* plant developed at Fayetteville State University. Beetles were exposed to the formulation through choice tests and feeding experiments. Following feeding experiments, adult beetles were measured for changes in acetylcholinesterase activity by a modified Ellman's Assay. Choice tests included giving 60 *Tribolium* adults and 30 *Tenebrio* larvae a choice between flour containing hemp seed and flour without hemp seed over a period of two weeks. Feeding experiments involved placing 50 *Tribolium* adults and 40 *Tenebrio* larvae in cups containing wheat flour alone or with various amounts of hemp seed formulation and monitoring amounts of flour consumed over a period of two weeks. Acetylcholinesterase activity was measured in 25 *Tribolium* adults from feeding experiments. All statistical analyses were assessed by one-way ANOVA and post hoc Tukey test. Choice tests revealed that over 70% of *Tribolium* adults and *Tenebrio* larvae tested preferred food without hemp seed by Day 2 of choice experiments. After two weeks of feeding experiments, insects consumed approximately 90% of flour without hemp seed formulation compared to only 55% of flour containing hemp seed. Finally, hemp seed-exposed *Tribolium* adults displayed significantly lower acetylcholinesterase activity compared to controls that were not exposed to hemp seed. The hemp seed formulation appears to have repellant and anti-feeding properties with possible mechanism of action targeting the cholinergic system.

ABSTRACT FINAL ID: 3804 Poster Board: P496
TITLE: A Pilot Study of Malathion, Atrazine, Carbaryl, and Chlorpyrifos in the Breast Milk pf Women in Suburban and Agricultural Communities of Central Florida

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M. Bourgeois[1], E. Pulster[2]. *[1]EOH, USF COPH, Tampa, FL; [2]USF College of Marine Science, St. Petersburg, FL.*
KEYWORDS: Pesticides; Children'S Health; Developmental Toxicity; Post-Natal; Lactational Transfer

ABSTRACT BODY: Breastfed infants may be exposed to a variety of environmental and pharmaceutical contaminants via lactational transfer. Chronic exposures to environmental contaminants like pesticides may have disruptive physiological consequences as nursing occur during critical periods of infant development. Diet is the largest contributor by pathway to total pesticide intake from ubiquitous background sources for the general public and other situations where airborne levels are not remarkably high. The occupational contribution to the exposure pathway for agricultural workers dwarfs dietary influence. This means breastfed neonates in agricultural areas may be disproportionately vulnerable to the effects of pesticides. We examined the expressed breast milk of nursing mothers with a child < 3.0 years of age from suburban and agricultural/rural populations to assess the concentration of four commonly used Floridian pesticides–Malathion, Atrazine, Carbaryl and Chlorpyrifos. Individual breast milk samples were extracted using the QuEChERS sample preparation method. The GC/MS/MS response was monitored using a continuing calibration standard passing acceptable criteria (%RSD ≤20 for all compounds with the exception of carbamates). Two of 28 samples, both from donors living in rural locations, were found to have elevated Malathion levels in their breast milk. No other specimens had values exceeding the limits of detection (LOD) for any of the four study pesticides. An additional 5 specimens, also from rural donors, fell just below the 10 ng/mL LOD for Malathion. Breast milk is a pesticide excretion route for lactating mothers and an exposure route for nursing infants. Assessments such as this capture a 'snapshot in time'. Considering the vulnerability of nursing infants and the potential for disparate exposures based solely on occupational and socioeconomic status, an ongoing breast milk biomonitoring program may be warranted.

ABSTRACT FINAL ID: 3805 Poster Board: P497
TITLE: Using OP Pesticide Metabolites for Determination of Genetic Variability in Farmworkers

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KEYWORDS: Pesticides; Epigenetics; Exposure Assessment

ABSTRACT BODY: We assessed the utility of comparing urinary metabolite fractions of organophosphate (OP) pesticides in occupationally exposed populations as a new biomarker parameter to assess human genetic variation in OP metabolism. To do so, we utilized data obtained from the longitudinal examination of the potential for farmworker exposure to dimethyl OP pesticides, including azinphos methyl (AZ), in our Community Based Participatory Research Project (CBPRP) located in the Yakima Valley, Washington State. OP pesticides are metabolized via two main pathways, dearylation and desulfuration, both facilitated by cytochrome P-450 (CYP450s) and glutathione-s-transferase (GSTs) families of enzymes. We determined the genotypes for these metabolizing enzymes in our study population using the Affymetrix DMET multiplexed genotyping platform. We then assessed whether the metabolite fractions were significantly different between groups varying by these genotypes. Significant differences in the dimethyl phosphate (DMP) metabolite fractions (associated with desulfuration) were observed based on the number of CYP1A2 *1 and CYP1A2*1L and CYP2D6*1 and CYP2D6*2 alleles. Significant differences in the dimethyldithio phosphate (DMDTP) metabolite fractions (associated with dearylation) were observed based on the number of CYP1A2*1A and CYP1A2*1F and CYP2D6*1 and CYP2D6*2 alleles. This is the first time that it has been suggested that genetic variation in these CYP450 enzymes have an effect on the metabolism of AZ in humans. We propose that the fraction of urinary metabolites can be used to examine the effect of genetic variation in CYP450s and other metabolizing enzymes on the metabolism of OP pesticides *in vivo*. This work, therefore, builds on the limited amount of *in vitro* research that has evaluated the metabolism of AZ and other dimethyl OP pesticides.

ABSTRACT FINAL ID: 3806 Poster Board: P498
TITLE: Combined Application of Bacterial Products to Enhance Toxicity Against Fungal Pathogens

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KEYWORDS: Undergraduate Student

ABSTRACT BODY: Entomopathogenic nematodes (in the genera Steinernema and Heterorhabditis) are obligate lethal parasites of insects. They are mutualistically associated with Xenorhabdus spp. and Photorhabdus spp. bacteria, respectively. The bacterial cells multiply in the hemocoel of insects, produce various toxins and kill the host within 48 h. To protect the insect cadaver from secondary invasion by contaminating organisms, these bacteria produce a variety of antimicrobial compounds. The exudates and metabolites of Photorhabdus spp. and Xenorhabdus spp. bacteria exhibited toxic effects on various harmful plant pathogens. Our hypothesis was that combined applications of different bacterial exudates and/or metabolites would exhibit a stronger toxic effect on fungal plant pathogens than single bacterial treatments. In this study we used 3 different bacterial supernatants and one bacterial metabolite (Trans-Cinnamic-Acid) alone and in paired combinations (total 11 different treatments). The treatments were applied against two important fungal pathogens, Monilinia fructicola and Glomerella cingulate. Ten percent of bacterial suspension or 1% TCA was incorporated into Potato Dextrose Agar (PDA) media. A 5 mm diameter mycelia plug from a fungal culture was placed on the center surface of each agar dish. After incubation at 25oC for 7 days, the area of vegetative fungal growth was measured. The toxicity of single and combined applications was determined. The results indicated that 10% X. szentermaii exudate alone or especially when combined with 1%TCA was very effective in suppressing fungal pathogens. This combined application can be potentially used as a natural bio-fungicide against fungal pathogens of plants in the future.

ABSTRACT FINAL ID: 3807 Poster Board: P499
TITLE: Physiologic, Metabolomic and Proteomic Profiling of Mouse Neuroblastoma Cells Following Exposure to the Pesticide Sodium Fluoroacetate

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KEYWORDS: Metabolism; Neurotoxicity; Pesticides; Proteomics; Sodium Fluoroacetate

ABSTRACT BODY: The pesticide sodium fluoroacetate (SMFA) is a highly toxic, metabolic poison. The goals of this study were to 1) identify drug targets for post-exposure treatment options by quantitative metabolomics and proteomic profiling, and 2) develop a model system for testing and exploring physiological function in combination with proteomics/metabolomics. Mouse neuroblastoma N2A cells were exposed to 0.01-10 mM SMFA to determine the dose-response parameters. For determination of metabolic activity, cells were immediately assessed for evaluation of State 3, State 4, and uncoupler-stimulated respiration in a Seahorse XF Bioanalyzer. For quantitative metabolomics or proteomics, whole cells were treated with methanol or lysed and trypsin-digested followed by liquid chromatography-mass spectrometric (LC-MS) analysis. SMFA exposure affected mitochondrial respiration, including basal respiration, ATP production, proton leak, spare respiratory capacity, and coupling efficiency in a dose-dependent manner. Metabolites were identified and quantified with a Mass Spectrometry Metabolite Library of Standards of 619 high quality small biochemical molecules showing that SMFA exposure affected a broad-range of primary metabolism. The LC/MS analysis quantitated approximately 100 significantly regulated proteins in the N2A cells. The protein groups that were identified included those associated with energy metabolism, redox signaling, protein synthesis, and ATP/ADP transport. The quantitative profiling of specific protein sequences in combination with physiological testing provides further understanding of the mechanisms of SMFA toxicity and may aid in identification of new post-exposure treatment targets.

ABSTRACT FINAL ID: 3808 Poster Board: P500
TITLE: Chlorpyrifos Induces Airway Hyperreactivity in Rats

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KEYWORDS: Organophosphates; Pesticides; Lung; Pulmonary or Respiratory System; Chlorpyrifos

ABSTRACT BODY: Organophosphorus pesticides have been associated with increased incidence of human asthma. The canonical mechanism of OP neurotoxicity is inhibition of acetylcholinesterase (AChE), but we previously demonstrated that the OP chlorpyrifos (CPF) causes airway hyperreactivity in non-sensitized guinea pigs at levels that do not inhibit AChE in the lung or brain. Rather the mechanism appears to involve inflammatory mediators. While these findings are significant, they cannot be readily generalized to other species, including humans, because the guinea pig is a highly reactive species with a more robust inflammatory response to asthmatic stimuli than humans or other rodents. Therefore, in this study, we determined whether CPF similarly influences the airway physiology of rats, a far less reactive species, to provide cross-species validation of these findings. Pulmonary function was measured in 8 week old male Sprague Dawley rats during electrical stimulation of the vagus nerves on a Flexivent mechanical ventilator 24 h after subcutaneous injection of 30 mg/kg CPF. At the conclusion of physiological measurements, the lungs, cerebella and peripheral blood were collected and assayed for AChE activity. CPF increased airway resistance and elastance, and decreased lung compliance. CPF significantly decreased AChE activity in peripheral blood but not in extracts of lung or cerebellar tissues. These data indicate that CPF induces airway hyperreactivity in rats at levels that do not inhibit AChE activity in the lung or brain, suggesting that OP-induced airway hyperreactivity is a cross-species phenomenon. This work was supported by the NIH (grants R01 ES017592 and T32 HL07013).

ABSTRACT FINAL ID: 3809 Poster Board: P501
TITLE: Potential Mechanism of Neuronal Entry of a Glyphosate-Containing Herbicide in *C. elegans*

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: C.L. Corona, K.M. Montgomery, K.A. McVey, I.B. Snapp, V.A. Fitsanakis. *Biology, King University, Bristol, TN.*
KEYWORDS: Neurotoxicity; Pesticides; Undergraduate Student; GFP Reporter Constructs; Glyphosate

ABSTRACT BODY: Previous work in Caenorhabditis elegans (*C. elegans*) treated at the egg or the second larval (L2) stage with the glyphosate-containing herbicide TouchDown® (TD) showed evidence of dopaminergic (DAergic) neurodegeneration similar to that observed in Parkinson's disease. Given these data, we hypothesized that a potential mechanism of entry of TD into specific neuronal populations could be through pre-synaptic neurotransmitter transporters. This mechanism of entry is consistent with other toxicants known to target DAergic neurons, i.e. MPP+ and 6-OHDA, through the DA transporter (DAT). In order to test this hypothesis, two genetically modified *C. elegans* strains were used. The BZ555 strain had only DAergic neurons tagged with green fluorescent protein (GFP; [dat-1p::GFP]), while the NW1229 strain had all neurons pan-neuronally tagged with GFP (neuron-specific Ras::GFP). The resulting GFP fluorescence in these strains was used a surrogate for neuronal degeneration in visual and plate reader assays. In order to determine whether pre-synaptic neurotransmitter transporters may facilitate intracellular TD transport, NW1229 worms were pre-treated with the 5-HT transporter (SERT) antagonist Clomipramine, while BZ555 worms were pre-treated with the DA transporter (DAT) antagonist, GBR 12909 (Vanoxerine). As a control, groups of worms from the same strains were treated with TD in the absence of an antagonist pre-treatment. Following pre-treatment with the respective antagonists, both strains were treated chronically (24 h) with the TD formulation (commercially available) at low (2.7% glyphosate)-, mid (5.5% glyphosate)-, or high (9.8% glyphosate)-concentrations. Data from the BZ555 strain pre-treated with GBR12909 showed a statistically significant ($p = 0.02$) increase in neuron-associated fluorescence at 5.5% glyphosate in the TD formulation when compared to the corresponding non-antagonist treated group. This was not the case for the low- or high-levels of TD, although a trend towards increased fluorescence was noted in the 9.8% glyphosate group ($p= 0.08$). When studies were repeated in NW1229 worms pre-treated with Clomipramine, no statistically significant change in fluorescence were detected between pre-treated and non-pretreated groups at any TD concentration. Taken together, these data support the hypothesis that DAT, but not SERT, may play a role in the entry of TD into DAergic, but not other, neuronal populations.

ABSTRACT FINAL ID: 3810 Poster Board: P502
TITLE: Incubation of the Mn/Zn-Containing Fungicide Manzate with Dopamine Does Not Catalyze Redox Reactions

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KEYWORDS: Neurotoxicity; Pesticides; Undergraduate Student; Structure-Function Analysis; Manzate

ABSTRACT BODY: The measurement of reactive oxygen species (ROS) is frequently used as a technique for assessing ROS production resulting from direct interaction of a toxicant with its respective target molecules. One way to determine the formation of ROS is by using a sealed, water-jacketed oxygen consumption chamber fitted with a Clark electrode. This electrode is specific for detection of oxygen, but not for ROS. Previous work from this lab has shown that Caenorhabditis elegans (*C. elegans*) treated with manzate, a fungicide in which both Zn(II) and Mn(II) are coordinated with ethylene-bis-dithiocarbamate, show dopaminergic (DAergic) neurodegeneration, which is observed in Parkinson's disease (PD) patients. Since it is well-documented that oxidative stress can play a role in neuron death, we hypothesized that one mechanism of manzate toxicity could involve the direct interaction of manzate with DA. Furthermore, it is also known that catechols can autoxidize, and in the process catalyze the formation of hydrogen peroxide. The resulting hydrogen peroxide can participate in Fenton reactions in the presence of multiple transition metals, of which Zn(II) and Mn(II) are examples. Thus, we wanted to determine if the individual metals and/or the parent compound manzate, as found in commercial formulations, could react with the neurotransmitter DA leading to ROS production. Using a structure-function approach, DA or catechol was incubated for 10 min with the well-characterized Fenton reagent, Fe(II) chloride. Both studies (DA or catechol) resulted in a 10% decrease in oxygen in the chamber over this time frame. This suggested that the ethylamine group on DA had no effect on the ability of Fe(II) to contribute to ROS generation under these conditions. In contrast, when the studies were repeated using the chloride salts of Zn(II) or Mn(II), or the commercial formulation of manzate, no significant oxygen consumption occurred. These results indicate that catechol and DA can participate in a Fenton-like reaction with Fe(II), but that the Mn and Zn ions in manzate are not sufficiently redox active to initiate ROS in the presence of DA. These data also suggest that the ethylamine moiety present in DA, but absent in catechol, plays no significant role in the production of ROS in this assay. Finally, these studies provide evidence that our observed neurodegeneration in *C. elegans* may be independent of the direct interaction of manzate with DA.

ABSTRACT FINAL ID: 3811 Poster Board: P503
TITLE: ADHD in Mexican Children and Its Relationship With Pb or Cd Levels and MAO-A, COMT and 5HTT Genetic Polymorphisms

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KEYWORDS: Neurotoxicity; Metals; Genetic Polymorphisms; Neurotoxicity; Developmental; Cd

ABSTRACT BODY: The developing brain of a child is sensitive to lead (Pb) and cadmium (Cd) exposure. Behavioral problems are 15-20% of consultations in mental health care for children. Inappropriate behavior include attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD) and dissocial disorder (DD). Aggressiveness is often associated to ADHD as well as genetic polymorphisms of COMT, MAOA and 5HTT. We performed an exploratory study to determine the frequency of ADHD and disruptive behavior in elementary school children in Veracruz, Mexico, Pb and Cd levels and genotyping. One of 284 elementary schools was randomly selected to participate. Parents signed informed consent. Diagnostic structured interview (M.I.N.I.KID) was applied to participating children. Blood samples for complete chemistry and cells count, Pb and Cd levels and DNA extraction were obtained. Cd and Pb levels were analyzed by atomic absorption spectroscopy. Of 42 children (43% male, 57% female; age range 6-11 years old), eleven had ADHD, 2 of whom also had ODD and 1 had both ODD and DD. Only 1, without ADHD, had ODD. There were 7 with mild anemia including 4 children with ADHD, 1 with DD and 4/6 criteria for attention deficit and 2 of the control group. All children had Pb levels ≤ 1.4 µg/L, except a 7 years old girl (5.36µg/L) (control group without anemia). Range Cd levels was between 0.3034-3.5840 µg/L and only 6 kids had Cd levels ≤ 1.5 µg/L. The distribution of genotypes between cases and controls were not different. In conclusion, levels of Cd were higher in these children compared with children in other countries. We did not observe positive correlations between cases and controls with respect to Pb or Cd levels and distribution of genotypes, but anemia was more frequently found in children with ADHD than in controls. We will start a larger study to confirm or rule out the relationship between prevalence of these disorders with genetic polymorphisms and heavy metals exposure.

ABSTRACT FINAL ID: 3812 Poster Board: P504
TITLE: Acute Methylmercury Exposure Alters mRNA Expression of Glutamate Receptor Subunits in NSC34 Cells

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KEYWORDS: Neurotoxicity; Metals; Metals; Receptor

ABSTRACT BODY: Acute exposure to methylmercury (MeHg) causes disturbances in sensation, hearing, balance and movement. MeHg neurotoxicity exhibits pronounced cell specificity; motor neurons are one potential target. MeHg toxicity in motor neurons causes dysregulation of [Ca^{2+}_i] that contributes to cell death. These effects are mediated in part by alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate (NMDA)-type glutamate receptors. AMPA receptors (AMPARs) comprise 4 subunits in dimeric dimers, and include the GluA1-GluA4. Receptor subunit composition markedly affects their Ca^{2+} permeability. In this study we evaluated effects of acute MeHg exposure on relative mRNA expression levels of AMPAR subunits in a motor neuron cell line (NSC-34). Differential effects of MeHg on expression of AMPAR subunits could contribute to the understanding of its toxicity on motor neurons. Day *in vitro (*DIV 2) differentiated NSC34 cells were exposed to 0, 1, 2, or 5 µM MeHg for 24 hrs. RNA was isolated from 1 ml of NSC34 cells and reverse transcribed. Quantitative real-time PCR was used to determine the mRNA expression levels of GluA1, 2, 3, and 4. Acute exposure to 1µM MeHg led to downregulation of the GluA3 and GluA4 but had no effects on the GluA1 and GluA2 subunits, compared to control. Exposure to 2 or 5 µM MeHg led to downregulation of all the subunits. A previous study demonstrated that MeHg LC50 for NSC34 cells at 24 hrs is 1.7 µM, thus the effects observed could be an indication that the cells are unable to compensate for MeHg damage at higher concentrations (2 and 5 µM). As for the 1 µM MeHg exposure, the levels close to control observed for the GluA1 and GluA2 and not for GluR3 and GluA4 could indicate that at this concentration there could be a subunit composition alteration after acute MeHg exposure. However reducing expression of GluA3 and GluA4 would be expected to reduce Ca^{2+} permeability of motor neuron AMPARs. This work was supported by NIEHS grant R01ES024064 and NIEHS Training Grant T32ES007255-26.

ABSTRACT FINAL ID: 3813 Poster Board: P505
TITLE: Effects of Lead and Prenatal Stress on Post-Translational Histone Modifications in the Embyonic Brain

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KEYWORDS: Neurotoxicity; Metals; Neurotoxicity; Developmental; Epigenetics; Lead

ABSTRACT BODY: Epigenetic gene regulation is crucial for normal brain development and its misregulation can result in neurodevelopmental disorders. Early life exposure to lead (Pb) and prenatal stress (PS) are known to contribute to alterations in brain development and hypothalamic-pituitary-adrenal axis function resulting in cognitive/behavioral disturbances that may occur by epigenetic alterations. Here we explored the effects of Pb, PS and Pb+PS on post-translational histone modifications (PTHMs: H3K9ac, H3K4me3, H3K9me2 and H3K27me3) at a global level using multiplex quantitation during fetal development (E18) in frontal cortex (FC) and hippocampus (HIPP) in male and female mouse embryos. Female C57/Bl6 mice were randomly assigned to receive drinking water containing 0 or 100 ppm Pb acetate from 2 months prior to breeding through lactation and to non-stress (NS) or PS (i.e., restraint stress 3x/day for 30 min/day from gestational day 15-18), yielding 4 treatment groups: 0-NS, 0-PS, 100-NS and 100-PS. Significant effects of Pb, PS and/or Pb+PS were found in FC and HIPP, however, the pattern of changes differed by sex and brain region. For example, similar PS-related increases in H3K9me2 and H3K27me3 were observed in both sexes in FC, whereas H3K9ac was increased by Pb, PS and Pb+PS in females, but only by Pb in males, with reductions produced by PS and Pb+PS. Similarly, Pb and PS each increased FC H3K4me3 in males, but reduced levels in females. Pb alone produced pronounced increases in HIPP H3K9ac, whereas PS effects were modest, as were Pb and PS effects on this marker in female HIPP. Reductions in the repressive mark H3K9me2 in HIPP were produced by Pb, PS and Pb+PS in males, but only by PS in females. Additional studies found changes in expression of histone methyl and acetyl transferases and demethylases and deacetylases that may at least partially underlie the changes in PTHMs observed. Together these data suggest that early developmental exposure to Pb, PS and Pb+PS can significantly affect the brain epigenome, potentially leading to abnormal brain development and function. This approach could also facilitate understanding of relationships of epigenetic marks to both normal and abnormal cognitive functions. Supported by NIH grant ES021534.

ABSTRACT FINAL ID: 3814 Poster Board: P506
TITLE: An Investigation of Developmental Neurotoxicity by Inhalation Exposure to Manganese Dichloride in Rats

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KEYWORDS: Neurotoxicity; Developmental; Manganese

ABSTRACT BODY: Over the past ten years there has been an increase in the number of published epidemiological studies which suggest that environmental exposure to manganese, including by contaminated drinking water, may impair intellectual performance and behaviour in children. The purpose of this study was to investigate any potential functional and morphological effects on the developing nervous system of the rat offspring that may arise from exposure *in utero* and during early life. The study was performed in accordance with the OECD Guidelines for the Testing of Chemicals, No. 426. Three groups of 27 females were treated with manganese dichloride ($MnCl_2$) via inhalation (nose only), at concentrations of 3.5, 12.3 and 17.6 µg/L. The control group was treated with air under the same conditions. Animals were exposed for 6 hours daily from day 6 to day 19 *post coitum* and from day 1 to day 20 *post partum*. Pups were only treated indirectly via maternal blood and milk during pre- and postnatal neurological development. Measurement of $MnCl_2$ in maternal milk during lactation showed a clear presence of the test item. Offspring were randomly selected from within litters for neurotoxicity evaluation, which consisted of observations to detect gross neurologic and behavioral abnormalities, including behavioral ontogeny, motor and sensory function, learning and memory, and neuropathology during postnatal development and adulthood. At high dose level, up to 70% of the dams showed breathing noises with a reduction in mean food consumption and body weight. All dams recovered at the end of gestation. Exposure to $MnCl_2$, *in utero* and in early life produced no behavioral abnormalities, no neuropathological effects and, most importantly, no learning impairments at any of the doses. Based on these findings the NOAEL (No Observed Adverse Effect Level) for dams and pups was established at 17.6 µg/L air $MnCl_2$.

ABSTRACT FINAL ID: 3815 Poster Board: P507
TITLE: Effects of Developmental Lead (Pb) and Prenatal Stress (PS) on Glucocorticoid Receptor Gene Chromatin Modifications in Adult Brain

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: G. Varma[1], M. Sobolewski[2], D. W. Anderson[1], J. S. Schneider[1], D. A. Cory-Slechta[2]. [1]*Thomas Jefferson University, Philadelphia, PA;* [2]*University of Rochester, Rochester, NY.*
KEYWORDS: Epigenetics; Neurotoxicity; Metals; Neurotoxicity; Developmental; Lead

ABSTRACT BODY: Dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis and of brain glucocorticoid function can result from early life exposures to Pb and to PS, leading to cognitive and behavioral disturbances associated with these risk factors. Recent evidence suggests that such effects could reflect dynamic epigenomic remodeling. To determine effects of Pb, PS, and Pb+PS on enrichment of post-translational histone modifications (PTHMs) on the glucocorticoid receptor gene, Nr3c1, female C57/Bl6 mice received 0 or 100 ppm Pb acetate drinking water from 2 mos prior to breeding through lactation and to non-stress (NS) or PS (restraint stress 3x/day for 30 min/day from gestational days 15-18), yielding 4 offspring treatment groups: 0-NS, 0-PS, 100-NS and 100-PS. PTHMs H3K9ac, H3K4me3, H3K9me2 and H3K27me3 were analyzed in PND60 frontal cortex (FC) and hippocampus (HIPP) across the Nr3c1 gene encompassing regulatory regions including promoters and non-coding exons at 5'UTR, coding exons, introns and 3'UTR using ChIP assay. Immunoprecipitated DNA was quantified using a high-throughput microfluidic qPCR system. A significant main effect of PS was seen across the Nr3c1 gene with the repressive modification H3K9me2 in FC showing increased enrichment for both sexes. For both activating marks, H3K9ac and H3K4me3, only males had significant interaction effects with Pb+PS showing decreased levels in HIPP compared to either Pb or PS. However, significant differences were also observed in FC and HIPP within and between males and females in several locations across the Nr3c1 gene regions analyzed, particularly for the repressive PTHM H3K27me3 in males. These sequence specific changes suggest that the epigenetic responses to Pb, PS and Pb+PS includes not only promotor regions, but also transcriptional and intragenic sites along the entire Nr3c1 gene. These data also suggest that the epigenetic response to Pb, PS and Pb+PS is coordinated across the Nr3c1 genomic region and brain regions. The changes in PTHMs further suggest that developmental exposure to Pb, PS and Pb+PS uniquely modified Nr3c1 in a sex-specific manner that persisted into adulthood. These results provide mechanistic support for the well-known sex-specific cognitive/behavioral deficits observed following developmental Pb, PS and Pb+PS exposures.
Supported by NIH grant ES021534.

ABSTRACT FINAL ID: 3816 Poster Board: P508
TITLE: Vectors of Co-Morbidity: MicroRNAs Mediate the Transgenerational Inheritance of Addictive Behavior in Response to Early Alcohol-Nicotine Co-Exposure

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: F. Taki, B. Zhang. *Biology, East Carolina University, Greenville, NC.*
KEYWORDS: Epigenetics; Developmental Toxicity; Post-Natal; Exposure, Environmental; Alcohol, Nicotine, microRNAs; Drugs of Abuse

ABSTRACT BODY: Alcohol and nicotine are the most co-abused drugs among adolescents and are linked to increased health risks in future generations. However, little is known about the molecular mechanism for this transgenerational inheritance. In this study, we investigated the role of microRNAs as mediators of alcohol-nicotine co-abuse across generations using *C. elegans*. Parent worms were treated with alcohol with or without nicotine prior to sexual maturity from the L1 to L4 larval stages. Worms were then washed off and grown on treatment-free media for two generations. Several endpoints were assessed in three generations and included reproduction, behavior, and global miRNA profiles. Worm behavior was analyzed via MBF WormLab software. Real-Time PCR (Applied Biosystems ViiA™ 7) and ddCt method were used to study the expression of 231 miRNAs. miRNA-target prediction was based on TargetScan and miRanda, while functional annotation was based on KEGG enrichment via DAVID. Heat maps and principal component graphs were constructed via Mev4. Our results showed that early alcohol impacted fertility only in the parent generation [$F(6,21)=9.495$; $p=0.001$]. Interestingly, behavior and global miRNA profiles were altered across three generations in response to the single parental drug exposure. However, co-treatment with nicotine reversed behavioral clustering as well as miRNA profiles in comparison to alcohol treatment alone. Partial infertility and behavioral changes in grand-offspring worms were likely due to miRNA-dependent dysregulation of calcium, progesterone, Wnt, MAPK signaling and sphingolipid-methionine metabolisms. 8 miRNAs (miR-35-3p, miR-360-3p, miR-64-3p, miR-51-3p, miR-59-3p, miR-59-5p, miR-60-5p, miR-65-3p) were impacted in response to early alcohol and nicotine treatments in at least two generations. Unsupervised hierarchical clustering showed that alcohol alone impacted behavior, but was not enough to induce a true miRNA transgenerational response. However, when combined with nicotine, the impact remained evident in the grand-offspring generation. In conclusion, nicotine rendered grand-offspring worms more resistant to alcohol's toxicity through aberrant miRNA expression. Thus, our study provides insights on the role of miRNAs in increasing the susceptibility of binge drinking among adolescents across generations.

ABSTRACT FINAL ID: 3817 Poster Board: P509
TITLE: Low-Dose Developmental Benzo[a]pyrene Neurotoxicity In Zebrafish Was Found to Significantly Alter Juvenile and Adult Behavior but Effects Were Not Detected By A Test of Larval Motility

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KEYWORDS: Neurotoxicity; Developmental; Behavior; Polycyclic Aromatic Hydrocarbons

ABSTRACT BODY: The polycyclic aromatic hydrocarbon benzo[a]pyrene (BaP) is an ubiquitous environmental contaminant arising from both natural and anthropogenic sources. Developmental exposures to BaP have been linked to later behavioral effects in both humans and animal models. Zebrafish have become widely used to assess neurodevelopmental effects of pharmacological compounds and environmental toxicants; however, this model has been minimally applied to understanding the lifelong consequences of early-life BaP exposure. We investigated the impact of early-life exposures to BaP on zebrafish behavior at larval (6 days post-fertilization (pf)), juvenile (1.5 months pf), and adult (6 months pf) stages. Zebrafish were exposed to 0, 1, 10, 100 and 500 ug/L BaP from 6 hours to 5 days pf. A locomotor assay under alternating dark and light conditions was performed on a subset of larvae at 6 days pf. The remainder were grown to adulthood and submitted to a behavioral testing battery at 1.5 and 6 months pf. No significant differences in locomotor response to changing light conditions were detected at 6 days pf. In contrast, we observed significant differences in juvenile and adult behavior. During the juvenile stage, developmental 100 ug/L BaP exposure caused reduced exploration behavior in a novel tank exploration task, but no other significant effects were observed. More prevalent differences were seen in zebrafish tested at 6 months pf. At this age developmental BaP exposure caused increased exploration in a novel tank when zebrafish were treated with a low dose of BaP (1 ug/L) ($p<0.05$). 10 and 500 ug/L exposure during development caused an enhanced startle response compared to controls ($p<0.001$ and $p<0.05$, respectively) in a tap/startle habituation task. Additionally, zebrafish treated with a moderate dose of BaP (10 ug/L) during development had a significantly greater flee response than both low (1 ug/L; $p<0.005$) and high (500 ug/L; $p<0.05$) dose groups in a predator escape task. Although larval zebrafish motility in response to light/dark conditions has proven a useful assay for screening the potential for chemicals to alter neurodevelopment, this test may lack the sensitivity to detect subtle changes that impact complex behaviors later in life. Research supported by the Duke University Superfund Research Center (ES010356) and a National Institute of Environmental Health Sciences training grant (T32-ES021432).

ABSTRACT FINAL ID: 3818 Poster Board: P510
TITLE: Combined Effects of Gestational Exposures to Arsenic and Deprivation Stress in Mice

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: K. Conrad, E. Marvin, C. Klocke, M. Sobolewski, D.A. Cory-Slechta, J.L. Allen. *Environmental Medicine, University of Rochester Medical School, Rochester, NY.*
KEYWORDS: Behavioral; Neurotoxicity; Developmental; Neurotoxicity; Metals; Stress; Arsenic

ABSTRACT BODY: Humans are concurrently exposed to multiple environmental risk factors. Some target common biological substrates and thus have the potential to produce enhanced or cumulative adverse effects, as we have previously shown in both mice and rat offspring exposed developmentally to lead (Pb) or methylmercury (MeHg) combined with prenatal restraint stress (PS) on neurodevelopmental outcomes. This study extended this hypothesis to assess combined effects of maternal arsenic (As) exposures (0 or 50 ppb in drinking water from 2 weeks prior to breeding until pup birth) in conjunction with a deprivation stress (DS) imposed daily throughout gestation. The DS model was designed to provide a better translational animal model of stressors associated with poverty, particularly differential access to resources. Pair-housed dams were first provided with daily access to a highly preferred food, mealworms. Upon presence of a vaginal plug (GD0.5), dams were housed in adjacent cages separated by wire mesh, and only one dam received mealworms (non-stress), while the other dam could smell, see, etc. but never access mealworms (DS). This yielded 4 treatment groups of offspring per sex: 0-NS (no arsenic, no DS), 0-DS (no arsenic, DS), 50-NS (As only) and 50-DS (As+DS). DS and As+DS produced a female specific doubling of serum corticosterone measured at postnatal day (PND) 0 relative to controls. Behavioral evaluation of offspring began at PND 60 on a fixed interval (FI) 60 sec schedule of food reward, a behavioral baseline proven sensitive to combined Pb and MeHg with prenatal stress, carried out for 33 daily (M-F) test sessions. As expected, FI overall response rates increased across test sessions. However, the increase was significantly reduced in males by As and even further reduced by As+DS, consistent with cumulative toxicity. Effects of DS per se were also seen in males during early FI test sessions on measures of timing on the FI schedule, with increases in postreinforcement pause time and median inter-response time values. These data further support the hypothesis that metals and stressors targeting common biological substrates can enhance neurotoxicity relative to these risk factors in isolation and underscore the importance of furthering cumulative risk assessments. In addition, these findings support the efficacy of our newly established DS model that has the potential to be utilized multigenerationally.

ABSTRACT FINAL ID: 3819 Poster Board: P511
TITLE: A Polymer-Halloysite Formulation for Postnatal Methylmercury Delivery: A Pilot Study

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KEYWORDS: Neurotoxicity; Developmental; Neurotoxicity; Metals; Nanotechnology; Halloysite, Pluronic F127

ABSTRACT BODY: The developing nervous system is particularly sensitive to methylmercury (MeHg). To model human gestation, rodent models must include a postnatal exposure regimen (often via breast milk) because the first week of rodent life models the last trimester of human gestation. Unfortunately, the bioavailability of MeHg in breast milk is negligible so an alternative route is necessary. We developed an injectable polymer-halloysite formulation for subcutaneous injection during postnatal days 1-7. This solution is liquid when chilled and congeals, so release rate is slow, at body temperature. In Experiment 1, halloysite loadings under vacuum produced two different MeHg concentrations. Loaded halloysite was dispersed in Pluronic F127. Two 5 g mixtures of polymer-halloysite (2 and 4 MeHg loadings) were placed in separate conical vials, topped up with deionized water, and suspended in a water bath (34 C). Water samples were taken at 1.2, 6.3, 30, 48, 74, and 125 h. Cumulative release was calculated for each time point. In Experiment 2, C57BL/6 mouse pups were injected (*s.c.*) on PND 1 with MeHg-loaded polymer-halloysite formulation; halloysite was loaded with MeHg four times. Pups were sacrificed after 24, 72, 120, and 168 h to determine brain Hg concentration by ICP-MS. Vehicle controls included pups injected with halloysite in corn oil , halloysite in mineral oil , Pluronic alone, Pluronic + halloysite, and control. Body mass and simple behavioral measures served as the main biomarkers of health. In Experiment 1, the two loadings (2 or 4) released MeHg over 120 h but at different release rates. The higher loading was used in Experiment 2. Brain Hg concentration increased after 24 h to 1.33 ppm, peaked after 72 h to 2.41 ppm, decreasing slowly to lower levels after 168 h (0.96 ppm). There were body weight differences between the MeHg and control/vehicle control groups. An injectable polymer-halloysite formulation may be beneficial for the study of gestational exposure to MeHg. Further studies will be conducted to extend the utility of this model. [Funded by NIH ES024564]

ABSTRACT FINAL ID: 3820 Poster Board: P512
TITLE: Effect of Repeated Juvenile Exposure to Δ9-Tetrahydrocannabinol on Social Behavior in Adolescent Rats

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: R.L. Hybart, A. N. Mohammed, N.Alugubelly, B.L. Kaplan, R.L. Carr. *Center for Environmental Health Sciences, Mississippi State University, Mississippi State, MS.*
KEYWORDS: Neurotoxicity; Developmental; Behavior; Undergraduate Student; Marijuana

ABSTRACT BODY: Cannabinoid compounds are derived from marijuana, the most widely abused illicit drug in the world. The main psychoactive constituent of marijuana is Δ9-tetrahydrocannabinol (Δ9-THC). It has been reported that perinatal exposure to Δ9-THC results in altered social behavior in adult rats. However, exposure of older children to cannabinoids is increasing but little work has been done to investigate the effects of childhood exposure on social behavior especially during the adolescent period. The goal of the present study was to investigate the effects of developmental exposure to Δ9-THC on social behavior in adolescence. Ten day old rat pups were exposed orally to either corn oil or 10 mg/kg Δ9-THC daily for 7 days. On day 38, following a 24 hour isolation period, two unfamiliar rats of the same treatment, sex, age and size were placed together in an empty litter-filled cage. Social behaviors of the rats were observed for 10 min. The parameters recorded were: time to first interaction; episodes of grooming, chasing, investigative sniffing, nape attacks, crawling over/under, play fighting, and pinning; and the time spent playing. The rats exposed to Δ9-THC exhibited increased the episodes of investigative sniffing, nape attacks, and pinning and spent more time playing than did the control rats. Thus, activation of the endocannabinoid system by exposure to Δ9-THC during late preweanling ages can lead to altered social interaction and play behavior in adolescent rats.

ABSTRACT FINAL ID: 3821 Poster Board: P513
TITLE: Effect of Repeated Juvenile Exposure to Permethrin and Deltamethrin on Social Behavior in Adolescent Rats

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: G.C. Parker, N. Alugubelly, A. N. Mohammed, R. L. Carr. *Center for Environmental Health Sciences, Mississippi State University, Mississippi State, MS.*
KEYWORDS: Neurotoxicity; Developmental; Behavior; Undergraduate Student; Pyrethroid

ABSTRACT BODY: There is concern that children are more at risk to the negative effects of pesticides because of their ongoing nervous system development. The pyrethroid insecticides are frequently used for insect control in both households and in agriculture thereby increasing the risk of childhood exposure. Some studies have linked developmental exposure to pyrethroid insecticides to greater incidence of attention deficit/hyperactivity disorder (ADHD) of which social difficulties are a common feature. The goal of the present study was to investigate the effects of developmental exposure to permethrin, a Type I pyrethroid, and deltamethrin, a Type II pyrethroid, on social behavior in adolescence. Ten day old rat pups were exposed orally to either corn oil, 25 mg/kg permethrin, or 0.25 mg/kg deltamethrin daily for 7 days. On day 38, following a 24 hour isolation period, two unfamiliar rats of the same treatment, sex, age and size were placed together in an empty litter-filled cage. Social behaviors of the rats were observed for 10 min. The parameters recorded were: Time to first interaction; episodes of grooming, chasing, investigative sniffing, nape attacks, crawling over/under, play fighting, and pinning; and the time spent playing. Both pyrethroids decreased the episodes of chasing while increasing the episodes of investigative sniffing. In addition, the episodes of play fighting and the time spent playing were also slightly increased with both pyrethroids. These data suggest that juvenile exposure to both Type I and Type II pyrethroids can result in altered social behavior in adolescent rats.

ABSTRACT FINAL ID: 3822 Poster Board: P514
TITLE: Inhibition of Endocannabinoid Metabolism in Brain Regions Linked to Emotionality in Juvenile Rats Exposed to Chlorpyrifos

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M.E. Andrews, R.W. Buntyn, R.L. Hybart, C.A. Nail, H.R. Nelson, M.K. Ross, R.L. Carr. *Center for Environmental Health Sciences, Mississippi State University, Mississippi State, MS.*
KEYWORDS: Neurotoxicity; Developmental; Neurotoxicity; Pesticides; Juvenile Toxicity; Chlorpyrifos

ABSTRACT BODY: There is a concern that chlorpyrifos (CPF), an organophosphorus insecticide, may cause developmental neurotoxicity in children leading to long term effects. Our laboratory has reported that developmental exposure of rat pups to low levels of CPF disrupts endocannabinoid metabolism in the brain through the inhibition of fatty acid amide hydrolase (FAAH). At juvenile and adolescent ages, CPF-exposed rats exhibit altered emotional behavior in several tests (e.g, a light-dark emergence test, the elevated plus maze, and social play). In addition, the altered behavior was similar to the behavior of rats exposed to PF-04457845, a specific inhibitor of FAAH. This study investigated the alteration of endocannabinoid metabolism immediately following exposure in brain regions known to be strongly associated with emotionality. Ten day old rat pups were exposed orally to either 0.5, 0.75, or 1.0 mg/kg CPF or 0.02 mg/kg PF-04457845 daily for 7 days. At 12 hrs following the last administration, brains were collected, sliced, and punches were taken to obtain the hippocampus, amygdala, and nucleus accumbens. Activity of the endocannabinoid metabolizing enzymes, FAAH and monoacylglycerol lipase (MAGL), were determined in each region. For CPF, MAGL activity was inhibited (10-15%) only by the high dosage, whereas FAAH activity was inhibited in a dose-dependent manner in all three brain regions [% inhibition: hippocampus (20%, 34%, and 45%, respectively), amygdala (27%, 42%, and 58%), nucleus accumbens (29%, 45%, and 69%)]. For PF-04457845, inhibition of FAAH activity was comparable to the middle and highest dosage of CPF in all three brain regions [% inhibition: hippocampus (40%), amygdala (56%) and nucleus accumbens (53%)]. Regardless of compound used, the level of inhibition of FAAH was higher in the amygdala and the nucleus accumbens than in the hippocampus, suggesting that there may be regional differences in the effects of covalent FAAH inhibitors.

ABSTRACT FINAL ID: 3823 Poster Board: P515
TITLE: Assessment of The Potential Therapeutic Effect of Intranasal Administration Stem Cells in Rotenone Model of Parkinson's Disease in C57bl/6 Mice

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KEYWORDS: Neurotoxicology; Nose; Intranasal Administration Stem Cells

ABSTRACT BODY: Parkinson's disease (PD) is the second most common neurodegenerative disorder in the elderly affecting approximately 1% of the population over 60 years old. While, the prevalence of PD in a certain area of Egypt, Upper Egypt, is more than double of the worldwide prevalence of PD. PD motor defects result from degeneration of dopaminergic neurons that project from the substantia nigra pars compacta to the striatum. As the disease progresses, motor manifestation worsens. Until now there is no causal therapy available to slow down or halt disease progression just symptomatic intervention are available. Reduction of progression of PD to half will reduce PD cost to about third. Cell therapy may represent one of the promising therapeutic modalities for PD, yet, the best route of administration of the cells to the brain is still a subject of decades of scientific trials. In this study we assessed the potential beneficial effect of intranasal administration of mesenchymal stem cells (MSCs) to rotenone model of PD in C57bl/6 mice. Thirty mice were divided into three equal groups; control, rotenone and rotenone plus intranasally administrated MSCs. Tested mice were subjected to behavioral assessment and after 70 days, ten days of administration of MSCs, animal were sacrificed under deep anesthesia. Photomicrographs for coronal sections of the sustantia nigra and striatum were evaluated immunohistochemically regarding tyrosine hydroxylase antibodies binding. Results indicated the effective delivery of MSCs to the brains of the mice. Together with that, MSCs were capable to counteract the toxic effect of rotenone on both pathological and behavioral levels. Thus, this study warrants further investigation of intranasal route as therapeutic option for Parkinson's disease patients.

ABSTRACT FINAL ID: 3824 Poster Board: P516
TITLE: 3,4-Methylenedioxypyrovalerone (MDPV) Induce Cytotoxicity and Alter the Tight Junctions Proteins in Blood-Brain Barrier Endothelial Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: H. Rosas-Hernandez[1], E. Cuevas[1], S.M. Lantz[1], K.C. Rice[2], B.M. Gannon[3], W.E. Fantegrossi[3], M.G. Paule[1], C. Gonzalez[4], S.F. Ali[1]. *[1]Division of Neurotoxicology, National Center for Toxicological Research, Jefferson, AR; [2]Drug Design and Synthesis Section, Chemical Biology Research Branch, NIDA/NIAAA, Bethesda, MD; [3]Department of Pharmacol & Toxicol, UAMS, Little Rock, AR; [4]FCQ, UASLP, SLP, Mexico.*
KEYWORDS: Neurotoxicology; Blood-Brain Barrier; MDPV

ABSTRACT BODY: In recent years, the use of synthetic cathinones has grown rapidly as an alternative to classic amphetamine-like drugs. These drugs are usually referred to as "bath salts", with 3,4-methylenedioxypyrovalerone (MDPV) being the most prevalent constituent. MDPV exerts its neurotoxic effects by increasing extracellular levels of dopamine, serotonin and norepinephrine in a way similar to methamphetamine (Meth). Recently, we reported that Meth increased the permeability of capillaries in the blood-brain barrier (BBB). As there is currently no information about the effects of MDPV on brain vasculature, the main aim of this study was to evaluate if MDPV affects the BBB *in vitro*. Using primary cultures of rat brain microvessel endothelial cells (BMVECs) as an *in vitro* model of the BBB, we analyzed whether MDPV (0- 2.5 mM) modified LDH release as an index of cytotoxicity, bromodeoxyuridine (BrdU) incorporation as an index of cellular proliferation, DNA fragmentation as an index of apoptosis, and the expression of the tight junction (TJ) proteins occludin and ZO-1 through western blot. The results indicated that MDPV exposure decreased cellular proliferation and an increased apoptosis at 1 and 2.5 mM. However, MDPV increased LDH release beginning at 0.25 mM, which correlated with a decrease in the expression of the TJ proteins occludin and ZO-1. These results suggest that MDPV is cytotoxic to brain endothelial cells via apoptosis, inhibition of cellular proliferation and decrease in expression of TJ proteins; cumulatively, these events may lead to the alteration of membrane integrity and increase BBB permeability, which may in turn contribute to its neurotoxic effects. Further studies are needed to elucidate the cellular mechanisms involved in MDPV neurotoxicity and its effects *in vivo*.

ABSTRACT FINAL ID: 3825 Poster Board: P517
TITLE: Acute Neurotoxic Effects of Tri-cresyl Phosphates (TCPs) on Glutamate Receptors

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KEYWORDS: Organophosphates; *In Vitro* and Alternatives; Nervous System

ABSTRACT BODY: We recently showed that the ortho-isoform of tri-cresyl phosphates (TCPs) ToCP impaired glutamate signaling in mouse primary cortical neurons (pCNs) at levels fare below its cytotoxic concentrations (Hausherr et al. 2014). Additional experiments confirmed that this new mode of action of ToCP could not be observed for the two other isomers TmCP, TpCP, a commercial TCP mixture and cresyl saligenin phosphate (CBDP) the metabolite of ToCP. While these experiments used a 24h preincubation before assessing the responsiveness of the pCNs for the main excitatory neurotransmitter glutamate by means of fluorescence-based life-cell Ca^{2+} imaging, we here investigated the direct modulation of the glutamate-evoked signals by the TCPs. Moreover, we specifically blocked the NMDA and AMPA receptors to analyze the involvement of these ionotropic glutamate receptors. None of the non-ortho TCP isomers, nor the mixture or the metabolite CBDP decreased the response amplitude when simultaneously applied with 30 µM glutamate. In contrast, ToCP dose-dependently reduced the glutamate-evoked signals and 100 µM resulted in a reduction of the responses by 70 %. When blocking the responses of AMPA receptors with ZK200775 no further "blocking" effects of the co-application of 10 or 100 µM ToCP could be observed. In contrast, an additional and significant reduction of the glutamate-evoked signals by ToCP could be observed when blocking the NMDA receptor with MK801. This comparative analyses of the two individual receptor responses revealed that ToCP might modify the ability of the AMPA receptor to respond to its natural agonist glutamate. This perturbation of the AMPA receptor function could be the molecular initiating event of ToCP affecting various processes of neuronal plasticity. Here, further research will investigate the impact of ToCP on AMPA receptor subunits as well as AMPA receptor trafficking an important neurobiological process underlying long-term potentiation and depression.

ABSTRACT FINAL ID: 3826 Poster Board: P518
TITLE: Sensitivity of the M-(Water) Maze for Assessment of Learning and Memory in the Adult Sprague Dawley Rat

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KEYWORDS: Neurotoxicology

ABSTRACT BODY: The M-shaped water maze (MM) has been in use for >20 years in our laboratory to assess the effects (if any) of compounds on learning and memory (L&M) in order to meet various regulatory guidelines (EPA OPPTS 870.6300 and OECD 426) for Agrochemical products. We have demonstrated the sensitivity of the MM as a standardized test that satisfies the requisite regulatory criteria by comparing the MM with the Cincinnati water maze using Scopolamine as a positive control in the Wistar rat. The current study was performed to assess the sensitivity of the MM for perturbation of L&M by inducing alterations in neurotransmitter systems that are most likely affected by pharmaceutical products in the Sprague Dawley rat, the strain of choice for pharmaceutical testing. Reference compounds known to affect acquisition and/or retention of spatial memory in the Morris water maze (MWM) were given to 10 males/group, prior to acquisition testing, and re-tested seven days later for retention. Results indicate reference substance-related changes in L&M with Scopolamine (Muscaranic receptor antagonist at 0.6 mg/kg; s.c.), MK-801(glutamate receptor antagonist at 0.15 mg/kg; i.p.) or Baclofen (GABA receptor agonist at 7 mg/kg; i.p.). In the acquisition phase, longer latency, more errors, and a greater number of animals failing to meet criterion were observed. In the retention phase, all treated groups required more trials to criterion and had increased average latency and number of errors, relative to controls. These results are congruent with the published results using MWM, which supports the utility of the MM for assessment of L&M.

ABSTRACT FINAL ID: 3827 Poster Board: P519
TITLE: Automated High-Throughput Measurement of Neuronal Calcium Transients and Action Potentials with Kinetic Image Cytometry

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KEYWORDS: Neurotoxicology; Clinical Toxicology; Neurotransmitter

ABSTRACT BODY: Neurons experience transient depolarizations (action potentials) that propagate along the plasma membrane enabling communication with other neurons or target tissues. The action potentials elicit release of neurotransmitters at synapses, and for excitatory neurons of the central nervous system (CNS), the neurotransmitter (glutamate) activates calcium transients in the post-synaptic cells. Vala Sciences Inc is developing methods to quantify the effects of chemicals on the function of primary neurons and neurons derived from induced pluripotent stem cells (iPSCs). To do this, the neurons are cultured on 96- or 384-well dishes and loaded with fluorescent dyes that report changes in either intracellular calcium (e.g., fluo-4), or membrane voltage (e.g., fluovolt). The plates are then loaded on to Vala's Kinetic Image Cytometer (KIC), an automated microscopy work-station designed to collect digital movies (frame rates of 30 to 800 fps) from excitable cells (e.g., neurons, muscle, cardiac myocytes). The digital movies are then analyzed for changes in calcium or voltage utilizing Vala's CyteSeer cell image analysis software. With the KIC, we have found that tamoxifen, a commonly used anti-breast cancer therapeutic, reduces the occurrence of spontaneous calcium transients in primary rat hippocampal neurons, which is consistent with the reduced cognitive function in cancer survivors treated with this compound (e.g., "chemo-brain"). We have also recorded action potential activity in iPSC-derived peripheral neurons relevant to peripheral neuropathies (e.g., associated with chemotherapies and diabetes) and iPSC-dopaminergic neurons relevant to Parkinson's Disease. Thus, quantification of chemical effects on neuronal function using primary and iPSC-neurons with KIC, will have numerous applications in both toxicity and drug-discovery relevant to afflictions of the central and peripheral nervous system.

ABSTRACT FINAL ID: 3828 Poster Board: P520
TITLE: Transgenic Expression of a Designer Ubiquitinase Against the Light Chain of Botulinum Toxin a Delays Toxin-Induced Paralysis of *Ex Vivo* Mouse Neuromuscular Junctions

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: A.B. Bradford[1], T. Russo[1], J. Machamer[1], M. Eisen[1], D. Nguyen[1], M. Lyman[1], M. Nelson[1], G. Oyler[2], P. McNutt[1]. *[1]Cell and Molecular Biology, US Army Medical Research Institute of Chemical Defense, Gunpowder, MD; [2]Synaptic Research, LLC, Baltimore, MD.*
KEYWORDS: Chemical and Biological Weapons; Muscle Toxicity; Neurotoxicology

ABSTRACT BODY: Botulinum neurotoxin serotype A (BoNT/A) is an extremely potent toxin that specifically cleaves the presynaptic SNARE protein SNAP-25, blocking cholinergic neurotransmission. Resulting muscle paralysis becomes fatal once respiratory muscles are impaired. Although passive immunization removes neurotoxin from circulation, there are no treatments that neutralize toxin within presynaptic terminals. Due to the persistence of catalytic BoNT/A light chain (LC/A), victims may remain paralyzed for months before toxin is cleared and synaptic function resumes. Our goal is to design novel treatments that target later stages of disease by blocking or reversing toxin activity within pre-synaptic terminals. Here we describe development and testing of a recombinant protein that both inhibits SNAP-25 proteolysis and enhances LC/A ubiquitination for accelerated proteolysis. This designer ubiquitinase (DesUbA) is a modular protein that uses a single-chain antibody (B8 VHH) to localize a recombinant ubiquitin ligase domain (TrCP) to LC/A *in situ. In vitro* studies conducted in neuroblastoma cells display increased ubiquitination and clearance of LC/A in the presence of DesUbA. To test *in vivo*, transgenic mice were created expressing YFP-DesUbA under control of a pan-neuronal Thy1.1 promoter. In stable breeding lines, DesUbA is expressed throughout the central and peripheral nervous systems, with distribution to motor neuron axons and neuromuscular junctions. Acute inhibition of LC/A toxicity by B8 VHH was assessed by monitoring nerve-evoked muscle twitches in *ex vivo* phrenic nerve-diaphragm preparations (PNDs). After establishing baseline twitch strength, PNDs from transgenic and age-matched wild-type animals were treated with 6.7 pM BoNT/A and progression of paralysis was quantified. Time-to-half paralysis and full paralysis were significantly extended by over 50% in transgenic preparations. These results suggest that the B8 VHH component of DesUbA acutely blocks LC/A function, thereby reducing effective presynaptic toxin load. *In vivo* assays are ongoing to evaluate the duration of paralysis, which presumably reflects enhanced LC/A ubiquitination.

ABSTRACT FINAL ID: 3829 Poster Board: P521
TITLE: Nerve Agent-Induced Alterations in Plasticity in the CA1-Schaffer Collateral Synapse following Acute Soman Exposure

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: K. Hoffman, M. Nelson, P. McNutt. *USAMRICD, Gunpowder, MD.*
KEYWORDS: Agents; Chemical and Biological Weapons

ABSTRACT BODY: Organophosphorus nerve agents (NAs) irreversibly block acetylcholinesterase function, causing the accumulation of excess acetylcholine in cholinergic synapses. However, in multiple seizure models it has been shown that persistent cognitive changes can also result from mechanisms that are independent of neuronal cell death. The observed alterations in circuit activity initiate biochemical and genetic programs that lead to abnormal synaptogenesis, resulting in a maladaptive form of plasticity that exploits the mechanisms of synaptic plasticity to effect short- and long-term changes. Here, we explored the acute effects of soman-perfusion on synaptic plasticity in field recordings within hippocampal coronal slices. Perfusion of 10 μM soman rapidly elicited a stable depression of fEPSP amplitude and an increase in paired pulse ratio ($n=6$, $p < 0.01$ for both), suggesting pre-synaptic involvement. Soman-induced long-term depression (sLTD) was blocked by co-administration of the muscarinic receptor antagonist atropine ($n = 7$, $p < 0.01$), confirming the role of cholinergic overstimulation. To further investigate pathways responsible for muscarinic-initiated sLTD, we pharmacologically dissected pre- and post-synaptic components. sLTD was refractory to APV treatment, demonstrating it was not mediated by the post-synaptic NMDAR. Alternatively, sLTD was completely blocked by the cannabinoid receptor type 1 (CB1R) antagonist AM-251. These findings suggest that retrograde endocannabinoid signaling in response to soman perfusion is an essential step in reducing the probability of release at the presynaptic compartment. We are currently characterizing pre- and post-synaptic signaling mechanisms in glutamatergic and GABAergic synapses to determine whether CB1R signaling is an adaptive attempt by the brain to prevent seizure onset or a maladaptive response to excessive muscarinic signaling. Based on these findings, we hypothesize that NA-induced hippocampal seizures initiate pre-synaptic LTD in the CA1 area, representing a novel co-morbidity that may contribute to acute and persistent neurological responses to NA exposure.

ABSTRACT FINAL ID: 3830 Poster Board: P522
TITLE: Aβ25-35 Induces Formation of AD-Like Plaques in Rat Microvascular Endothelial Cells

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KEYWORDS: Toxicity; Acute; Neurotoxicology; Amyloid-Beta

ABSTRACT BODY: Previously, we have demonstrated that microinjection of the amyloid β-peptide fraction 25-35 (Aβ25-35) into rat hippocampus produced neurotoxicity mediated by advanced glycation end products (RAGE). It has been demonstrated that RAGE interacts with Aβ and mediates Aβ transport across the blood-brain barrier (BBB). However, the molecular mechanisms underlying this interaction of Aβ with RAGE and the resulting alterations in BBB transport and its role in formation of Aβ plaques characteristic of Alzheimer's disease (AD) are poorly understood. In order to determine whether the interaction of Aβ25-35 with RAGE is involved in the formation of AD-like plaques, primary cultures of rat brain microvessel endothelial cells (rBMVECs) were used as an *in vitro* model of the BBB. rBMVECs were treated with activated Aβ25-35 or Aβ35-25 (control) at 20 μM for 24 hours and the formation of plaques was evaluated immunocytochemically. RAGE, occludin (OC), claudin-5 (C-5), and zonula occludens-1 (ZO-1) expression were measured using Western blot. Cytotoxicity was evaluated using the XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] assay for mitochondrial function and the detection of 2', 7'- dichlorofluorescin diacetate (DCFH-DA) to measure production of reactive oxygen species (ROS). The integrity of the rBMVEC monolayer was also measured using trans-epithelial electrical resistance (TEER) electrodes and a permeability assay was performed. Exposure to the Aβ25-35 fragment induced formation of AD-like plaques and Aβ was co-localized with RAGE. This exposure also decreased mitochondrial function, increased oxidative stress (ROS), and induced the expression of RAGE, OC, and C-5, while decreasing the expression of ZO-1.TEER readings were also decreased and the permeability of the monolayer was increased. Together, these data suggest that Aβ25-35-induced toxicity is mediated by oxidative stress coupled with up-regulation of RAGE leading to AD-like amyloid plaque formation, which may result in cell death and disruption of rBMVEC integrity. Further studies are undeway in order to better define the time-course of the formation of the AD-like plaques.

ABSTRACT FINAL ID: 3831 Poster Board: P523
TITLE: Base Excision Repair Variants and Pesticide Exposure Increase Parkinson's Disease Risk

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KEYWORDS: Neurotoxicity; Pesticides; DNA Repair; Pesticides; Parkinson'S Disease

ABSTRACT BODY: We recently reported selective mitochondrial DNA (mtDNA) damage in the form of abasic sites in the vulnerable nigral neurons in Parkinson's disease (PD). The persistence of abasic sites suggests an ineffective base excision repair (BER) response in PD. In addition, we recently showed that pesticide exposure, which has been linked to PD risk, can cause mtDNA damage. The objective of this study was to investigate the joint impact of variations in genes involved in the BER system and pesticide exposure. To determine whether either APEX1 rs1130409 or OGG1 rs1052133 polymorphisms was independently associated with PD, 619 PD patients early in disease and 854 population controls were examined. PD risk was not influenced by either genotype alone. We next investigated whether there were interactions for either BER polymorphism and pesticides. We detected statistically significant (p=0.05) or near significant (p=0.07) interactions for both APEX1 rs1130409 and OGG1 rs1052133 and ambient oxidative stress exposure. At both loci, for pesticide exposed risk allele carriers we estimated more than multiplicative risk increase. Specifically, for OGG1 rs1052133, the risk was increased by almost 80% in exposed variant carriers (OR=1.79, 95% CI=1.22-2.64). Similarly, for APEX1 rs1130409, in exposed T allele carriers experienced a 67% increase in risk (OR=1.67, 95% CI=1.13-2.47). The strongest interactions we identified were for the combined BER genetic risk score and pesticide exposures; we detected more than a multiplicative risk with both oxidative stress inducing pesticide exposure (p=0.01) and mitochondrial inhibiting pesticide exposure (p=0.07). The highest risk was estimated for the joint effect of pesticide exposure and the two risk genotypes (mitochondrial inhibitor exposure: OR=2.32, 95% CI=1.44-3.75; oxidative stressor exposure: OR=2.21, 95% CI=1.45-3.38). One of the pesticides analyzed in our study, paraquat, induces oxidative stress by redox cycling. We found mtDNA damage was increased and mitochondrial function impaired following acute paraquat exposure in *in vitro* neuronal cultures. This is the first study to discover variants in BER enzymes interact with pesticide exposure to increase the risk of PD.

ABSTRACT FINAL ID: 3832 Poster Board: P524
TITLE: Intranasal Delivery of the Antioxidant Carnosine to the Thy1-aSyn Mouse Model of Parkinson's Disease Reduces its Progression

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KEYWORDS: Antioxidants; Behavioral; Intranasal Drug Delivery

ABSTRACT BODY: Parkinson's disease (PD) is the second leading neurodegenerative disease, which affects millions of people worldwide. PD is characterized by several motor and prior onset non-motor deficits, including gait instability and decreased olfactory function. No cure exists and this devastating disease presents with hallmark protein aggregates and evidence of oxidative stress. Our studies focus on the evaluation of a novel mechanism-based treatment regimen for PD, and on effective routes of administration in the Thy1-aSyn mouse model of PD. Carnosine is an endogenous dipeptide abundant in muscle, brain and the olfactory system, but concentrations decline with age and in pathological conditions such as PD. Carnosine is known to reduce protein aggregation and protect against oxidative stress. Hypothesis: Intranasal (IN) administration of carnosine will significantly reduce disease progression in the Thy1-aSyn mouse model of PD. Methods: Wild-type (WT) and Thy1-aSyn mice were treated IN with 2 mg/day carnosine or sterile water (as control) for 2 mo. Sensorimotor function was evaluated using the buried food pellet and the challenging beam traversal (CBT) tests at the beginning and end of treatment. Results: Carnosine administered IN was not toxic, based on clinical and histological observations of treated mice. IN carnosine did not cause decreased olfactory function in the buried food pellet test. At the initial CBT evaluation, the average number of errors per step, as expected, was significantly higher in Thy1-aSyn compared to WT mice. After IN carnosine treatment, the number of errors per step was lower in the carnosine treated Thy1-aSyn group compared to the untreated Thy1-aSyn group ($p<0.05$). Conclusions: The lower number of errors per step in the carnosine treated Thy1-aSyn mice suggests a protective role for carnosine in the progression of PD. Future Directions: Results will be validated and the mechanism of action of carnosine will be evaluated by examining changes in protein aggregation, mitochondrial function, and expression of PD associated genes.

ABSTRACT FINAL ID: 3833 Poster Board: P525
TITLE: Neuroinflammatory Responses following Inhalation of Fine and Ultrafine Carbonaceous Particulates Combined with Vehicular Gases

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KEYWORDS: Particulates; Nervous System; Volatile Organic Compounds

ABSTRACT BODY: Epidemiological evidence has emerged showing a relationship between neurodegenerative diseases and particulate matter exposure. However, toxicological support for this association is lacking. The present study explored whether vehicle engine (diesel and gasoline combined)-derived particulate matter, separated as fine (FP) and ultrafine (UFP) sizes, could induce markers of neuroinflammation in WT and Apolipoprotein E knockout mice. ApoE-/- mice were fed high-fat chow in order to model atherosclerosis, while WT mice were fed a normal diet. The mice were exposed to 300 µg/m3 of either UFP or FP for 6h/d for 1-d or 30-d by whole-body inhalation and brains were perfused with ice-cold saline and snap frozen for measurement of transcriptional responses and histopathology 24 hours after final exposures. Two additional groups were included which received the FP or UFP combined with gaseous copollutants derived from fresh gasoline and diesel emissions, to examine the toxicological contribution of gas-particle interactions. Cortex, frontal cortex, and hippocampus were dissected for the isolation of RNA and qPCR determination of inflammatory transcripts. Increasing trends for IL-6, CCL2, and CCL5 were noted in varying regions of the brain following exposure. No robust and consistent differences between particle size and gas co-exposure could be discerned for neuroinflammatory markers, despite differences observed in pulmonary macrophage particle uptake. Increased transcription of the vascular inflammatory marker, VCAM-1, was noted in UFP and FP groups, but this effect was lsot with the addition of copollutant gases. Regardless, these findings provide compelling evidence for PM-related neuroinflammatory responses following a subchronic inhalation exposure.

ABSTRACT FINAL ID: 3834 Poster Board: P526
TITLE: Diverse Effect of Glucose and Insulin on the Viability of SH-SY5Y Neuroblastoma Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: A. Basak Engin[1], R. Karakus[2]. *[1]Department of Toxicology, Gazi University, Faculty of Pharmacy, Ankara, Turkey;[2]Department of Immunology, Gazi University, Faculty of Medicine, Ankara, Turkey.*
KEYWORDS: Neurotoxicology; Oxidative Injury; Cell Culture

ABSTRACT BODY: Type 3 diabetes has been proposed for Alzheimer's disease which is related to insulin resistance in the brain. However, comprehensive experimental models that evaluate the mechanism of insulin resistance are limited. The aim of this study was to clarify the effect of insulin on neuronal cell glucose transport and oxidative stress in a state of insulin resistance. At various time points SH-SY5Y cells were incubated with or without 40 µU/ml insulin and 25 uM L-NAME, in addition to 150 - 250 mg/dl glucose concentrations that were determined by International Diabetes Federation as hyperglycemic conditions. The metabolic activity of the cells was determined by MTT assay. GLUT3 and nitrite+nitrate (NOx) were measured spectrophotometrically. 40 uU/ml insulin in addition to the high glucose concentrations decreased NOx synthesis of SH-SY5Y cells by 60.5%, leading to the membrane depolarization and decrease of GLUT3 membrane translocation and fusion by 61%. Thus the reduction of glucose transport into the neurons prevented the regeneration of antioxidant capacity and increased the mitochondrial oxidative stress by 209%. The mitochondrial metabolic activity decreased by 58.2%, leading to insulin resistance in neuronal cells. Addition of L-NAME into high glucose exposed SH-SY5Y cells dropped the NOx synthesis by 57.2% in the second cell cycle. In SH-SY5Y neuroblastoma cells, insulin augments high glucose provoked oxidative stress which can be partially improved by the prevention of NO synthesis.

ABSTRACT FINAL ID: 3835 Poster Board: P527
TITLE: Cholinergic Dysfunction and Muscarinic Receptor Uncoupling in Alzheimer's Disease

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KEYWORDS: Neurotoxicology

ABSTRACT BODY: The major goal of this study was to characterize the mechanism(s) underlying muscarinic receptor uncoupling in Alzheimer's disease (AD) in a neuroblastoma cell model (SH-5YSY cells) and an AD mouse model (3xTG-AD mouse). Muscarinic receptor signaling is terminated by GRK phosphorylation, followed by β-arrestin binding, which begins the process of receptor uncoupling and internalization We have demonstrated that muscarinic receptors were uncoupled from G-proteins in brains of patients with Alzheimer's disease (AD), as well as in non-demented controls with substantial β-amyloid deposition and neuritic plaque formation. Levels of β-arrestin were examined in four groups: patients diagnosed with Alzheimer's (AD), age matched controls with many amyloid plaques (MP), age matched controls with sparse plaques (SP), and age-matched controls with no plaques (NP). The extent of plaque formation was measured using an ELISA kit specific for β-amyloid and was positively correlated with loss of cholinergic neurons as assessed by choline acetyltransferase (ChAT) activity. Western analysis sowed increased β-arrestin levels. In the current study, using a neuroblastoma cell line that overexpresses APP695 shows that exposure to β-amyloid for 24 hrs caused a both a decrease in GRK-2 and an increase in β-arrestin levels indicating alterations in the coupling of the muscarinic receptor to its g-protein. Using a fluorescently-labeled protein, it was shown that β-amyloid disrupted normal cellular trafficking of arrestin. The extent of uncoupling is positively correlated with an increase in β-amyloid levels as assed by ELISA. Cells overexpressing APP695 had greater levels of muscarinic uncoupling and amyloid levels indicating that increased levels of processing of β-amyloid may contribute to the uncoupling of the muscarinic receptor. Finally, we examined muscarinic receptor uncoupling in the 3xTg-AD mouse model of AD. Results indicate differences in both ChAT activity and muscarinic uncoupling at 6 and 9 months of age compared to non-TG control mice. It is likely that alterations in GRK, coupled with a decrease in β-arrestin, could impair muscarinic receptor and g-protein recycling and contribute to the cholinergic dysfunction associated with AD. Thus, it may be beneficial to circumvent impairment of signal transduction by addressing cholinergic dysfunction in the treatment of Alzheimer's disease.

ABSTRACT FINAL ID: 3836 Poster Board: P528
TITLE: 5-Hydroxymethylation Plays an Epigenetic Role in Early State of Carcinogenesis

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KEYWORDS: Epigenetics; Carcinogenesis; Rt-Pcr; Riddelliine

ABSTRACT BODY: DNA methylation at the 5-carbon position of cytosine is a critical epigenetic mechanism in the regulation of the genome that impacts on a broad range of biological functions and pathological processes. 5-Methylcytosine (5-mC) can be further converted to 5-hydroxymethylcytosine (5-hmC) by the product of ten-eleven translocation (TET) family genes. Recent studies have shown that loss of 5-hmC is found in different types of cancers and is associated with poor survival of cancer patients. In addition, decrease of TET gene expression or TET mutation is associated with carcinogenesis. However, whether 5-hmC level is perturbed in early stages of carcinogenesis caused by genotoxic carcinogens and whether the TET gene is involved in the process are not defined. In this study, 5-hmC levels and TET2 expression were evaluated in liver of rats treated with two genotoxic carcinogens, riddelliine and aristolochic acid. Riddelliine has been classified as a potentially human carcinogen while AA is a human carcinogen. Our previous studies indicate that both of them can bind to DNA and induce mutations in rat liver. Levels of 5-hmC were measured using immuno-dot blot and immunohistochemical methods; and levels of TET2 expression were accessed using real-time PCR. Both 5-hmC and TET2 expression significantly decreased in the liver of the carcinogens-treated rats. Loss of 5-hmC correlates well with documented induction of genetic mutations by the carcinogens, suggesting that TET2-mediated 5-hydroxymethylation plays an epigenetic role in early state of carcinogenesis.

ABSTRACT FINAL ID: 3837 Poster Board: P529
TITLE: Role of DNA Methylation in Altered Gene Expression Patterns in Adult Zebrafish (*Danio rerio*) Exposed to 3,3',4,4',5-Pentachlorobiphenyl (PCB 126)

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KEYWORDS: Polychlorinated Biphenyls; Toxicogenomics; Receptor; Aryl Hydrocarbon; DNA Methylation

ABSTRACT BODY: The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor well known for mediating the toxicity of environmental chemicals such as polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs). There is extensive knowledge on the range of target genes regulated by AHR ligands, yet there is limited information regarding the effect of AHR ligands on DNA methylation. In this study, we investigated genome-wide changes in DNA methylation concomitant with altered gene expression patterns in response to PCB126 exposure. Adult zebrafish were exposed to 10 nM PCB126 for 24 hours (water borne exposure) and were reared in clean water for 7 days before tissue sampling. DNA methylation and transcriptional changes in the liver and brain tissues were quantified by Reduced Representation Bisulfite Sequencing (RRBS) and RNAseq, respectively. RRBS analysis revealed DNA hypomethylation in response to PCB exposure in both liver and brain tissues. We observed 482 differentially methylated regions (DMRs) in the liver and 476 DMRs in the brain. In both tissues, majority of the DMRs are localized to the intergenic regions and more than 20 kilobases upstream of transcriptional start sites of the nearest neighboring genes. Only 12-14% of DMRs are localized to the promoter regions (5000 bp upstream of TSS). Majority of the DMRs do not overlap with the CpG islands defined in the zebrafish genome. RNAseq results from the liver revealed differential expression of genes related to xenobiotic metabolism, oxidative stress and carbohydrate metabolism in response to PCB exposure. In the brain, PCB exposure altered the expression of genes involved in myelination and glutamate signaling. Our initial results suggest that there is very little correlation between DNA methylation and gene expression patterns among the differentially expressed genes (DEGs). We are continuing the investigation of the relationship between DMRs and DEGs.
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ABSTRACT FINAL ID: 3838 Poster Board: P530
TITLE: Butyl Benzyl Phthalate (BBP) Induces Adipogenesis via Mirna-34a Mediated Regulation

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S. Meruvu, M. Choudhury. *Pharmaceutical Sciences, Texas A&M Health Science Center, Kingsville, TX.*
KEYWORDS: Phthalates; Cell Culture; Epigenetics; Obesogens; Phthalates

ABSTRACT BODY: The obesogen hypothesis states that exposure to certain environmental chemicals during critical developmental stages of life can alter gene expression through epigenetic mechanisms leading to diabesity (diabetes and obesity). Phthalates, a plasticizer group, are used extensively in food contact materials. Recent studies have implicated butyl benzyl phthalate (BBP) as an obesogen that has raised public health concerns. However, BBP induced epigenetic regulation during adipogenesis is still unknown. We investigated if BBP altered miR-34a, a key miRNA involved in obesity, and regulated its downstream pathway. We exposed differentiating 3T3-L1 cells to various doses of BBP without exogenous adipogenic stimuli and tested for adipogenesis markers (PPARγ and aP2) and stained for lipid accumulation with Oil Red O staining. We then determined miR-34a expression and its target genes, Nampt, and Sirt1, along with another significant epigenetic modulator, Sirt3. Furthermore, using antagomiR, we investigated whether miR-34a knockdown attenuated adipogenesis and altered the protein expression levels of Nampt, Sirt1, and Sirt3 in BBP-treated adipocytes. BBP exposure resulted in increased expression levels of miR-34a with an associated increase in adipogenesis. BBP significantly decreased the mRNA expression levels of Nampt, Sirt1, and Sirt3. However, a decrease in the protein expression was observed only for Nampt indicating that miR-34a under BBP exposure may regulate Sirt1/Sirt3 only at the transcriptional level. Interestingly, knockdown of miR-34a decreased adipogenesis in the differentiating 3T3-L1 cells in the presence of BBP. Furthermore, miR-34a knockdown increased the protein expression levels of Nampt indicating that miR-34a regulates Nampt during BBP exposure. BBP exposure demonstrated the involvement of epigenetic regulation by altering the expression patterns of miR-34a, Nampt, Sirt1, and Sirt3, thereby perturbing the energy homeostasis of the differentiating adipocytes resulting in increased adipogenesis. These *in vitro* findings are of considerable biological relevance since the human population is continuously exposed to BBP which can be associated with the increased incidences of metabolic diseases in our industrialized society.

ABSTRACT FINAL ID: 3839 Poster Board: P531
TITLE: Benzyl Butyl Phthalate Induces Epigenetic Stress to Enhance Adipogenesis in Mesenchymal Stem Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: R. Sonkar, C. A. Powell, M. Choudhury. *Pharmaceutical Sciences, TAMHSC Rangel College of Pharmacy, Kingsville, TX.*
KEYWORDS: Phthalates; Endocrine Disruptors; Endocrine Toxicology; Benzyl Butyl Phthalate

ABSTRACT BODY: Endocrine disruptors, phthalates, may have contributed to recent global obesity health crisis. Our study investigated the potential of benzyl butyl phthalate (BBP) to regulate the mesenchymal stem cell epigenome to drive adipogenesis. BBP exposure enhanced adipogenesis in a dose-dependent manner with a 3.5-fold greater accumulation of lipid content compared to control ($P<0.001$). Adipogenesis markers, PPARγ ($P<0.001$), C/EBPα ($P<0.01$), and aP2 ($P<0.001$) were significantly upregulated by increasing concentrations of BBP when compared to control. BBP enhanced the H3K9 acetylation while decreasing H3K9 dimethylation. Fifty μM BBP increased histone acetyltransferases, p300 ($P<0.05$) and GCN5 ($P<0.01$) gene expression by 5-fold and 2.5-fold at day 8, respectively. Furthermore, histone deacetylases (HDACs), HDAC3 ($P<0.01$) and HDAC10 ($P<0.01$, 10 μM BBP; $P<0.001$, 50 μM BBP) and histone methyltransferases, SETDB1 ($P<0.01$) and G9a ($P<0.01$), were also significantly downregulated by 10 and 50 μM BBP exposure. In conclusion, BBP regulated MSCs towards adipogenesis by tipping the epigenomic balance.

ABSTRACT FINAL ID: 3840 Poster Board: P532
TITLE: The Plasticizer BBP Selectively Inhibits Epigenetic Regulator Sirtuin During Stem Cell Differentiation

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J. Zhang, M. Choudhury. *TAMHSC, Kingsville, TX.*
KEYWORDS: Phthalates; Lipids; Endocrine Disruptors; Stem Cell; BBP

ABSTRACT BODY: Exposure to environmental chemicals can perturb an individual's metabolic set point, especially during critical periods of development, and as a result increase his or her propensity towards obesity that is manifested later in life and possibly in successive generations. We hypothesized that benzyl butyl phthalate (BBP), a widespread endocrine disruptor, may impair one important epigenetic regulator, sirtuin, in mesenchymal stem cells and induce adipogenesis. Our results showed that gene expression of two well-known adipogenic markers, aP2 and PPARγ, were significantly increased from day 2 to day 8 under 50 μM BBP exposure when compared to control in C3H10T1/2 stem cells ($p<0.05$). Consistent with transcriptional expression, Oil Red O staining was significantly increased after 8 days of treatment ($p<0.01$). Sirt1 gene expression was significantly decreased at day 2, 4, 6, and 8 under 50 μM BBP exposure ($p<0.05$). However, Sirt7 gene expression was decreased only at day 8 ($p<0.05$) while other sirtuin transcriptional levels remained unaltered throughout. Interestingly, Sirt1 and Sirt3 protein expression was decreased at day 8 under BBP exposure ($p<0.05$). Overall protein hyperacetylation was observed at day 8 under 50 μM BBP exposure. Furthermore, FOXO1 and β-catenin, Sirt1 targets and adipogenesis regulators, were hyperacetylated at day 8. In addition, gene expression of mitochondrial biogenesis regulators, as well as downstream targets of Sirt1 and -3, namely PGC1α, NRF1, NRF2, and Tfam, were significantly decreased after 8 days of 50 μM BBP exposure ($p<0.05$). This is the first report to demonstrate that BBP selectively disrupts specific sirtuin in stem cells. In conclusion, our study suggests that BBP, acting as a potential epigenetic disruptor, can lead to increased adipogenesis and metabolic dysregulation by impairing vital epigenetic regulators during critical windows of development and can lead to obesity.

ABSTRACT FINAL ID: 3841 Poster Board: P533
TITLE: Double Trouble—Plastic and Fats: Combination of Benzyl Butyl Phthalate and Palmitic Acid Enhance Adipogenesis by Epigenetic Regulation

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: C.A. Powell, R. Sonkar, S. Meruvu, M. Choudhury. *Pharmaceutical Sciences, Texas A&M University Health Science Center, Kingsville, TX.*
KEYWORDS: Phthalates; Endocrine Disruptors; Epigenetics; Benzyl Butyl Phthalate

ABSTRACT BODY: Obesity has reached epidemic proportions in the United States with more than 78 million Americans considered obese. While poor diet and a lack of physical activity are fundamental components of obesity, emerging evidence supports the role of endocrine disruptors (EDs) in obesity development and progression. Our study investigates the combinatorial effect of an ED, benzyl butyl phthalate (BBP) and palmitic acid (PA) on epigenetic regulation of adipogenesis. We hypothesized that BBP synergistically increased high-fat diets' effect via adipogenic microRNA (miRNA) regulation via promoter methylation. The preadipocyte cell line, 3T3-L1 was treated with BBP, PA, or a combination of both to induce adipogenesis in the absense of an adipogenic cocktail. Adipogenic gene markers and adipogenisis-associated miRNA-34a and miRNA-103 expressions were analyzed in differentiating and mature adipocytes. BBP (1 μM, 10 μM, 50 μM) induced adipogenesis without an adipogenic cocktail in a dose-dependent manner. PA (100 μM) induced adipogenesis above control in 3T3-L1 cells, however, not to the extent of BBP. Furthermore, the combination of BBP and PA significantly increased adipogenesis over individual treatments with a significant upregulation of PPARγ and aP2 gene expressions. BBP significantly upregulated miRNA-34a ($P<0.05$) and miRNA-103 ($P<0.01$) expression at day 8 of differentiation. BBP in combination with PA significantly upregulated miRNA-34a expression ~8-fold and miRNA-103 expression ~3-fold compared to control or BBP alone ($P<0.001$). We are currently investigating miRNA promoter methylation in adipogenesis. In summary, BBP and PA alone can induce adipogenesis. However, the combination further enhanced adipogenesis by inducing key epigenetic regulators miRNA-34a and miRNA-103. In conclusion, BBP can synergistically augment high fat diet-induced adipogenesis by altering the epigenome program.

ABSTRACT FINAL ID: 3842 Poster Board: P534
TITLE: Whole-Genome Bisulfite Sequencing Reveals Genomic Regions Vulnerable to Epigenetic Perturbation

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S.N. Martos[1], M. Susiarjo[2], M.S. Bartolomei[2], Z. Wang[1]. *[1]Environmental Health Sciences, Johns Hopkins University, Baltimore, MD; [2]Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.*
KEYWORDS: Epigenetics; Mechanisms; Endocrine Disruptors; Whole-Genome Bisulfite Sequencing; Bisphenol A

ABSTRACT BODY: Epigenetic mechanisms of DNA methylation, histone modification, and noncoding RNA regulate genomic processes in a cell-, tissue-, and developmental-stage specific manner by influencing chromatin structure and modulating protein-genome interactions. Early life exposures that alter epigenetic marks can have phenotypic consequences for multiple generations. Because of this, it is increasingly important to understand how epigenetic states are established and maintained to determine how the plasticity and heritability of epigenetic marks, such as DNA methylation, contribute to mechanisms of toxicity from *in utero* exposures. We applied whole-genome bisulfite sequencing (WGBS) to characterize the methylomes of one wild-type (WT) mouse embryonic stem cell (mESC) line and four mutant mESCs with disrupted DNA methyltransferase (DNMT) enzymes. The mutant mESCs are (1) 1KO: $Dnmt1^{-/-}$ loss of "maintenance" DNMT, (2) rescued-1KO (r1KO): $Dnmt1^{-/-}$, with exogenous DNMT1 expression, (3) DKO: $Dnmt3a^{-/-}$/$Dnmt3b^{-/-}$, loss of both "*de novo*"DNMTs, and (4) TKO: $Dnmt^{-/-}$/$Dnmt3a^{-/-}$ /$Dnmt3b^{-/-}$, loss of "maintenance" and "*de novo*" DNMTs. Comparison of WT, 1KO, and DKO indicates a division of labor between DNMT1 and DNMT3a/3b to suppress distinct types of retrotransposons. Comparison of WT, 1KO, and r1KO reveals a feature of imprinted differentially methylated regions (DMRs); loss of methylation in 1KO cells is not rescued by exogenous DNMT1 expression at known imprinted DMRs. We identified additional non-rescuable regions that appear similarly vulnerable to DNA methylation loss in mESCs. Bisphenol A (BPA), an endocrine disrupting compound, has been demonstrated to alter DNA methylation patterns in mice exposed *in utero*. To determine whether the previously described genomic regions of interest are affected by maternal exposure to BPA, we use WGBS to compare methylomes of fetal livers of male offspring from pregnant C57BL/6J mice exposed to 10 μg/kg bw/d (n=3) or 10 mg/kg bw/d (n=3) BPA through BPA supplemented diet (control diet not supplemented, n=3) from two weeks prior to mating through embryonic day 18.5 (E18.5). We found that DNMT1-dependent and non-rescueable genomic regions are, in general, more susceptible to environmental perturbation and have altered CpG methylation in response to BPA exposure.

ABSTRACT FINAL ID: 3843 Poster Board: P535
TITLE: Global DNA Methylation and Exposure to Ambient Air Pollution

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KEYWORDS: Exposure, Environmental; Epigenetics; Epidemiology

ABSTRACT BODY: Ambient air pollution is a complex mixture of particulate matter (PM) and gases. It has been associated with several adverse health effects although underlying mechanisms are not well understood yet. Advances in technologies such as sensor systems and molecular analytical approaches, such as epigenetics, provide new opportunities to improve the understanding of how the environment impacts disease outcomes. The aims of this study are to investigate the effects of low-level exposure to different air pollutants by exploring the association of global DNA methylation with ambient air pollution in healthy individuals. We used data from 2 studies nested in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Our data include participants from Italy (n=457) and the Netherlands (n=167), in which methylation profiles were acquired from prospective blood samples using the Illumina 450K Methylation assay. Long-term air pollution exposure estimates of PM2.5, PM10, PM2.5abs, NOx and NO2 were calculated using exposure models developed within the European Study of Cohorts for Air Pollution Effects (ESCAPE). We assessed the association between global methylation and air pollution estimates using beta-regression controlling for technical variations (and confounding factors (age, gender, smoking, being a cancer case later in life). Exposure to NO2 and NOx was associated with a global hypomethylation on the CpG island's shores -significant in Netherlands (β±se = -3.5E-03 ± 1.6E-03, p-value=0.03 and β±se = -1.7E-03 ± 7.4E-04, p-value=0.02 for NO2 and NOx respectively) and borderline significant in Italy (β±se = -1.8E-04 ± 1.1E-04, p-value=0.09, and β±se = -8.4E-05 ± 5.1E-05, p-value=0.09 for NO2 and NOx, respectively). The promoter regions were borderline significant for NO2 in the Dutch cohort. Our results suggest an association between outdoor NOx and NO2 levels and DNA hypomethylation at CpG island's shores in healthy adults.

ABSTRACT FINAL ID: 3844 Poster Board: P536
TITLE: *In Vitro* and *In Vivo* Dermal Absorption of Bromochlorophene, a Cosmetic Preservative Ingredient in Rats

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KEYWORDS: Bioavailability; Pharmacokinetics; Dermal Absorption; Bromochlorophene

ABSTRACT BODY: Recently, rapid growth of cosmetic industry has increased interest for safety of cosmetic ingredients. Bromochlorophene is the cosmetic ingredient which is used as a preservative. In this study, two types of cosmetic formulation (cream and skin emulsion) were constructed with 1% bromochlorophene to evaluate its dermal absorption as a cosmetic ingredient. The dermal absorption of the bromochlorophene formulations was assessed by *in vitro* dermal absorption study and *in vivo* pharmacokinetics (PK) study according to Korea Ministry of Food and Drug Safety (MFDS) guideline and Organization for Economic Cooperation and Development (OECD) guideline 417 and OECD guideline 427 respectively. *In vitro* skin absorption of each bromochlorophene formulation was determined by Franz diffusion cell system using rat skin. When bromochlorophene from Franz diffusion cell system was measured in receptor fluid, stratum corneum, epidermis and dermis using LC/MS/MS, 105.43 ± 11.07 % for cream and 109.12 ± 8.79 % for skin emulsion were totally recovered after the dermal application and total dermal absorption rate was 1.5 ± 0.9% for cream and 7.42 ± 0.74 % for skin emulsion. To determine *in vivo* dermal absorption of bromochlorophene, it was dissolved in glycerol formal and injected by tail vein (n=3) at doses of 1 and 0.2 mg/kg and the PK parameters were analyzed by non-compartmental method. Subsequently, dermal PK parameters were determined by dermal application of cream and skin emulsion formulation (234 mg/kg, bromochlororphene of 2.34 mg/kg) to dorsal skin of male rats. The bioavailability of dermal application of bromochlorophene was calculated in 4.65 ± 0.60 % for cream and in 12.20 ± 2.63 % for skin emulsion. Conclusively, the dermal absorption of bromochlorophene was finalized to be 12.20 ± 2.63 % based on *in vivo* PK study.

ABSTRACT FINAL ID: 3845 Poster Board: P537
TITLE: Determination of Dermal Penetration Efficiency of [14C] Fenbutatin Oxide in Wistar Rats

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KEYWORDS: Pesticides; Dermal Penetration; Fenbutatin Oxide Technical

ABSTRACT BODY: This study was conducted following guidelines of US EPA OPPTS 870.7600 to evaluate the extent of penetration of Fenbutatin oxide (FBTO) into the skin of Wistar rats at different time points post dosing. A pilot study was conducted at single dose of 2 mg/cm2 of FBTO prepared in corn oil with ≈10 µCi of the radioactivity which was applied on the skin of 2 male rats. The site of application was covered using specially designed 'O' ring to avoid the direct contact of test item with adhesive tape. The total recovered radioactivity through exhaled air in first 24 h of pilot study was <1% of total applied radioactivity. So, main study was conducted without exhaled air collection. A single dose of 0.02 (phase 1), 0.2 (phase 2) and 2.0 (phase 3) mg of FBTO /cm2 of skin with ≈10 µCi of the radioactivity was applied to 24 male rats per dose level. Urine, faces, cage wash, cotton swab, 'O' ring, applicator, whole blood, exposed skin, brain, muscle, heart, kidney, lung, liver and residual carcass were collected at 0.5, 1, 2, 4, 10 and 24 h post dosing. A fix quantity of each sample was weighed, processed and analyzed for radioactivity using liquid scintillation analyzer. Percent absorbed, absorbable and dose of test item not absorbed through skin was determined. The mean dose absorbed at all three dose levels through skin was around 1.837 ± 0.846 %. The mean dose which could get absorbed through skin, if dermal exposure increased (absorbable dose) was 13.18 ± 1.18 % whereas approximately 84.98% dose of FBOT remained unabsorbed on the rat skin. Based on the results obtained in this study, it was concluded that maximum quantity of FBOT was remained unabsorbed during 24 hours of dermal exposure. However, dermal penetration of FBTO was increased in time dependant manner from 0.5 to 24 hours after dermal application which may be continued with increased time of exposure considering the absorbable dose level in skin.

ABSTRACT FINAL ID: 3846 Poster Board: P538
TITLE: Phenotypic Profiling in Human Primary Cell-Based Systems Elucidates Mechanisms of Skin Irritation

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KEYWORDS: Cutaneous or Skin Toxicity; Receptor; Nuclear Hormone; *In Vitro* and Alternatives

ABSTRACT BODY: We are applying a chemical biology approach for elucidating the toxicity mechanisms of drug adverse effects (AEs). In previous work with the EPA for the ToxCastTM program and subsequent studies, we tested large numbers of compounds across a panel of human primary cell-based tissue and disease models, and defined a signature profile of biomarker activities for retinoid receptor agonists. It was of interest to determine if any of these biomarker activities are associated with the skin irritation side effect of retinoids. Prominent biomarker activities for retinoid receptor agonists were found to include increased prostaglandin E2 (PGE2) and decreased TNFalpha in a primary human endothelial cell co-culture model with human peripheral blood monocytes stimulated with lipopolysaccharide. Evaluation of the biomarker profiles for 28 previously developed mechanism classes, identified four mechanism classes that share these activities: Aryl Hydrocarbon Receptor (AhR) agonists, RAR/RXR agonists, Prostaglandin EP Receptor agonists and Vitamin D Receptor (VDR) agonists. AhR and VDR agonists, like retinoids, are known to cause skin irritation. We then extended this analysis to a large reference dataset to identify additional agents and mechanisms that share this activity signature. For this a reference database of more than 3400 test agents (drugs, experimental chemicals, etc.) was searched to identify agents that increase levels of PGE2 in the BioMAP LPS system and decease the level of TNFalpha (>20%) at two or more concentrations, without causing overt cytotoxicity. Only 30 test agents (0.9%) of 3400 agents were found to share this signature. In addition to compounds from the four mechanism classes identified previously, we identified 2-Chloroethyl Ethyl Sulfide, a chemical vesicant, and inhibitors of Thromboxane A2 synthetase. We have identified an *in vitro* signature, increased PGE2 and decreased TNFalpha in a monocyte driven model of vascular inflammation, which is shared by certain compound classes known to cause skin irritation. PGE2 is known to regulate immune responses, supporting Th2-type immune responses, suggesting that the mechanism of irritation by these agents may involve promotion of Th2-type immune responses. These data suggest that one mechanism by which retinoids, VDR agonists, RAR/RXR agonists, Thromboxane A2 synthetase inhibitors, and chemical vesicants induce skin irritation is by promoting Th2 immune responses through PGE2.

ABSTRACT FINAL ID: 3847 Poster Board: P539
TITLE: Induction of Metal-Responsive and Oxidative Stress Gene Biomarkers in Placental JEG-3 Cells by Arsenic and Cadmium Mixtures from Polluted Waste Sites

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KEYWORDS: Gene Expression/Regulation; Metals; Risk Assessment

ABSTRACT BODY: Exposure to elevated levels of the toxic metals inorganic arsenic (iAs) and cadmium (Cd) represents a major global health problem. These metals often occur as mixtures in the environment, creating the potential for interactive or synergistic biological effects different from those observed in single exposure conditions. In the present study, environmental mixtures collected using a passive sampling device from two waste sites in China, and identical mixtures prepared in the lab were tested for toxicogenomic response in placental JEG-3 cells. These cells serve as a model for evaluating cellular responses to exposures during pregnancy. One of the mixtures was predominated by iAs and one by Cd. The gene biomarkers heme oxygenase 1 (*HO-1*) and *metallothionein isoforms* (MT1F and MT1G) previously shown to be preferentially induced by exposure to either iAs or Cd, and metal transporter genes aquaporin-9 *AQP9* and ATPase, beta polypeptide ATP7B were measured in order to evaluate the effects from the metals mixtures using dose and time course experiments. There was a significant increase in the mRNA expression levels of *HO-1, MT1F* and *MT1G* in mixture-treated cells compared to the iAs or Cd only-treated cells. Notably, the genomic responses were observed at concentrations significantly lower than levels found at the environmental collection sites. These data demonstrate that metal mixtures increase the expression of gene biomarkers in placental JEG-3 cells in a synergistic manner. Taken together, the data suggest that toxic metals that co-occur may induce detrimental health effects that are currently underestimated when analyzed as single metals.

ABSTRACT FINAL ID: 3848 Poster Board: P540
TITLE: The Role of TMEM135 in Manganese-Induced Death in *C. elegans* and Primary Dopaminergic Cultures

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KEYWORDS: Metals; Neurotoxicity; Metals

ABSTRACT BODY: Manganese (Mn) is a ubiquitous trace element involved in proper neuronal and glial function, but excessive exposure is associated with dopaminergic dysfunction and parkinsonian symptoms. Several mechanisms may underlie these neurotoxic effects, including oxidative stress and mitochondrial dysfunction. Studying genes involved in these processes can enhance our understanding of Mn-induced pathophysiology. We have shown that the novel protein TMEM135 is associated with cold and fasting stress response in mice, and mitochondrial activity and longevity in *C. elegans*. Given their common molecular pathways, we sought to determine whether TMEM135 contributes to Mn neurotoxicity. Thus, we assessed the effects of Mn exposure and TMEM135 expression on health and survival in two models. L1 larval *C. elegans* deletion mutant tmem135(ok1646) (tmemKO) and wild-type (WT) *C. elegans* were acutely exposed to 0-50 mM MnCl2. All worms were counted on day 1 and surviving worms were counted daily to calculate percentage survival. Compared to WT worms, survival was significantly reduced in tmemKO worms exposed to Mn at 10, 25, and 50 mM on day 3 and in all Mn-exposed tmemKO worms in concentration- and time-dependent manners starting on day 5. Dissociated rat primary cultures of ventral tegmental area and substantia nigra neurons and/or microglia were exposed to 0-1000 µM MnCl2 to probe the involvement of TMEM135 in Mn effects on dopaminergic cell health. Neuronal cultures exhibited decreased viability by MTT assay at 1000 µM Mn, and the addition of microglia significantly enhanced sensitivity to Mn-induced cell death from 10-300 µM Mn. Western blots of Mn-exposed cultures revealed that TMEM135 expression is reduced in neuronal-microglial co-cultures compared to neuron-only cultures at Mn concentrations that reduce viability. Taken together, these data suggest that TMEM135 may be an important factor in survival of exposure to toxic levels of Mn. This work was supported by NIH (grant ES025415-01A1).

ABSTRACT FINAL ID: 3849 Poster Board: P541
TITLE: Unique Tactics in Tracing, Monitoring, and Remediating Mercury Contamination

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KEYWORDS: Metals; Exposure Assessment; Regulatory/Policy; Mercury

ABSTRACT BODY: Spilled liquid mercury is easily spread via contaminated shoes and clothing, which can lead to widespread contamination in buildings with sizeable populations (e.g. schools/industrial facilities). When a spill occurs, mercury evaporates and becomes an invisible, odorless vapor, which may pose a long-term health risk. In a recent school spill, breathing zone air concentrations were below the Agency for Toxic Substances and Disease Registry (ATSDR) recommended action levels, yet accumulation of mercury on shoe soles was evident upon entry into the building. This indicated persistent mercury contamination on surfaces that was not identified by air monitoring. Unique monitoring and remediation techniques were necessary in order to continue remediation activities. The ATSDR recommends that headspace readings for personal belongs in the range of 3-6 µg/m3 be considered protective of human health. We developed an activity based study (ABS) to examine floor contamination. Workers donned clean footwear, entered the school, and walked for a specified time period in classrooms and hallways. Afterward, footwear was sealed and heated passively in the sun. Headspace analysis was performed, and footwear with mercury vapor readings >3 µg/m3 were considered indicative of the continued presence of mercury contamination in the area. Multiple mercury remediation techniques were used to assess the most effective remediation based upon mercury vapor readings. Using the ABS, we were able to characterize the migration of mercury throughout the school. This allowed for targeted clean-up efforts prior to re-entry of students to prevent further contamination and off-site migration. Clean-up using multiple remediation techniques proved effective without having to destroy building materials. During mercury spills, even though air concentrations may remain below regulatory levels, the potential may exist for mercury contamination on personal belongings that could produce a source of vapor when stored in cars, closets, or homes. The use of an ABS in these circumstances provides a detailed examination in order to establish appropriate remediation efforts for the continued safety of students and workers. Furthermore, varied and new remediation techniques may be needed to quickly and effectively mitigation widespread mercury contamination.

ABSTRACT FINAL ID: 3850 Poster Board: P542
TITLE: An Exposure and Health Risk Assessment of Lead in Beverages

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KEYWORDS: Metals; Food Safety/Nutrition; Beverages; Lead

ABSTRACT BODY: Lead (Pb) content in beverages such as wine, beer, and soda has been recently reported in the published literature. Acute and chronic Pb exposure may lead to a range of human health effects, including nausea, headaches, cognitive changes, and renal effects. This study modeled national dietary consumption data from NHANES (National Health and Nutrition Examination Survey) with reported concentrations of Pb in wine, beer, and soda from published literature to determine if ingestion of these beverages may pose a health risk due to Pb. Blood Lead Levels (BLLs) were predicted using the Adult Lead Methodology (ALM) model, assuming background blood concentration in adults of 0 µg/dL or 1 µg/dL. Median consumption data was modeled with median and 95th percentile Pb concentrations, and findings were compared to guideline values of +1.0 µg/dL above background set by California's Office for Environmental Health Hazard Assessment (CA OEHHA) and maximum level of 5 µg/dL set by the Centers for Disease Control and Prevention (CDC). Pb concentrations ranged from 0-0.453 ppm (n=168) for wine, 0.000004-0.290 ppm (n=109) for beer, and 0.171-3.39 ppm (n=23) for soda. Wine consumption was associated with BLL increases of +0.2 µg/dL and +2.0 µg/dL for the median and 95th percentile Pb concentrations, respectively. When limited to wines purchased or originating in the United States, consumption was associated with only +0.2 µg/dL at the 95th percentile Pb concentration. Beer consumption was associated with BLL increases of +1.1 µg/dL at the median Pb concentration and +4.4 µg/dL at the 95th percentile Pb concentration, while soda consumption was associated with BLL increases of +11.4 µg/dL and +93.9 µg/dL at the median and 95th percentile Pb concentration, respectively. Beer and wine consumption was not associated with BLLs above the CDC guideline value, assuming background blood levels of 0 µg/dL. While findings suggests that the consumption of certain beverages from outside the U.S. have the potential to raise BLLs above guideline values, it should be noted that Pb exposure data was limited by sample size and geographic region of origin. According to our analysis, Pb content in beverages from the U.S. do not pose a health risk.

ABSTRACT FINAL ID: 3851 Poster Board: P543
TITLE: Exposure to Inorganic Arsenic in Drinking Water May Increase Plasma Concentrations of Natriuretic Peptides in Children

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KEYWORDS: Metals; Children's Health; Cardiovascular System; Natriuretic Peptides; Arsenic

ABSTRACT BODY: The exposure to inorganic arsenic (iAs) is frequent in several parts of the world. Prolonged exposure to iAs has been associated with the development of cardiovascular diseases such as hypertension and altered hemodynamic and morphological parameters associated with cardiac function, even in children. Furthermore, the cardiac dysfunction have been associated in adults with increased plasma concentrations of natriuretic peptides (BNP). However, in children the use of these markers have been used only in cases of congenital heart abnormalities and not in cases of exposure to environmental contaminants. The aim of this study was to evaluate plasma concentrations of natriuretic peptides in 167 children in iAs endemic area in Zimapán, Hidalgo, Mexico. The sum of the urinary concentrations of iAs+MMA+DMA was considered as total concentration of arsenic (tAs) ranged of 5.7-370 ng/mL. In total, 79% of children had tAs values higher than the Biological Exposure Index (BEI®) of 35 ng/mL. The plasmatic levels of BNP, evaluated by ELISA, ranged of 1.44-967.76 pg/mL, presenting high biological variability. The tAs urinary concentration was associated positively and significantly with the plasma concentration of BNP. Comparing plasma concentrations of BNP with the group of children with urinary concentrations within the biological exposure limit (BEI®; <35 ng/mL of tAs) with groups between 35 to 70 ng/mL of urinary tAs and those >70 ng/mL (β =45.94, p=0.001 and β =65.74, p =0.001, respectively). The BNP concentration could be used as a biomarker of cardiac damage from to the metalloid exposure. Further studies on the possible role of natriuretic peptides as biomarkers of chronic exposure to iAs are required.

ABSTRACT FINAL ID: 3852 Poster Board: P544
TITLE: Heavy Metals Exposure and Oxygen Blood Levels in Children From Mexican Industrial Area

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KEYWORDS: Metals; Children's Health; Respiratory Toxicology; Heavy Metals

ABSTRACT BODY: Respiratory adverse effects has been describe in subjects occupationally exposed to heavy metals like: Chromium (Cr), Nickel (Ni), Silver (Ag) and Cobalt (Co), consequently it causes a lower oxygen content in capillary blood. Hypoxic conditions had been reported in children exposed to Ag by medical treatments as well as in adults by occupational co-exposure whit Cr and Ni. In Tula, Hidalgo, México, government reported emissions of heavy metals (Ni, Co, Cr and Pb) to environment from industrial activity; children who living in study area, are at risk of exposure to heavy metals from industrial sources. In addition, Ag is commonly used in medical treatments and sanitation products. The aim of this study was to evaluate the oxygen blood levels in children exposed to heavy metals. Blood samples were obtained from 30 children (6-10 years old). Exposure assessment to heavy metals (Cr, Co, Ag, Pb and Ni) was carried out by ICP-MS (Thermo, X series II). Oxygen blood saturation (OBS) was determined using a pulse oximeter (Deluxe SM-110). Exposure assessment indicates that children are exposed to next metals above guidelines or levels reported in references for human exposure: Cr, 93%> 3.0 µg/dL (CDC guidelines); Cd, 30%> 0.035 µg/dL (CDC guidelines); Co, 73%>0.5 µg/dL (CDC guidelines); Ag, 30% above non exposed (<0.005 µg/dL), but lower that exposure adults (≥0.1 µg/dL) reported. All children showed levels below CDC guidelines for Pb (≤10 µg/dL). Our results confirms the exposure to heavy metals in the area, caused by the emissions of industrial activities reported before. Ag source is not clear. For blood oxygen assessment, only 21% of children showed normal OBS (≥95%); 36% showed low OBS (<95≥90) and 43% of the evaluated children shows hypoxia (<90%). No statistical significance between heavy metals exposure and OBS was found.

ABSTRACT FINAL ID: 3853 Poster Board: P545
TITLE: Toxicity of Mobile Phone Recharge Card Coating Films in Swiss Albino Rats

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KEYWORDS: Metals; Histopathology; Hematotoxicity; Hepatotoxicity; Recharge Card Coatings Films and Potassium Dichromate

ABSTRACT BODY: The advent of the Global System of Mobile (GSM) telecommunication in Nigeria in year 2001 not only brought changes in the telecommunication sector, but also increased the social economic status of Nigerians. However, people became inadvertently exposed to the opaque thin film coatings used in concealing the re-charge card Personal Identification Number as they are rich in heavy metals. There is a dearth of information on the coatings' *in vivo* toxicity. Coatings were collected from hundred naira price denomination cards of a major mobile telecommunication company and fed to albino rats at 5, 50 and 100 mg/kg body weight thrice per week for four weeks. Positive control rats were injected with 12 mg/kg body weight of K2Cr2O7 thrice per week, while the negative controls were given deionised water. Twenty-four hours after the last treatment, the animals were sacrificed and the levels of serum liver and kidney function markers specifically, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase, urea, and creatinine, were monitored. Limited histopathology and hematological parameters such as hematocrit, white blood cell and platelet count were also evaluated. Exposure to K2Cr2O7 and the coatings led to a significant ($P< 0.05$) increase in AST, ALT ALP, urea and creatinine levels as compared with the control. Similarly, K2Cr2O7 and the coatings led to alteration in hematological parameters as well as liver and kidney cell architecture. Our results suggest that exposure to coating film from GSM cards could exert damaging effect on the liver, kidney and hematopoietic cells.

ABSTRACT FINAL ID: 3854 Poster Board: P546
TITLE: The Role of Autophagy in Arsenic-Induced Transform Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: L. Wang[1], J. A. Hitron[2], Y. Son[1], J. T.f. Wise[3], P. Pratheeshkumar[1], Z. Zhang[2], X. Shi[1]. *[1]Center for Research on Environmental Disease, University of Kentucky, Lexington, KY; [2]Toxicology and Cancer Biology, University of Kentucky, Lexington, KY; [3]Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY.*
KEYWORDS: Metals; Carcinogenesis; Cell Culture

ABSTRACT BODY: Carcinogenesis induced by environmental arsenic exposure is a major public health concern. However, the exact mechanisms underlying arsenic carcinogenesis remain elusive. Recent studies indicate that autophagy is likely play an important role in metal carcinogenesis. Here, we hypothesize that autophagy is involved in arsenic-induced malignant transformation. In the present study, we found that arsenic-transformed cells exhibit autophagy deficiency. In arsenic-transformed cells, there are only a limited number of autolysosomes generated by fusion between autophagosomes and lysosomes. Our data also show that the autophagy deficiency is caused by inhibition of transcription factor EB (TFEB). TFEB is a master gene for lysosomal biogenesis. Under arsenic exposure, TFEB is inhibited and followed by lysosomal biogenesis aberrant. The lack of lysosome contributes to autophagy flux blocking. Arsenic-transformed cells exhibit an increase of p62 and hypoxia-inducible factor 1 α (HIF-1α) because autophagy deficiency limited the cells to eliminate this protein. The accumulation of p62 consequently increases the level of NF-E2-related factor 2 (Nrf2). HIF-1α and p62/Nrf2 in transformed cells further contribute to up-regulate antioxidant enzymes, antiapoptotic proteins, inflammatory factors, and angiogenesis proteins, which could play important roles in arsenic-induced carcinogenesis.

ABSTRACT FINAL ID: 3855 Poster Board: P547
TITLE: Manganese Exposure Promotes a Compensatory Protective Response by Upregulating the Prokineticin-2 Gene through Transcriptional Activation of HIF1 And NRF1 in Cell Culture and Animal Models

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J. Luo, H. Jin, M. Neal, M. Langley, V. Anantharam, A. Kanthasamy, A. Kanthasamy. *Biomedical Sciences, Iowa State University, Ames, IA.*
KEYWORDS: Metals; Neurotoxicity; Metals; Transcription Factors; HIF1, NRF1, Prokineticin

ABSTRACT BODY: Although chronic exposure to excessive manganese (Mn) produces a neurotoxic insult to the basal ganglia neurotransmitter system that culminates in Parkinsonism, the cellular and molecular mechanisms underlying Mn-induced neurotoxicity are largely unknown. While much focus has been placed on cell death processes underlying the neurotoxic effect of Mn, early neuronal response to Mn exposure deserves more attention. In a gene expression analysis, we unexpectedly discovered that the prokineticin-2 (PK2) gene is greatly upregulated during early stages of Mn neurotoxic stress in a dopaminergic cell model. Functional studies revealed that recombinant PK2 and overexpression of PK2 protect against Mn-induced dopaminergic neurotoxicity. To further study the molecular mechanisms underlying Mn-induced PK2 upregulation, we performed *in silico* analysis of the PK2 promoter and revealed the presence of both HIF1 and NRF1 putative binding sites. Importantly, we observed Mn exposure increased HIF1 and NRF1 levels at early stages, suggesting that Mn regulates PK2 expression via an HIF1/NRF1-dependent pathway. Overexpression of HIF1 or NRF1 upregulated PK2 expression. Finally, we tested the *in vivo* effect of Mn on PK2 expression using a mouse model. A toxicologically relevant dose of Mn exposure by an oral route (30 mg/kg for 30 days) significantly upregulated PK2 levels in the substantia nigra with concomitant increases in HIF1 and NRF1 transcription factors. Taken together, these results suggest that Mn upregulates PK2 levels to counter the early neurotoxic stress and that Mn-induced upregulation of PK2 expression is transcriptionally regulated by HIF1 and NRF (NIH grants NS78247and ES10586).

ABSTRACT FINAL ID: 3856 Poster Board: P548
TITLE: Global Assessment of Cadmium Concentrations in the Skin of Free-Ranging Sperm Whales (*Physeter macrocephalus*)

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KEYWORDS: Metals; Environmental Toxicology; Cadmium

ABSTRACT BODY: Cadmium is a toxic, non-essential metal that is a recognized environmental pollutant. Cadmium has been found to be accumulated in the organs of stranded cetaceans. Currently, there are no baseline cadmium concentrations reported in a free-ranging cetacean. Studies have established that skin can be used as a non-invasive sampling method to evaluate trace element concentrations in free-ranging cetaceans. The sperm whale (*Physeter macrocephalus*) has a global distribution, high trophic level and is an indicator species of oceanic health. The aim of this study was to determine cadmium concentrations in the skin of free-ranging sperm whales (n = 340) collected from 16 regions around the world during the voyage of the *Odyssey* (2000 - 2005) considering region, gender, and age in males. Cadmium was detected in 82% of skin biopsies with a mean of 0.3 ± 0.04 ug/g ww (0.02 to 12.4 ug/g ww). These concentrations were higher than reported in toothed whale skin in literature that ranged from 0.002 - 0.1 ug/g ww. Concentrations by region were significantly different ($p < 0.0001$) with the highest mean in the waters near the Maldives and Sea of Cortez with 0.8 and 0.6 ug/g ww, respectively. There was no significant difference in cadmium concentration by gender ($p > 0.35$). Cadmium is known to have a long biological half-life, and cadmium concentrations in males were significantly higher in adults compared to subadults ($p = 0.03$). Selenium, an element that binds to cadmium inhibiting its toxicity, had a moderately positive correlation with cadmium ($r = 0.41$). Mercury, a toxic metal that positively correlates with cadmium in cetacean tissue, had a weakly positive relationship ($r = 0.20$). The regional baselines reported in this study may be used to develop residue criteria for prediction of toxicological risk in sperm whale skin. Additionally, this study depicts the extent of cadmium exposure in a pelagic cetacean that has global distribution.

ABSTRACT FINAL ID: 3857 Poster Board: P549
TITLE: Heavy Metal Detoxification Profiles Between Vieques (Military Bombarding Range) and Mainland Puerto Rico Population

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KEYWORDS: Metals; Environmental Toxicology; Exposure, Environmental; Trace Metals

ABSTRACT BODY: Vieques is a 145-km2 island located 9.6 km from the archipelagos of Puerto Rico. Over 60 years, more than half of Vieques territory was used by the US Navy for storage and live ammunition practice. Environmental studies have shown the presence of toxic metals within Vieques target zone. High levels of Arsenic (As) were found above the FDA tissue limit in several fish species of Vieques. Since Vieques has a very active fishing community, and its proximity to the target range, a great concern exists that the community has become exposed to higher levels of metals as compared to the mainland population and hence are at a higher health risk. Several metals are considered cumulative poisons, and are toxic and pose health risk even at low concentrations. Recent studies have correlated exposure with many developmental and neurodegenerative disease, however, no research has been done to characterize metal exposure in the Vieques community. We collected a 24 hr urine sample from 58 patients from Puerto Rico mainland (Isla Grande) and 32 from Vieques. Urine samples were collected after iv injection with Ca^{++} EDTA and analyzed at Cincinnati, OH: Environmental Monitoring Systems Laboratory, using EPA 1991a Method 200.8: Determination of trace elements by inductively coupled plasma-mass spectrometry (ICP-MS). These results were normalized to urine creatinine and expressed in ug/g. A total of 16 metals were analyzed (Rb, Al, As, Cs, Ba, Hg, Ni, Sn, Cd, Pb, Sb, Tl, W, Ga, Pt, U). The toxicological data obtained shows that heavy metals released by patients in urine using this protocol increases with age. In general the depuration process employed was able to remove more trace elements in patients from Vieques as compared to those from mainland. Aluminum (Al), Arsenic (As), lead (Pb) and Uranium were depurated at the higher concentrations in urine by patients from Vieques as compared to patients from mainland at the age range of 34-54 and 55-75. Concentrations of these metals in urine are dramatic when analyzed by age groups, reflecting increases of two-to-three times higher. These results show that the Vieques population has been exposed to higher levels of these four elements when compared to mainland. We discuss the sources of exposure to these metals in the Vieques population.

ABSTRACT FINAL ID: 3858 Poster Board: P550
TITLE: Zinc-Dependent Targets of Uranium and Aresenic in Immune Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: E.J. Dashner, K.L. Cooper, D. Jackson, D. MacKenzie, J. Lewis, L. G. Hudson. *Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*
KEYWORDS: Metals; Exposure, Environmental

ABSTRACT BODY: Members of tribal communities in the western region of the United States are in frequent contact with metal mixtures from unremediated abandoned uranium (U) mines through contaminated dusts, drinking water, and food. Both arsenic (As) and U co-occur in mine waste and adjacent soils in these communities at levels that are orders of magnitude greater than crustal averages. Results from the Diné Network for Environmental Health project indicate that chronic, low-level community exposure to U waste contributes to immune dysfunction. We hypothesize that certain zinc-dependent proteins are vulnerable to disruption by As and U. Among the zinc binding E3 ubiquitin ligases involved in immunological responses Casitas B-lineage lymphoma proto-oncogene-b (Cbl-b) is an important gatekeeper of immune activation. Cbl-b-deficient T-cells have lower activation thresholds and cbl-b knockout mice are prone to spontaneous and experimental autoimmunity. In this study, we report that low, non-cytotoxic, concentrations of As (≤1 μM) and U (≤3 μM) are able to displace zinc from Cbl-b RING finger motifs of THP-1 cells without altering expression levels, suggesting an attenuation of Cbl-b enzymatic activity. Co-incubation with zinc largely overcame the loss of zinc from the RING domain as measured by zinc release assay. The susceptibility to loss of zinc from the RING domain is less sensitive than the previously reported zinc finger DNA repair protein poly(ADP-ribose)polymerase (PARP)-1. Despite confirming PARP-1 as a target for As and U in THP-1 cells, there is no significant increase in DNA damage by comet assay. Most notably, there is evidence of As and U interaction when THP-1 cells are co-exposed to the metals at concentrations meeting the maximum contaminant level for regulated drinking water. These findings suggest that multiple proteins with zinc-dependent functional motifs may be targets of environmental metals associated with uranium mine waste.

ABSTRACT FINAL ID: 3859 Poster Board: P551
TITLE: Potential Mechanism of the Stimulatory Effect of Low Dose Cd on Lung Fibrosis

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KEYWORDS: Metals; Lung; Pulmonary Or Respiratory System; Signal Transduction; RT-PCR; Cadmium

ABSTRACT BODY: Chronic low-level exposure of cadmium (Cd) presents a previously underestimated health hazard. Our previous study of low dose Cd effects on mouse lung fibroblasts and HeLa cells showed that Cd in submicromolar concentration is sufficient to alter cell signaling and redox control mechanism, e.g., oxidation of actin cytoskeleton proteins, stimulation of thioredoxin-1 translocation from cytoplasm to nuclei, activation of NF-κB transcription factor, and stimulation of actin polymerization. To study low dose Cd effect further, in the present study, we hypothesized that low-dose Cd disturbs cytoplasmic-nuclear redox signaling and stimulates fibrosis signaling. In this study, we used human fetal lung fibroblasts to examine DNA binding activity of transcriptional factors in respond to low level Cd treatment (1 μM and 2 μM). The result of firefly luminescence signal as a measure of DNA binding activity of transcription factor showed that among 45 transcription factors, 6 were significantly elevated by Cd while 2 were decreased ($p < 0.05$). Of 6 transcription factors activated by Cd, metal response element-binding transcription factor-1 (MTF-1) was most highly affected by Cd (5-fold increase by Cd). In addition, activating transcription factor (ATF) 2/3/4, myocyte enhancer factor-2 (Mef2), signal transducer and activator of transcription 3 (STAT3) and recombination signal binding protein for immunoglobulin kappa J region (RBP-Jκ) were activated 2-fold or higher by Cd compared to control cells with no Cd treatment. With respect to fibrosis signaling, we found that SMAD 2/3/4 transcription factor was activated by 2 μM Cd (1.4-fold). To support Cd-stimulated activation of transcription factors, we examined subsequent gene expression targeted by MTF-1 using quantitative real time (qRT)-PCR analysis. The data of qRT-PCR show that mRNA levels of metallothionein as target gene of MTF-1, were significantly elevated by Cd (8-fold, $p < 0.001$). The results suggest that low level Cd stimulates lung fibrosis by activating SMAD2/3/4 transcription factor. Whether the low doses of Cd cause upregulation of fibrosis markers and whether the perturbation of redox-dependent signaling pathways involving translocation of thioredoxin-1 to nuclei are being studied. Together, our data supports potential mechanism of the stimulatory effect of low dose Cd on lung fibrosis and can contribute to advancing our understanding of pulmonary fibrosis disease mechanism.

ABSTRACT FINAL ID: 3860 Poster Board: P552
TITLE: A Comparison of the Cytotoxicity and Genotoxicity of Particulate and Soluble Hexavalent Chromium in Human and Leatherback Sea Turtle (*Dermochelys coriacea*) Skin Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: R.M. Speer[1], J.L. Young[1], M. Martin Bras[2], M. Barandiaran[3], L. Marquez[2], J.P. Wise[1]. *[1]Pharmacology and Toxicology, University of Louisville, Louisville, KY; [2]Vieques Conservation and Historical Trust, Vieques, Puerto Rico; [3]US Fish and Wildlife Service, Vieques, Puerto Rico.*
KEYWORDS: Cytotoxicity; Genotoxicity; Hexavalent Chromium; Hexavalent Chromium

ABSTRACT BODY: Monitoring the health effects of environmental contaminants such as heavy metals can be achieved using sentinel species as models and a means for establishing baseline comparison data. Leatherback sea turtles are an endangered marine species that may experience prolonged exposures to environmental contaminants such as Cr(VI). Cr(VI) is a ubiquitous global contaminant of the marine environment as a result of human activities. While Cr(VI) has been identified as a known human carcinogen the health effects in marine species are poorly understood. In this study we assessed the cytotoxic and genotoxic effects of particulate and soluble Cr(VI) in leatherback sea turtle skin cells and compared these results with data in human skin cells. Both particulate and soluble Cr(VI) induced a concentration-dependent increase in cytotoxicity that was comparable to experiments in human skin cells. We have shown Cr(VI) is clastogenic to human skin cells. Therefore, using a chromosome aberration assay we assessed the genotoxic effects of Cr(VI) in leatherback sea turtles. Particulate and soluble Cr(VI) induced a concentration-dependent increase in clastogenicity. These data indicate that Cr(VI) may be a health concern for leatherback sea turtles and other long-lived marine species. Additionally, the data indicate leatherback sea turtles can serve as a model species for monitoring the health effects of Cr(VI) in the environment and therefore serve as an indicator species for environmental human exposures.

ABSTRACT FINAL ID: 3861 Poster Board: P553
TITLE: Global Assessment of Manganese Concentrations in the Skin of Free-Ranging Sperm Whales (*Physeter microcephalus*)

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S.S. Wise[1,2], L.C. Savery[2], J.T.F. Wise[2,3], C. Gianios[1,2], J. Buonagurio[4], C. Perkins[5], T. Zheng[6], C. Zhu[7], J.P. Wise[1,2]. *[1]Pharmacology and Toxicology, University of Louisville, Louisville, KY; [2]Wise Laboratory Field Research Program, Washington, DC; [3]Pharmacology and Toxicology, University of Kentucky, Lexington, KY; [4]Exponent, Alexandria, VA; [5]University of Connecticut, Storrs, CT; [6]Department of Epidemiology, Brown University, Providence, RI; [7]West China School of Public Health, Sichuan University, Chengdu, China.*
KEYWORDS: Metals; Environmental Toxicology; Ecotoxicology

ABSTRACT BODY: Manganese is a naturally occurring, ubiquitous element. Manganese is an essential trace element but is also known to accumulate to toxic levels in mammals, including marine species. Currently, there are no baseline manganese concentrations reported in a free-ranging cetaceans. Studies have established that skin biopsies can be used as a non-invasive method to evaluate trace element concentrations in free-ranging cetaceans. The sperm whale (*Physeter macrocephalus*) has a global distribution and as an apex predator serves as an indicator species of oceanic health. The aim of this study was to determine manganese concentrations in the skin of free-ranging sperm whales collected from around the world during the voyage of the Odyssey (2000 - 2005). Skin biopsies (n = 298) were collected in 16 equatorial regions and analyzed for manganese via inductively coupled plasma-mass spectrometry (ICP-MS) considering region, gender, and age in males. Manganese was detected in all of the skin biopsies with a mean of 6.8 ± 1.0 ug/g ww (0.2 to 208.1 ug/g ww). These values were much higher than muscle values reported for other large whales and dolphins. Concentrations by region were significantly different ($p < 0.001$) with the highest mean in the Pacific crossing and the Sea of Cortez (16.2 and 12.6 ug/g ww, respectively). The biopsies with the highest manganese concentrations were three females found in the waters of the Sea of Cortez with 208.1 ug/g ww, in the Pacific crossing with 74.2 ug/g ww, and in the Maldives with 85.6 ug/g ww. There was no significant difference in manganese concentration by gender ($p = 0.22$) or age in males ($p = 0.89$). Manganese concentrations in two sloughed skin samples from adult males in Seychelles, 1.6 and 3.1 ug/g ww, were more than two times lower than the average concentration found in the skin biopsies, 7.2 ug/g ww. This is the first study to address manganese levels in a free-ranging cetacean on a global scale.

ABSTRACT FINAL ID: 3862 Poster Board: P554
TITLE: Acute Toxicity of Recycled Water from the Sewage Treatment Plant “La Morita” from Tijuana, B.C. Mexico on Guppies *Poecilia reticulata*

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: L.R. Lara Jacobo[1], T. M. Oropeza Guzman[2], E. Hoh[3], A.D. Re Araujo[4]. *[1]School of Chemical Science and Engineering, University Autonomous of Baja California (UABC), Tijuana, Mexico;[2]Center for Research and Technological Development in Electrochemistry (CIDETEQ), Tijuana, Mexico;[3]Public Health, San Diego State University (SDSU), San Diego, CA;[4]Ensenada Center for Scientific Research and Higher Education (CICESE), Ensenada, Mexico.*
KEYWORDS: Environmental Toxicology; Aquatic Toxicology; Exposure, Environmental; Recycled Water

ABSTRACT BODY: The need to seek alternatives for water supply in Baja California, Mexico, has led us give a direct water use and the research of this water quality. Recycled water has been used in the last years for irrigation streets, industrial use, baseball fields, football, and other recreational activities. The guppy fish was selected for the toxicity experiment. There were 3 groups of fishes with 3 differences concentrations Control (0% of recycled water) 50% (half of recycled water) and 100% (entire recycled water), the temperature of water was regulated at 24 ± 1 0C. The experiments were repeated twice and they were monitoring during 48 h after the exposure, in order to determine LC50 for the guppies. Data obtained from this acute toxicity tests were evaluated and it was not possible to analyze the data, at the end of the 48 h the fish were still alive. We conclude that the recycled water complies with the Mexican regulations and is not toxic to fish. It is noteworthy that this study will form the basis for further studies that allow us to know more about the quality of this recycled water, like chronic toxicity experiments and other kind of essays.

ABSTRACT FINAL ID: 3863 Poster Board: P555
TITLE: Sublethal Responses of the Indicator Mussel Species *Unio mancus eucirrus* to Selected Phthalate Esters

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: O. Kuzukiran[1], B. Yurdakok Dikmen[2], B. Erkmen[3], C. A. Karasu Benli[4], Z. Yildirim[5], P. Arslan[6], E. Totan[2], A. Filazi[2], F. Erkoc[7]. *[1]Etlik, Veterinary Control Central Research Institute, Ankara, Turkey; [2]Department of Pharmacology and Toxicology, Ankara University Faculty of Veterinary Medicine, Ankara, Turkey; [3]Department of Biology, Aksaray University Faculty of Science and Letters, Aksaray, Turkey; [4]Department of Environmental Sciences, Institute of Natural and Applied Sciences, Gazi University, Ankara, Turkey;[5]Etimesgut Public Health Laboratory, Ankara, Turkey; [6]Department of Biology, Ankara University Faculty of Science, Ankara, Turkey; [7]Department of Biology Education, Gazi University, Ankara, Turkey.*
KEYWORDS: Phthalates; Non-Mammalian Species; Biomarkers

ABSTRACT BODY: High commercial interest di-isononyl phthalate-DiNP, di-isodecyl phthalate-DiDP (with known TDIs) and dimethyl phthalate-DMP (subchronic toxicant) were studied for aquatic toxicity to nontarget fresh water mussels. Hand collected 59.7 mm shell length adult *Unio mancus eucirrus* Bourguignat, 1857 (Bivalvia, Unionidae) were exposed to DiNP and DiDP at 100 µg/L; DMP at 1 mg/L in DMSO. After 96 h exposure total hemocytes (THCs, increased as biomarker of host defense); LPO were determined as MDA and GSH in gill and digestive gland tissue. THCs were found to have changed in the DMP group due to stress and/or enhancement of the mussel resistance. MDA levels were higher in DMP group in both tissues ($p<0.05$); whereas DiDP caused increase only in gills. Gill GSH levels were lower in the DiDP group while higher in the DMP group in both tissues ($p<0.05$). Differences in xenobiotic bioavailability may cause dissimilar response eventhough these dialkyl ortho-phthalates have low solubility, changes in LPO biomarkers indicate possible initial physical absorption by the mussel. Phthalate exposure induced biological and oxidative stress mainly on gills where low molecular weight DMP caused the highest damage. Unio is a novel alternative, economic bioindicator species easily and reliably used for ecotoxicology risk assessment studies for emerging contaminants of concern.

ABSTRACT FINAL ID: 3864 Poster Board: P556
TITLE: Occurrences, Distributions, and Monthly Variations of Some Persistent Organic Pollutants in Water and Surface Sediments From Ankara River, Turkey

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: B. Yurdakok Dikmen[1], O. Kuzukiran[2], F.G. Aydin[1], H. Tutun[1], S. Sevin[1], A. Filazi[1]. *[1]Department of Pharmacology and Toxicology, Ankara University Faculty of Veterinary Medicine, Ankara, Turkey,[2]Veterinary Control Central Research Institute, Etlik, Ankara, Turkey.*
KEYWORDS: Polychlorinated Biphenyls; Persistent Organic Chemicals; Ecotoxicology

ABSTRACT BODY: Pollutants of domestic origin in urban areas and mainly pesticide in rural areas have the potential to bioconcentrate and disrupt endogenous control of development and sexual differentiation. These potential pollutants with endocrine disrupting properties such as persistent organic pollutants (POPs) are expected to be screened regularly by local authorities from environmental samples. Ankara creek is the third largest drainage area among all river basins in Turkey which pass through the capital, affected by municipal and industrial discharges; where irrigation is still present for agricultural production. In this study, surface water (12 stations) and sediment samples (6 station) collected from Ankara creek for 12 months were evaluated for its organic pollutant content (PCBs 28, 52, 101, 118, 138, 153, 180; PBDEs 17, 47, 66, 100, 153, 183 and OCPs α-HCH, HCB, γ-HCH, Heptachlor, p,p-DDD, p,p-DDE, p,p-DDT)using GC-MS; to examine the current situation and the effects of monthly variation and the distribution. Among PCBs, no contamination in water samples were present whereas PCB 28 (4 sample), 101 (13 sample), 138 (2 sample) and 153 (2 sample) (0.383-2.976 ppb) were found in sediment samples with higher concentrations in especially September. PBDE 17, 47, 100 (0.160-0.685 ppb) were found in water samples, whereas on PBDE 66 (1.007-6.941 ppb) was present in sediment samples in July and August. p,p-DDE was found in water samples for all months except December; where this month p,p-DDT was present. γ-HCH was present only in sediment samples. In general, the pollution was found to be profound in rural areas; meanwhile OCPs were found both in rural and urban areas. Governmental strategies should be implemented to evaluate the areas of risk, especially for water resources for irrigation purposes.

ABSTRACT FINAL ID: 3865 Poster Board: P557
TITLE: Toxicokinetics and Physiologically-Based Toxicokinetic Modeling of Tamoxifen in Rainbow Trout

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: K. Gillies[1], T. Cavileer[2], J. Nagler[2], C. Trostle[1], I. Schultz[1]. *[1]Pacific NW National Lab, Sequim, WA; [2]University of Idaho, Moscow, ID.*
KEYWORDS: Toxicokinetics; Endocrine; Estrogens; Endocrine Disruptors; SERM; Tamoxifen

ABSTRACT BODY: Tamoxifen is a selective estrogen receptor modulator exhibiting both weak agonistic and antagonistic behavior. In previous studies in rainbow trout, tamoxifen was observed to have an overall antagonistic effect towards estrogen signaling. We are currently using tamoxifen as one of a suite of model compounds to evaluate *in vitro* testing for predicting whole fish effects of contaminants. These studies are part of larger effort seeking the best approaches for implementing the adverse outcome pathway (AOP) concept in toxicity testing. In the present study, we first characterized tamoxifen toxicokinetics and metabolism in sexually mature female trout and then used these results to guide development of a physiologically based toxicokinetic (PBTK) model. The uptake clearance of tamoxifen into trout was measured using a static water expsoure system and observed to be 49 ml/hr/g, which is comparable to gill water flow. Tamoxifen tissue:plasma partition ratios was measured after continuous water exposures and observed to be between 5-15 for most well-perfused tissues except the brain and pituitary, which had ratios of 20 and 38, respectively. *In vitro* experiments using freshly prepared trout hepatocytes demonstrated formation of the 4-OH-tamoxifen metabolite (considered a potent anti-estrogen), which corroborated *in vivo* observations that trout could biotransform tamoxifen. Subsequent analysis of bile collected after water exposures to tamoxifen observed at least 90% of the contents was as the 4-OH-tamoxifen-glucuronide conjugate. A PBTK model was then developed for tamoxifen and 4-OH-tamoxifen in female rainbow trout that included separate compartments for the brain, liver, ovary, which are all potential target sites of anti-estrogen toxicity. The tamoxifen model inlcuded a description of formation of the 4-OH-tamoxifen metabolite. The 4-OH tamoxifen PBTK model included a description of the conjugation and entgerohepatic recirculation of the conjugate. The combined PBTK models were then used to reconstruct 4-OH-tamoxifen tissue levels after continous exposure to various water concentrations of tamoxifen. Supported by EPA-STAR grant R835167.

ABSTRACT FINAL ID: 3866 Poster Board: P558
TITLE: Linking Mitochondrial Dysfunction and Growth in the Fathead Minnow (*pimephales Promelas*)

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: D.G. Bolser, D.A. Dreier, E. Li, K.J. Kroll, J.T. Schmidt, C.J. Martyniuk, N.D. Denslow. *Physiological Sciences, University of Florida, Gainesville, FL.*
KEYWORDS: Aquatic Toxicology

ABSTRACT BODY: Several high-throughput screening assessments suggest that many environmental contaminants impact mitochondrial function. Changes in mitochondrial function, such as those related to basal metabolism and ATP production, have been implicated in diseases such as neurodegeneration and aging. In addition, changes in mitochondrial function can activate the mTOR pathway, leading to decreased protein synthesis for a wide range of physiological processes. Based on these studies, there are robust models to assess mitochondrial function for human health (e.g., mammalian cell lines, zebrafish), but there remains a need to address these effects in ecologically-relevant species like the fathead minnow, a species found throughout North America. Assessing mitochondrial dysfunction in these species may be critical for understanding effects on ecologically relevant apical endpoints, such as growth. Thus, the objective of this study was to assess mitochondrial function in fathead minnow embryos to develop adverse outcome pathways for mTOR activity and growth. Here, the Seahorse XFe24 Bioanalyzer was used to measure oxygen consumption rate (OCR) in 48hr. old embryos. Our optimization studies have determined that one embryo per well is sufficient to detect an OCR of up to 600 pmol/min. To test this bioassay, 2,4-Dinitrophenol and Octylamine were used as model toxicants. Results demonstrate these chemicals inhibit mitochondrial respiration, as evidenced by a decrease in OCR in both basal and maximal respiration phases. Thus, these chemicals are ideal candidates to assess adverse outcome pathways for mTOR activity and growth as they relate to mitochondrial dysfunction. The development of these adverse outcome pathways are crucial to understanding the mechanisms behind changes in growth, and may be important in the development of other ecologically relevant endpoints.

ABSTRACT FINAL ID: 3867 Poster Board: P559
TITLE: Cytotoxicity and Genotoxicity of 7-Glutathione-Pyrrole Metabolite in HepG2 Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: Q. Xia, X. He, P. P. Fu. *Biochemical Toxicology, NCTR, US FDA, Jefferson, AR.*
KEYWORDS: Glutathione; Genotoxicity; Mechanisms; Pyrrolizidine Alkaloids

ABSTRACT BODY: Pyrrolizidine alkaloids (PAs) require metabolic activation to form primary dehydro-PA metabolites that can bind to cellular DNA and proteins leading to cytotoxicity, genotoxicity, and tumorigenicity. However, dehydro-PAs are highly unstable and may not be the principal reactive metabolites to initiate PA-induced genotoxicity and carcinogenicity. Therefore, it is important to determine other reactive metabolites that can bind to cellular DNA and contribute to liver tumor initiation. We have previously determined that 7-glutathionyl-(±)-6,7-dihydro-1-hydroxymethyl-5H-pyrrolizine (7-GS-DHP), a known metabolite formed *in vitro* and *in vivo*, can bind to dG, dA and calf thymus DNA resulting in the formation of the same set of DHP-dG and DHP-dA adducts that have been shown to be responsible for PA-induced liver tumors. In this study, we determined that incubation of 7-GS-DHP in HepG2 cell culture resulted in the formation of the same set of DHP-dG and DHP-dA adducts in a dose-response manner. Under our experimental conditions, 7-GS-DHP did not exhibit cytotoxicity to the cells, which suggested that the formation of these DNA adducts was through the binding of 7-GS-DHP to DNA in viable HepG2 cells. These results suggest that: (i) 7-GS-DHP is a reactive metabolite that can induce DNA adducts formation, and possibly lead to liver tumor initiation; (ii) formation of 7-GS-DHP can be a metabolic activation pathway of PAs; and (iii) there are multiple metabolic activation pathways leading to PA-induced liver tumors. (This article is not an official guidance or policy statement of the US FDA. No official support or endorsement by the US FDA is intended or should be inferred).

ABSTRACT FINAL ID: 3868 Poster Board: P560
TITLE: 7-Cysteine-Pyrrole Conjugate Is a New DNA Reactive Metabolite of Pyrrolizidine Alkaloids

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: X. He, Q. Xia, L. Ma, P.P. Fu. *Biochemical Toxicology, NCTR, US FDA, Jefferson, AR.*
KEYWORDS: Metabolic Activation; Reactive Intermediate; DNA Adducts; Pyrrolizidine Alkaloid

ABSTRACT BODY: Pyrrolizidine alkaloid (PA)-containing plants are probably the most common poisonous plants affecting livestock, wildlife, and humans. PAs require metabolic activation to exert cytotoxicity, genotoxicity, and tumorigenicity. We previously determined that the four (±)-6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine (DHP)-derived DNA adducts, designated as DHP-dG-3, DHP-dG-4, DHP-dA-3 and DHP-dA-4 adducts, formed in the liver of rats are responsible for PA-induced liver tumor formation, and are potentially common biological biomarkers of PA exposure and carcinogenesis. In this study, we determined that metabolism of riddelliine and monocrotaline by human or rat liver microsomes with or without the addition of cysteine all resulted in the formation of 7-cysteine-DHP and DHP. In addition, metabolism of 7-glutathione-DHP, by human and rat liver microsomes also generated 7-cysteine-DHP. We further determined that reaction of 7-cysteine-DHP with 2'-deoxyguanosine (dG) and 2'-deoxyadenosine (dA) yielded DHP-dG-3, DHP-dG-4, DHP-dA-3 and DHP-dA-4 adducts as well as the reactive metabolite DHP; Reaction of 7-cysteine-DHP with calf thymus DNA at 37°C for 1, 3 and 5 days followed by enzymatic hydrolysis also yielded DHP-dG-3, DHP-dG-4, DHP-dA-3, and DHP-dA-4 adducts with DHP-dA 3/4 as the predominant adducts and in a time-dependent manner. Their structures were determined by comparison of the HPLC retention times, UV-visible absorption spectra, and LC/MS data with those of previously synthesized standards. This study represents the first report that 7-cysteine-DHP is a new active metabolite that can lead to DNA adduct formation. (This article is not an official guidance or policy statement of the US FDA. No official support or endorsement by the US FDA is intended or should be inferred).

ABSTRACT FINAL ID: 3869 Poster Board: P561
TITLE: Protective Effect of Quercetin Against Benzo[a]pyrene-Induced Dna Damage in Sprague-Dawley Rats

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KEYWORDS: Polycyclic Aromatic Hydrocarbons; Metabolism; Gastrointestinal

ABSTRACT BODY: Benzo[a]pyrene (BaP) is a very well-known as an environmental pollutant. It has been reported that dietary exposure to BaP can induce gastrointestinal cancer. However, there are a few data on the molecular mechanism on protective effects of phytochemicals during the gastrointestinal carcinogenesis. Therefore, the aim of this study is to investigate the preventive effects of quercetin on BaP-induced DNA damage in digestive organs of Sprague-Dawley rats. The flavonoid quercetin is naturally found in fruits and vegetables which have physiological properties such as antioxidant, anti-aging, and anti-inflammatory effects. For this study, BaP (20 mg/kg body weight) was administered to rats in corn oil via oral gavage over a 30 days period. Quercetin (2.5, 7.5, or 15 mg/kg) was coadministered with BaP (20 mg/kg) for 30 days. All rats were anesthetized and major organs, including liver, kidney, stomach, intestine and colon were removed at 24 h after the last treatment. In the biochemical analysis, blood urea nitrogen (BUN) and aspartate aminortansferase (AST) levels were significantly increased in the BaP-treated group when compared to control group. Expression levels of CYP1A1 and CYP1B1 were significantly increased in the liver of rats treated with BaP. However, cotreatment with quercetin markedly decreased CYP1A1 expression in a dose-dependent manner. Furthermore, plasma concentration of benzo[a]pyrene diolepoxie (BPDE) was significantly decreased by quercetin treatment compared with BaP alone. Furthermore, cotreatment with quercetin significantly decreased the BPDE-I-DNA adduct formation in liver, kidney, stomach and intestine. Overall, our observations reveal that quercetin inhibits gastrointestinal tumorigenesis when given together with BaP and holds promise as a chemopreventive agent.

ABSTRACT FINAL ID: 3870 Poster Board: P562
TITLE: Epigenetic Changes Associated With Chronic Oxidative Stress-Induced Malignant Transformation of Human Renal Epithelial Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: P.K.S. Mahalingaiah, L. Ponnusamy, K.P. Singh. *Environmental Toxicology, The Institute of Environmental and Human Health (TIEHH),Texas Tech University, Lubbock, TX.*
KEYWORDS: Kidney; Oxidative Injury; Epigenetics

ABSTRACT BODY: Renal Cell Carcinoma (RCC) in humans is positively influenced by oxidative stress status in kidneys. We recently reported that adaptive response to low level of chronic (6 months) oxidative stress induces malignant transformation of normal human renal tubular epithelial cells and is associated with acquisition of epithelial to mesenchymal transition (EMT) and stem cell characteristics. However, the molecular mechanism for oxidative stress-induced kidney carcinogenesis is not clear. Recent studies suggest that aberrant epigenetic modifications can cause changes in gene expression during carcinogenesis. Therefore, the objective of this study was to identify the epigenetic changes induced by chronic oxidative stress and determine their role in neoplastic transformation of HK-2, human renal tubular epithelial cells. The result of this study revealed aberrant expression of epigenetic regulatory genes/proteins associated with DNA methylation (DNMT1, DNMT3a and MBD4) as well as histone modifications (HDAC1, HMT1 and HAT1) in HK-2 cells malignantly transformed by chronic oxidative stress. In addition, significant decrease in the growth and tumorigenic potential of malignantly transformed cells as observed in *in vitro* soft agar assay and *in vivo* xenograft study in athymic mice in response to DNA demethylation by 5-aza 2' deoxycytidine treatment further suggested the potential role of DNA hypermethylation in chronic oxidative stress-induced malignant transformation of HK-2 cells. Taken together, the results of our study for the first time suggested potential role of epigenetic changes in chronic oxidative stress induced-neoplastic transformation of human kidney epithelial cells.

ABSTRACT FINAL ID: 3871 Poster Board: P563
TITLE: Polyphenolics From Mango (*mangifera Indica L*.) Suppress Breast Cancer Ductal Carcinoma by Targeting Both the mTOR and AMPK Pathway in Dcis.com Breast Cancer Xenografts in Mice

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M.J. Nemec. *Toxicology, Texas A&M University, College Station, TX.*
KEYWORDS: Carcinogenesis; Food Safety/Nutrition; Bioavailability

ABSTRACT BODY: It is estimated that 1 in 8 women will be diagnosed with breast cancer. Botanical-based alternative treatments and preventive regiment may contribute to decreasing this concerning incidence. Polyphenols are tumor-cytotoxic botanicals and in this study, a gallate-rich extract from mango (ML) and a major intestinal metabolite pyrogallate (PG) were used as treatments. 6-8 week female athymic nude mice, xenografted with ductal carcinoma cells MCF10DCIS.COM into the mammary pads and received either 0.8 mg/day of ML or 0.2 mg/day PG via oral gavage for 4 weeks. Tumor morphology, mRNA, total protein, and phosphorylated protein levels within the proliferation-involved AMPK-mTOR axis were investigated. A follow-up study comparing 0.2 mg/day and 0.8 mg/day PG was conducted focusing on determining bioavailability. Both, ML and PG significantly reduced tumor volume to less than 50% of control animals. *In vitro* results suggested that these treatments would produce a significant amount of ROS. In previous publication AMPK was upregulated by ROS and in this study sestrin protein levels were significantly elevated in both treatments along with phosphorylated AMPK levels. Treatments also upregulated LC3, a primary indicator of autophagy. In the follow-up study, PG decreased tumor volume by more than 40% at both doses. Data indicate that mango polyphenols and their major intestinal metabolite pyrogallate are effective in reducing tumor size in xenografted nude mice via modulation of the IGFR-1-AKT-mTOR axis and upregulation of AMPK, at least in part through ROS-induced sestrin. Future studies should investigate whether dietary levels of mango tannins and pyrogallol would not only reduce proliferation but also prevent the advancement of DCIS breast cancer to a metastatic state.

ABSTRACT FINAL ID: 3872 Poster Board: P564
TITLE: The Aryl Hydrocarbon Receptor Regulates Epithelial-mesenchymal Transition and Potentiates Cancer Stem Cells via Actin Reorganization

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: Y. Xie, S.E. Eltom. *Biochem & Cancer Biology, Meharry Medical College, Nashville, TN.*
KEYWORDS: Carcinogenesis; Receptor; Aryl Hydrocarbon; Signal Transduction; Epithelial; Mesenchymal; Actin Reorganization; Stress Fibers; Cancer Stem Cells

ABSTRACT BODY: Cytoskeleton reorganization is a key part of the morphology change seen during epithelial-mesenchymal transition (EMT). Here we report that the aryl hydrocarbon receptor (AhR) in a phosphorylation-dependent manner, regulates actin reorganization, thus linking phosphorylation events to dynamics of EMT and stemness. AhR, originally identified as a ligand-dependent transcription factor that regulates xenobiotics signaling pathways, is upregulated in many metastatic cancers. We previously showed that knockdown of AhR inhibits metastasis of breast cancer cells in experimental metastasis mouse model. Here we found AhR knockdown or overexpression induces actin dynamics through changes in the expression or activity of moesin and vasodilator stimulated phosphoprotein (VASP), as well as Rho GTPases and Rho kinase (ROCK). AhR-induced actin stress fiber formation requires its phosphorylation by AKT; thus mutation of the AhR conserved AKT phosphorylation site at T45 disrupted actin stress fiber formation. AhR cooperates specifically with polymerized F-actin to induce mammosphere formation. Most importantly, AhR knockdown, or combined inhibition of PI3K/AKT and ROCK, blocked AhR mediated-actin stress fiber formation and abolished AhR-dependent stem traits, underscoring the significant role of AhR phosphorylation. Independently, SILAC-based global phosphoproteomics identified sets of phosphopeptides affected by AhR knockdown, which are mapped to signaling pathways regulating cell adhesion, morphology, motility, and actin cytoskeleton signaling. Our novel findings reveal that AhR regulates cancer stem-like cells at least in part through phosphorylation-mediated actin reorganization, and introduce AhR as a new target for disrupting EMT and cancer stem cells potentially for the treatment of metastatic breast cancer.

ABSTRACT FINAL ID: 3873 Poster Board: P565
TITLE: Sunitinib-Induced Cardiotoxicity Is Associated with Changes in Expression Levels of Cardiac MicroRNAs in Human Induced Pluripotent Stem Cell (iPSC)-Derived Cardiomyocytes

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M.C. White, L. Ren, X. Yang. *Systems Biology, US FDA National Center for Toxicological Research, Jefferson, AR.*
KEYWORDS: Cardiovascular System; Pharmaceuticals; microRNA; Tyrosine Kinase Inhibitors

ABSTRACT BODY: Sunitinib (Sutent®) is an FDA-approved multi-kinase inhibitor with activity against vascular endothelial growth factor receptor (VEGFR) 1 and 2, platelet-derived growth factor receptor (PDGFR) α and β, and multiple other receptor kinases. Sunitinib is used to treat gastrointestinal stromal tumors (GIST), advanced renal cell carcinoma (RCC), and pancreatic neuroendocrine tumors (pNET). Despite clinical efficacy, a major concern associated with sunitinib therapy remains the development of cardiac dysfunction, which may include arrhythmia, decreased left ventricle ejection fraction (LVEF), and congestive heart failure. A better understanding of the molecular mechanisms underlying sunitinib-induced cardiotoxicity is needed. Here, we screened human induced pluripotent stem cell (iPSC)-derived cardiomyocytes with clinically-relevant concentrations of sunitinib and measured beating patterns, beating amplitude, and cytotoxicity. Because recent evidence has highlighted the role of microRNAs (miRNAs) in cardiomyocyte function, we then profiled changes in the miRNA transcriptome at corresponding concentrations and timepoints in order to explore potential biomarkers of functional toxicity. Sunitinib caused significant changes (fold-change ≥ 2; $p \leq 0.05$) in over 150 miRNAs, including a subset of miRNAs associated with cardiac function and cardiovascular disease (e.g. miR-133a/b, miR-320a/b, miR-1, miR-125a/b-5p). These data provide clues as to how the miRNA transcriptome reflects cardiomyocyte function following sunitinib exposure, and support further evaluation of miRNA changes as biomarkers for cardiotoxicity.

ABSTRACT FINAL ID: 3874 Poster Board: P566
TITLE: Effects of Physicochemistry of Ambient Particulate Matter on Blood Pressure, Heart Rate, and Heart Rate Variability in Rats

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: T. Cheng[1], H. Chuang[2], Y. Lin[1], C. CK Chou[3], J. Hwang[3]. *[1]National Taiwan University, Taipei, Taiwan; [2]Taipei Medical University, Taipei, Taiwan; [3]Academia Sinica, Taipei, Taiwan.*
KEYWORDS: Inhalation Toxicology; Particulates; Cardiovascular System

ABSTRACT BODY: The mechanisms underlying the effects of the physicochemical characteristics of PM are not well defined. Association between the cardiovascular effects of urban ambient PM constituents was investigated. A real-time non-concentrated PM2.5 exposure system located at Taipei city, an urban area with dominant traffic emissions, was constructed and tested. In the 7 weeks of experimental period, the Wistar-Kyoto rats were exposed to the whole air or HEPA-filtered air for 7 days followed by a wash-out period for 7 days for 4 repeats on a cross over design. The PM2.5 mass and number concentrations, and black carbon (BC) were continuously monitored, and components of PM including organic carbon (OC), elemental carbon (EC), and ions were also determined on a daily basis. Radiotelemetric data were subsequently obtained during the exposure period (PM and control) for analysis, including blood pressure (BP) [systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP)], heart rate (HR) and heart rate viability (HRV). The average concentration of PM2.5 during exposure period was 16.8 μg/m3. Exposure of PM2.5 mass concentration produced a significant reduction of SBP, DBP, MAP and HR ($p<0.05$). However, there was no change in BP and HR caused by PM number concentrations and BC. LnSDNN and LnRMSSD were increased by PM2.5 mass concentration ($p<0.05$), while there was no effect of PM number concentration and BC on HRV. The cardiovascular effects were observed at the beginning of exposure, reached the peak in 24 hours, and remained significant in 72 hours. Alteration in HR was sensitive to OC, EC, Na^+, Cl- and NO3-. Cl- was associated with DBP, MAP, HR, LnSDNN and LnRMSSD, whereas NO3- was correlated to SBP, MAP, HR, LnSDNN and LnRMSSD. Our results showed that mass concentrations were associated with cardiovascular responses in rats, while there was no effects for number and BC. We also found components of PM were correlated with the responses. The exact sources and components of PM responsible for the cardiovascular changes need further study.

ABSTRACT FINAL ID: 3875 Poster Board: P567
TITLE: Evaluation of Batch Variations in Induced Pluripotent Stem Cell Derived-Human Cardiomyocytes

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: L. Pang[1], J. H. Huo[1,2], A. Kamalakar[1], B. Word[1], B. Lyn-Cook[1], X. Yang[3], N. Stockbridge[4]. *[1]Division of Biochemical Toxicology, NCTR/US FDA, Jefferson, AR; [2]Department of Cardiovascular Medicine, First Hospital of Xi'an Jiaotong University, Xi'an, China; [3]Division of Systems Biology, NCTR/US FDA, Jefferson, AR; [4]Division of Cardiovascular and Renal Products, Office of New Drugs, CDER/US FDA, Silver Spring, MD.*
KEYWORDS: Cardiovascular System; Safety Pharmacology; *In Vitro* and Alternatives

ABSTRACT BODY: Drug-induced proarrhythmia is a major safety issue in drug development. Sensitive *in vitro* assays that can predict drug-induced cardiotoxicity in humans have been a focus of toxicology research for the past decade. Recently, induced pluripotent stem cell derived-human cardiomyocytes (iPSC-hCMs) have become a popular model because they largely replicate the electrophysiological behavior of human ventricular cardiomyocytes. "The Heart in the Petri Dish" has been proposed for personalized cardiac drug selection and adverse drug response prediction; however, many procedures are involved in the cardiomyocyte differentiation and purification process, and iPSC-hCMs are in a continuing state of maturation, which may result in large batch-to-batch variations. In this study, we examined the cardiac ion channel gene expression profile and electrophysiological response of three different batches of iCells from Cellular Dynamics International. We found that, based on field potential duration (FPD) data, the three batches of cells had similar sensitivities: the minimal concentration of hERG, sodium, and calcium channel blockers that elicited ± 10% changes in FPD compared to the vehicle control were identical among the three batches of cells; with an Iks blocker, there was a three-fold difference among the batches. Rate correction of FPD with either Bazett's or Fridericia's formula increased the variability. Most of the cardiac ion channel genes were expressed uniformly among three batches of cells, with the exception of CACNA1C, the expression of which was high when the FPD value was low. Careful evaluation of the performance of iPSC-hCMs and data analysis methods is warranted for the use of these cells in regulatory toxicity testing.

ABSTRACT FINAL ID: 3876 Poster Board: P568
TITLE: Calcium Sensitizing Effects on Hemodynamics and Left Ventricular Contractility Via Intravenous Administration in Both Conscious and Anesthetized Conditions in Telemetry Implanted Rats

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KEYWORDS: Cardiovascular System; Safety Pharmacology; Dose-Response; Anesthesia; Pimobendan

ABSTRACT BODY: Early in the drug discovery process it is critical to obtain sufficient pharmacological data while also not overinvesting resources (e.g., time and money) into each compound until a lead candidate is selected for further investigation. Anesthetized preparations offer a lower cost approach to lead compound selection compared to the alternative of investigating animals with chronic instrumentation. One of the greatest difficulties in considering the pharmacological responses during anesthetized preparations is interpreting the confounding effects of anesthesia on critical parameters. The objective of this study was to evaluate the effects of a known calcium sensitizing agent (pimobendan 2 mg/kg) on hemodynamic and left ventricular contractility (dP/dt_{max}) parameters in both conscious and anesthetized rats (n=3 male Sprague Dawley) via intravenous administration. Effects on mean arterial pressure (MAP) were not significant in relation to vehicle administration during anesthetized and conscious conditions. However, left ventricular dP/dt_{max} for the pimobendan group was nearly 66% higher and vehicle was 4% lower during anesthesia and remained significantly different throughout the collection period after cessation of anesthesia. These results demonstrate that the magnitude of change in MAP and dP/dt_{max} parameters from pimobendan administration is maintained in both anesthetized and conscious conditions.

ABSTRACT FINAL ID: 3877 Poster Board: P569
TITLE: Proarrhythmic Effects of Ouabain Continuously Assessed by Automated ECG Analysis in the Telemetered Beagle Dog

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: N. Sadekova[1], K. Norton[1], R. Kaiser[1], A. Mehendale[2], C. Kolin[2], H. Holzgrefe[1]. *[1]Charles River Laboratories, Senneville, QC, Canada; [2]Data Sciences International, St. Paul, MN.*
KEYWORDS: Risk Assessment; Cardiovascular System; Safety Pharmacology; Arrhythmia Detection

ABSTRACT BODY: While automated ECG arrhythmia (ARR) analysis has been possible for several years, extensive characterization of ARR analysis systems has been hampered by the low incidence of ARRs in typical preclinical species. Accordingly, we assessed the dose-dependent proarrhythmic effects of ouabain (O), a cardiac glycoside that rapidly inhibits Na^+/K^+-ATPase, in the isoflurane-anesthetized dog (n=5). O was administered as a 20 µg/kg iv bolus followed by a continuous infusion at 1(n=4) or 2 (n=1) µg/kg/min until ARR onset (119±25.3 µg/kg over 99±25.3 min). ECGs were digitized at 500 Hz and analyzed by Ponemah™ acquisition and analysis software including Data Insights™, a validated ARR detection module (ver. 5.2, SP7, Data Sciences International, St. Paul, MN.). All automated ARR variants were reviewed and verified by a trained operator. For the period preceding ARR onset, ECG intervals were normalized and presented as the % change from the individual predose period to address baseline differences between subjects. Prespecified ARR variants were tabulated for the duration of the experiment and were expressed as the respective sum for all subjects. Prior to ARR onset, O dose-dependently increased the RR, PR, and QRS intervals and decreased the rate-corrected QTcV interval (Van de Water method). RR intervals increased by 9.4, 23.7, 19.4, and 24.5%; PR intervals increased by 5.0, 13.9, 23.3, and 23.8%; QRS increased by -1.2, -1.8, -1.5 and 21.2% and the QTcV decreased by -7.7, -18.7, -20.0 and -20.7% at 40, 60, 80, and 100 µg/kg O, respectively. No ARRs were observed during the predose interval in any subject. ARRs persisted for 99.2±79.3 min prior to acquisition stop. Cumulative prespecified arrhythmias included 2° atrioventicular block (496), premature atrial contractions (841), junctional (2685) and ventricular ectopic beats (8164). The onset of atrial events generally occurred at lower doses than required to induce ventricular ectopy, consistent with the dose-dependent PR prolongation which preceded both QRS increases and ARR onset. The current data demonstrate, for the first time, unambiguous dose-dependence in both the type and frequency of ARRs employing a well-characterized control agent in conjunction with automated ARR analysis, confirming the utility and effectiveness of this analysis software in the drug-development process.

ABSTRACT FINAL ID: 3878 Poster Board: P570
TITLE: Inhibitors of Docosahexaenoic Acid Metabolism, an N-3 Polyunsaturated Fatty Acid, Do Not Block Its Antioxidant Activity

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KEYWORDS: Antioxidants

ABSTRACT BODY: The cardiovascular benefits of n-3 polyunsaturated fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are mediated, in part, by their antioxidant activity; however, the mechanism underlying these benefits have not been elucidated. We hypothesized that cytochrome P450-derived epoxide metabolites of EPA and DHA activate the antioxidant transcription factor, Nuclear factor (erythroid-derived 2)-like 2 (Nrf2). We treated a stable antioxidant response element reporter HepG2 cell line for 24 hr with 1 µM positive control L-Sulforaphane (LS), 30 µM DHA and EPA; 5 and 10 µM DHA peroxidation product 4-hydroxyhexanal (4-HHE), 1 µM EPA P450 epoxide metabolite 17,18-epoxyeicosatetraenoic acid (EEQ) and 1 µM DHA P450 epoxide metabolite 19,20-epoxydocosapentaenoic acid (EDP). Next, Nrf2 activation was assessed in cells treated with 10, 15, 20 and 30 µM DHA. Cells were also treated with inhibitors of cyclooxygenase (1 µM indomethacin, COX1 & 2; 1 µM NS398, COX2), lipoxygenase (20 µM baicalein, 12 & 15 LOX; 5 µM MK886, 5 LOX) and P450 metabolism (150 µM 1-aminobenzotriazole; 10 µM SKF525). While EPA, 4-HHE, 17,18-EEQ and 19,20-EDP failed to activate Nrf2, DHA resulted in a dose-dependent increase with a concentration as low as 15 µM increasing Nrf2 activity. These doses had no effect on cell viability. Interestingly, however, none of the enzyme inhibitors significantly blocked DHA-induced Nrf2 activity, including both P450 inhibitors. These data show that antioxidant activity by n-3 PUFAs via Nrf2 activation is limited to DHA but fail to support our hypothesis that a P450-epoxide metabolite mediates this activity. These data also suggest that non-enzymatic oxidation of DHA to 4-HHE does not contribute to DHA-dependent Nrf2 activation. Future studies will investigate the ability of DHA to activate mitochondrial ROS production, leading to Nrf2 activation. Supported by University of New Mexico, Cardiovascular and Metabolic Disease Signature Program.

ABSTRACT FINAL ID: 3879 Poster Board: P571
TITLE: Comparison of *In Vitro* and Clinical *In Vivo* Effects of Pimobendan A Canine Heart Failure Drug

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KEYWORDS: Safety Pharmacology; Cardiovascular System; Pharmaceuticals; Contractility; Pimobendan

ABSTRACT BODY: Heart failure is a common disease for which new drugs are constantly sought. Critical to the process are higher throughput *in vitro* systems that screen inotropic and lusitropic effects. We have validated the CardioECR instrument (ACEA Biosciences) by comparing contractile effects of pimobendan in human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) with well-known contractile effects in clinical canine studies. Pimobendan exerts inotropic effects by acting as a calcium sensitizer and phosphodiesterase inhibitor. Impedance was measured using iCELL cardiomyocytes2 paced at 0.67 Hz. As many as 12 drugs per day at two replicates/drug can be screened. For analysis we developed custom macros to calculate several contractile parameters including first derivatives (dΩ/dt). For the canine study we instrumented four naïve male Beagle dogs with Data Sciences International (DSI) Physiotel Digital L21 implants equipped with blood pressure, LVP catheters and ECG leads fixed in Lead 2 configuration. DSI Ponemah software was used to collect heart rate, dP/dt max dP/dt min, LVEDP, systemic arterial pressure, QT interval duration and ECG parameters for baseline (2 hours predose) and up to 24 hours post dose. We used 0.1-10 µM pimobendan for the impedance study and 0.1, 0.3 and 1 mg/kg pimobendan for the animal study. The total Cmax obtained with a single oral 0.25 mg/kg dose was 0.012 µM. At 90% PB the free Cmax was 0.0012 µM. Impedance results: at 10 µM pimobendan induced an increase in twitch amplitude (12 ± 1%, mean ± sem), dΩ/dt max (7 ± 2%), twitch area (25 ± 3%) and relaxation to 50% (6 ± 3%) after one hour exposure. The trend was apparent at 1 µM and became statistically significant between 3 -10 µM. Telemetered conscious dog model results: at 1 mg/kg pimobendan produced a mean increase of LV dP/dt max of 83% over a 7-hour period. The changes were statistically significant. The change in LVP dP/dt max correlated with a decrease in the QA interval. No changes were observed in LVEDP. These results support the impedance results. In conclusion, *in vitro* impedance screening of hiPSC-CMs at customary screening concentrations detected the contractility effects of pimobendan.

ABSTRACT FINAL ID: 3880 Poster Board: P572
TITLE: Are You Risking Data Integrity with Social-Housed Telemetry Animals? A Head-to-Head Comparison with Single-Housed Animals

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KEYWORDS: Safety Pharmacology; Telemetry

ABSTRACT BODY: Telemetric evaluations are largely conducted as part of the Safety Pharmacology core battery to satisfy ICH S7A and ICH S7B requirements for assessment of changes in hemodynamic and electrocardiographic measurements, respectively. Such evaluations have allowed for the collection of conscious ambulatory data from unanesthetized animal models. However; historically, acquisition limitations have precluded social housing of animals during the telemetric evaluation period due to signal interference. Technological advances in implantable telemetry devices and supporting hardware now offer benefits of greater signal quality, greater durations of implantation (due to upgraded battery usage and device construction), with the added capacity to offer group housing during the collection period. The current study was conducted to assess the enhancements offered by the PhysioTel Digital (digital) platform. Telemetric data were compared between moxifloxacin-treated animals using each telemetry platform. Both system evaluations were completed using a latin square cross-over dose design. Single-housed animals were used for the evaluation of the PhysioTelD70 (legacy) transmitter, while socially housed animals were used during the evaluation of the digital system. Head-to-head comparison of single vs social housed collections demonstrated remarkably similar results, thus demonstrating absence of compromise in data integrity for social housed animals. While both systems demonstrated equivalent changes in the potential for delayed ventricular repolarization (QTcV = >270 msec; up to ~45 msec from control at 15-16 hrs post dose). The digital telemetry system offers appeal by way of study setup efficiencies, reduced potential for collection error, and extended implant battery life, all while satisfying expectations for group housing of social animals. Under the current study condition, no meaningful differences in cardiovascular parameters were noted between individually and social housed animals. While no contamination issues were observed as a result of the social housing paradigm, considerations should be made to limit potential for contamination between treatment groups, and contamination of control, when evaluating socially housed animals.

ABSTRACT FINAL ID: 3881 Poster Board: P573
TITLE: An *In Vitro* Assay for Assessing Cardiac Contractility Effects of Test Compounds Using the Human Trabeculae Muscle Work Loop Assay

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M. Gharanei[1], R. Wallis[2], M. Bonner[2], A. Linekar[2], M. Babba[2], H. Maddock[1]. [1]*Applied Biological & Exercise Sciences Centre, Coventry University, Coventry, United Kingdom;* [2]*InoCardia Ltd, Coventry, United Kingdom.*
KEYWORDS: Risk Assessment; Pharmaceuticals; *In Vitro* and Alternatives; Predictive Toxicology

ABSTRACT BODY: Drug induced changes in cardiovascular function is a common cause of compound attrition throughout drug development. Although robust assays and procedures have been developed to detect adverse effects on the electrocardiogram, this is not the case for other adverse cardiovascular effects, such as cardiac contractility. Current drug testing paradigms rely on the use of animals which will not detect human specific toxicities. Development of a human heart contractility assay would improve the understanding of the human relevance of nonclinical findings. The Pharmaceutical, Cosmetic, Agrochemical and Regulators require an improved assessment of cardiovascular liability associated with drug/chemical-induced changes in cardiac contractility that is more predictive than existing assays. We have demonstrated that the Work-Loop (WL) cardiac contractility assay is more predictive of human findings than existing assays when using animal tissue. We have expanded this investigation to determine whether the human cardiac WL assay had the potential to provide a more predictive model of heart muscle dynamics. To validate this assay a range of inotropic agents were tested & effective concentrations compared with those known to affect human cardiac contractility. Methods: Trabeculae muscles were isolated from healthy donated human heart tissue & superfused with modified cardiac ringer. Muscle length and stimulation amplitude were optimised to develop maximum isometric developed force. Active WLs were undertaken & net power outputs (PO) calculated. Muscles were perfused in the absence or presence of relevant positive and negative inotropic agents. Results: The human cardiac muscle WL assay predicted inotropic effects at clinically relevant concentrations. The WL assay was able to demonstrate significant inotropy differences across a structural class of 3 positive inotropes (i.e PO increased by 80,104 & 209% respectively). Conclusion: The human cardiac WL assay is a new approach to the detection of drug/chemical effects on cardiac contractility, providing a superior predictivity of inotropy assessment & importantly identifying inotropy risk at clinically relevant concentrations. The WL assay for the first time can be used for assessing inotropic risk of drug/chemical-induced effects on contractility.

ABSTRACT FINAL ID: 3882 Poster Board: P574
TITLE: Diglycolic Acid Treatment Affects iPSC Cardiomyocyte Beating

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: K. Bailey, H. Toomer, R. Sprando, R. Calvert. *US FDA CFSAN, Laurel, MD.*
KEYWORDS: Cardiovascular System; Food Safety/Nutrition; *In Vitro* and Alternatives; Stem Cells; Diglycolic Acid

ABSTRACT BODY: Renal toxicity of diglycolic acid (DGA) has been described, but the effect of DGA on other organs has not been closely examined. An in house *in vivo* study detected elevated creatine kinase levels in rats treated with DGA, suggesting that damage may be occurring in the heart or skeletal muscle. This research focused on evaluating cardiac effects of DGA by treating human iPSC beating cardiomyocytes with increasing concentrations of DGA in media at pH 7.8 for 30 minutes. Cardiomyocyte beat rate (BPM) as well as estimates of relative cytoplasmic Ca^{2+} concentrations were measured using a fluorescent dye that detects Ca^{2+} in the cytoplasm during myocyte contraction. After 30 minutes of DGA treatment, BPM remained near control rates at concentrations of 2.5 mM and 5 mM. BPM decreased significantly at concentrations of 7.5 mM DGA and higher. No beats were detected in cells treated with 50 mM DGA. Peak amplitude was significantly decreased in all groups treated with DGA for 30 minutes, including concentrations below 5 mM where BPM did not change. Interestingly, BPM returned to control levels in cells previously treated with up to 25 mM DGA after replacing DGA containing media with fresh control media for 30 minutes. Though BPM returned to control levels, amplitude remained significantly lower in all cells except for those treated with 2.5 mM DGA. No beats were detected after fresh media replacement in cells exposed to 50 mM DGA. These *in vitro* findings indicating DGA cardiotoxicity in iPSCs correlate with the preliminary findings in our *in vivo* study where rats treated with 300 mg/kg of DGA developed abnormal cardiac pathology. The changes in beat rate and Ca^{2+} signal amplitude of the beating iPSC suggest that DGA affects Ca^{2+} flux in cardiac cells and that acute toxic effects of DGA are partially reversible.

ABSTRACT FINAL ID: 3883 Poster Board: P575
TITLE: Regulation of LPP3 in ROS Related Cardiac Injuries

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KEYWORDS: Cardiovascular System; Oxidative Injury

ABSTRACT BODY: Generation of reactive oxygen species (ROS) has been implicated in myocardial infarction (MI), stroke and sudden cardiac death. Mitochondrial respiration is a major source of ROS production and lipids regulate mitochondrial oxidative metabolism and homeostasis through effects on mitochondrial fusion and fission and on the activity of mitochondrial membrane proteins. Lipid phosphate phosphatases (LPPs) control the conversion of bioactive lipid phosphates to their dephosphorylated counterparts. These include phosphatidic acid (PA), and lysophosphatidic acid (LPA). Oxidative stress was identified to transactivate microRNA-92a, which is a negative regulator of LPP3. We found that LPP3 expression was markedly down regulated in ischemic regions after ischemia/reperfusion (I/R) injury. We observed a similar trend in the myocardium from patients with acute MI at 24h. Our *in vitro* studies indicate that overexpression of LPP3 protects the cardiomyocyte against ROS-induced cardiac injury and reduction of LPP3 by conditional specific cardiac knockout of the LPP3 gene in mice increases cardiac dysfunction and mortality. These mice showed early mortality ~8 months due to cardiac dysfunction. Whereas lack of LPP1 or LPP2 (global knockouts) didn't had any obvious phenotypic effect. Lack of LPP3 accounts for less than 10 percent activity in cardiomyocytes purified from the Myh6-LPP3$^{fl/fl\ \Delta}$, which augments our previous finding that the other two LPP isoforms have a lesser role in the cardiovascular system. Blood pressure was similar in LPP3$^{fl/fl}$ (96 ± 9 mmHg; n = 19) and Myh6-LPP3$^{fl/fl\ \Delta}$ (92 ± 7 mmHg; n = 19), although heart rates were significantly higher in Myh6-LPP3$^{fl/fl\ \Delta}$ 3-month old mice (642 ± 21 bpm, compared to LPP3$^{fl/fl}$ with 600± 17 bpm; P<0.001). Knockdown of LPP3 enhanced cardiomyocyte hypertrophy induced by LPA based on analysis of sarcomere organization, cell surface area, levels of fetal genes ANP and BNP, and ANF release from nuclei, which are hallmarks of cardiomyocyte hypertrophy, indicating that LPP3 negatively regulates cardiac dysfunction caused by LPA. We observed an increase in ATX levels accompanied by a decrease in LPP3 expression following infarction in the myocardium of LPP3$^{fl/fl}$ mice. Infarction induced expression of IL-6 and KC, were 3 ± 0.5-fold and 2 ± 0.6-fold higher, respectively, in Myh6-LPP3$^{fl/fl\ \Delta}$ mice. Analysis of plasma by cytokine antibody array confirmed the elevation in IL-6 and KC, whereas G-CSF and sICAM-1 appeared lower than in LPP3$^{fl/fl}$.

ABSTRACT FINAL ID: 3884 Poster Board: P576
TITLE: High-Throughput Microfluidic Platform for Culture of 3D Kidney Tissue Models

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: M. Vormann, B. Trietsch, R. van Vught, J. Joore, P. Vulto, H. Lanz.
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KEYWORDS: Kidney; Alternatives to Animal Testing; Predictive Toxicology

ABSTRACT BODY: Drug toxicity remains a major issue in drug discovery and stresses the need for better predictive models. Here, we describe the development of a perfused renal proximal tubule cell (RPTC) model in Mimetas' OrganoPlates™ [1,2] to predict kidney toxicity. The OrganoPlate™ is a microfluidic platform, which enables high-throughput culture of boundary tissues in miniaturized organ models. In OrganoPlates™, extracellular matrix (ECM) gels can be freely patterned in microchambers through the use of PhaseGuide™ technology. PhaseGuides™ (capillary pressure barriers) define channels within microchambers that can be used for extracellular matrix deposition or medium perfusion. The microfluidic channel dimensions not only allow solid tissue and barrier formation, but also perfused tubular epithelial vessel structures can be grown in the medium perfusion channel. The goal of developing a perfused RPTC model is to reconstruct viable and leak-tight boundaries for performing cytotoxicity and metabolism assays, as well as transport and efficacy studies. The proximal tubule cell line LLC-PK1 was grown against an ECM gel in a three channel OrganoPlate™, yielding access to both the apical and basal side. Confocal imaging revealed that the cells formed a tubular structure. The LLC-PK1 3D tubule stained positive for ZO-1 (tight junctions) and acetylated tubulin (polarization marker). Interestingly, cilia pointed in the direction of the lumen of tubules. The tightness of LLC-PK1 boundaries was assessed by diffusion of FITC-dextran dye added to the lumen of the tubule. Boundaries of LLC-PK1 showed leak-tight barriers and were maintained for several days. Furthermore, first experiments on primary human RPTEC resulted in partly leak tight tubules. The 3D proximal tubules cultured in the OrganoPlate™ are suitable for high-throughput toxicity screening, transport studies by real time imaging of transport and leakage, and complex co-culture models to recreate an *in vivo*-like microenvironment. 1.Trietsch, S. J., Israëls, G. D., Joore, J., Hankemeier, T. & Vulto, P. Microfluidic titer plate for stratified 3D cell culture. Lab Chip 13, 3548-54 (2013). 2.Moreno, E. L. et al. Differentiation of neuroepithelial stem cells into functional dopaminergic neurons in 3D microfluidic cell culture. Lab Chip 15, 2419-28 (2015).

ABSTRACT FINAL ID: 3885 Poster Board: P577
TITLE: Subchronic Exposure to Bismuth Trioxide Nanoparticles Causes Early Kidney Damage in Wistar Rats

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KEYWORDS: Kidney; Biomarkers; Nanoparticles; Np-Bi2O3

ABSTRACT BODY: The physicochemical characteristics of nanoparticles, and therefore their biological effects and potential toxicity, depends on the materials they are made from and their functionality. Bismuth trioxide nanoparticles (Np-Bi2O3) are studied for different applications including catalysis, X-ray imaging, and in sensors technology amongst others; however, the toxic health implications involved have not yet been entirely explored. We are interested in studying the Np-Bi2O3 toxic effect in the kidney. Np-Bi2O3 were characterized by Scanning Electron Microscopy (SEM) and the hydrodynamic size by dynamic light scatting (LDS). Male Wistar rats between 200-250g of body weight were orally exposed to 50 mg/kg/day of Np-Bi2O3 in suspension stabilized with albumin at 2.5 mg/ml for 28 days, and a control group received a sham suspension. During the exposure period, urine and blood were collected at 7, 14, 21 and 28 days. The urine samples were used for creatinine, osmolality and early kidney damage biomarkers (β-2-microglobulin, neutrophil gelatinase-associated lipocalin and Cystatin C) determinations. Creatinine and osmolality were measured in plasma. The SEM Np-Bi2O3 showed a primary size of 149.22 nmand a predominant spherical shape. The atomic spectrometry indicated that bismuth content in the Np-Bi2O3 suspension was70.18%. The hydrodynamic size measured after 15 minutes of sonication was 284.3 nm (Z-Average) and the zeta potential -10.7. We did not observe significant differencesin body weight, relative kidney weight, water intake or osmolar clearance between the exposed and control groups. Interestingly, we observed a significant increase in the glomerular filtration rate on days 21 and 28 as well as subtle changes in the urinary expression of early kidney damage biomarkers. In conclusion, our data suggests that exposure to Np-Bi2O3 is able to induce early tubular alterations, possibly through renal endothelial damages.
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ABSTRACT FINAL ID: 3886 Poster Board: P578
TITLE: Establishment of a Drug-Induced, Bile Acid-Dependent Hepatotoxicity Model Using HepaRG Cells

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KEYWORDS: Hepatocytes; Cell Lines, Transfected

ABSTRACT BODY: Bile acid (BA) retention within hepatocytes is an underlying mechanism of cholestatic drug-induced liver injury (DILI). We previously developed an assay employing sandwich-cultured human hepatocytes (SCHHs) to evaluate drug-induced hepatocyte toxicity accompanying intracellular BA accumulation. However, shortcomings are commonly associated with the use of primary human hepatocytes (e.g., limited availability, lot-to-lot variability, and high cost). To overcome this problem, the human hepatic stem cell line, HepaRG, was recently developed and revealed to stably express mRNAs for metabolic enzymes, transporters, and nuclear transcription factors, in contrast to other human hepatoma cell lines (e.g., HepG2). In the current study, we examined whether HepaRG cells might also be applicable to our assay system by comparing with SCHHs as a positive control and HepG2 cells as a negative control. SCHHs, HepaRG and HepG2 cells were exposed to cyclosporine A (CsA) (10 μM) or other test drugs (50 μM) with or without human serum components of BAs for 24h, and cytotoxicity was assessed. The inhibitory actions against the biliary excretion index (BEI) of [^{3}H]taurocholic acid (TC), intracellular amount of BAs, and mRNA expressions of BA transporters in each cell type were examined. The mRNA expression levels of BA uptake and efflux transporters were lower in HepaRG cells than in SCHHs, but higher than in HepG2 cells. CsA, a prototypical inhibitor of BA efflux transporters, was cytotoxic toward HepaRG cells in the presence of BAs. Consistently, CsA also reduced the BEI of [^{3}H]TC from 38.5% to 19.2% and increased intracellular amount of BAs in HepaRG cells. These effects were not observed in HepG2 cells. Overall toxicity response of HepaRG cells to 22 selected drugs in the presence of BAs was more similar to SCHHs than HepG2 cells. However, the prediction accuracy for cholestatic DILI risk was relatively poor for HepaRG cells compared to SCHHs, which was likely attributable to the false negative prediction for particular drugs in HepaRG cells. HepaRG cells are potentially applicable to our BA-dependent toxicity assay system. As a preclinical screening tool, however, further improvements are required for HepaRG cells to increase the prediction accuracy.

ABSTRACT FINAL ID: 3887 Poster Board: P579
TITLE: Development of a Mechanism-Based Evaluation Model for the Assessment of Cancer Risk Associated with Hepatic Hypertrophy

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KEYWORDS: Liver; Carcinogenesis; Receptor; Toxicogenomics

ABSTRACT BODY: Chemical exposure often results in increased liver weight accompanied by hepatocellular hypertrophy and/or cell proliferation. While most of these changes are considered as adaptive responses, some are considered to be associated with carcinogenesis. In the present study, we focused on the relationship between hypertrophy and hypertrophic carcinogenicity and developed a predictive model that can be used for risk assessment. We first analyzed microarray data of 134 chemicals (62 that induced hypertrophy) from the toxicogenomics database TG-GATEs that developed by Japanese Toxicogenomics Project, and extracted specific genes by Mann-Whitney U-test ($P < 0.01$). After applying logistic regression analysis, we selected 30 and 40 marker genes to develop predictive models that provided 93% and 100% concordance with hypertrophy and hypertrophic carcinogenesis by 10-fold cross-validation, respectively. Pathway analysis suggested that besides previously reported nuclear receptor (NR) activation, inflammatory events and genes (e.g., *Cidea* and *Esm1*) were expressed in the tumor necrosis factor (TNF)-α-related pathway indicating the involvement of a novel mode of action in hypertrophic carcinogenesis. Since toxicogenomic approach cannot identify the types of NR, which are important for human relevance, we then subjected the chemicals that were predicted as carcinogens to NR reporter gene assays (AhR, CAR, PXR, and PPARα) *in vitro*. Four chemicals were identified as PPARα agonists (e.g., clofibrate) that were specifically activated by rodents, indicating that these compounds presented no cancer risk to humans. In conclusion, our predictive model should prove useful in assessing the hypertrophic carcinogenic potential in rodents, and when combined with NR assays, it will be a promising model to evaluate risks to humans.

ABSTRACT FINAL ID: 3888 Poster Board: P580
TITLE: Automated Cyp Inhibition (IC_{50} Shift) Assays with LC-MS/MS Cocktail Analysis

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KEYWORDS: Cytochrome P450; Metabolism; Liver

ABSTRACT BODY: Inhibition of CYP remains integral to the DDI assessment of potential new drugs. Recently re-developed CYP inhibition assays to use of automated liquid handling, low microsomal protein concentrations, short incubation times and multiple IC_{50} curves to assess both TDI and MBI (IC_{50} shift analysis) using cocktail LC-MS analysis thereby markedly reducing costs. CYP inhibition assays were conducted using a Hamilton STAR automated liquid handling system to conduct the incubations of CYP substrates with pooled human liver microsomes. The stopped incubations were then combined for analysis using two separate LC-MS/MS methods. The substrates assayed were phenacetin (CYP1A2), bupropion (CYP2B6), amodiaquine (CYP2C8), diclofenac (CYP2C9), mephenytoin (CYP2C19), dextromethorphan (CYP2D6), testosterone (CYP3A4/5) and midazolam (CYP3A4/5) at a concentration of K_m for the relevant CYP enzyme. Incubations were conducted using both reversible and time-dependent chemical inhibitors namely α-napthaflavone (NAP), furafylline (FUR) (CYP1A2), sertraline (SET), thioTEPA (TT) (CYP2B6), montelukast (MON), gemfibrozil glucuronide (GG) (CYP2C8), sulphaphenazole (SUL), teinilic acid (TNA) (CYP2C9), benzylnirvanol (BEN), S-fluoxetine (FLU) (CYP2C19), quinidine (QUI), paroxetine (PAR) (CYP2D6), ketoconazole (KET)and troleandomycin (TAO) (CYP3A4/5). Three pre-incubation regimes were used: 0-minute and 30 minute pre-incubation in the presence of NADPH (to assess TDI) and 30 minute pre-incubation in the absence of NADPH (to assess MBI). Incubations contained microsomal protein concentrations ranging from 0.025-0.2 mg/mL and were incubated for 10 minutes or 45 minutes (mephenytoin only). The inhibitors MON, SUL, BEN, QUI and KET had IC_{50} shift values ranging between 0.7 and 1.3, confirming that these were reversible inhibitors of their respective CYP enzymes. The inhibitors FUR, SET, TT, GG, TNA, FLU, PAR and TAO had IC_{50} shifts ranging between 5.8 and 172, confirming that these were time dependent inhibitors of their respective CYP enzymes.

ABSTRACT FINAL ID: 3889 Poster Board: P581
TITLE: Suppression of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 Activity by a Cocktail of Inflammatory Cytokines

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J. Foster, T. Ormsby, G. Webber. *Metabolism, Envigo CRS Ltd, Cambridge, United Kingdom.*
KEYWORDS: Cytochrome P450; Cytokines; Metabolism

ABSTRACT BODY: Biological medicines comprise an increasing proportion of the drug development pipeline. In terms of potential hepatotoxicity assessment, potential down-regulation of cytochrome P450 (CYP) enzymes mediated by the release of inflammatory cytokines in response to a biological medicine is one area of concern. In this study, we assessed whether metabolic CYP activity is down-regulated in human hepatocytes following exposure to cytokines. Human hepatocytes from 3 individual donors (Hu4197, Hu8123 and Hu8125) were cultured in the presence of ranging concentrations of cytokine cocktails (CC) containing interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). Following either 24 or 48 hours the cytokine cocktails were removed and the CYP specific substrates: phenacetin (CYP1A2), tolbutamide (CYP2C9), mephenytoin (CYP2C19), bufuralol (CYP2D6) and midazolam (CYP3A4/5) were added. The samples were analysed for acetaminophen, 4-hydroxytolbutamide, 4-hydroxymephenytoin, 4-hydroxybufuralol and 1-hydroxymidazolam, respectively. CYP2C9, CYP2D6 and CYP2C19 were minimally or not suppressed by CCC (<20%). CYP2C19 and CYP1A2 were suppressed up to 30% and 53% by CCC, respectively. CYP suppression was cytokine concentration dependent with the greatest suppression observed following treatment with CCB. Maximal observed suppression of CYP1A2 activity was 96%, 90% and 73% in donors Hu4197, Hu8123 and Hu8125, respectively. Maximal observed suppression of CYP2C9 activity was 69%, 73% and 44% in donors Hu4197, Hu8123 and Hu8125, respectively. Maximal observed suppression of CYP2C19 activity was 47%, 69% and 32% in donors Hu4197, Hu8123 and Hu8125, respectively. Maximal observed suppression of CYP2D6 activity was 61% and 69% in donors Hu4197 and Hu8123, respectively. Maximal observed suppression of CYP3A4/5 activity was 71% and 54% in donors Hu4197 and Hu8123, respectively. Donor Hu8125 showed no suppression of CYP2D6 or CYP3A4/5 activities, Overall, these data show that exposure to cytokines can reduce CYP activity in human hepatocytes.

ABSTRACT FINAL ID: 3890 Poster Board: P582
TITLE: An Integrated Approach to More Accurately Assess Cholestatic Liability of Drugs

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: E. Novik[1], C. Cho[1], E. Pludwinski[1], A. Parekh[1], A. Shrirao[1], M. Warren[2]. *[1]Hurel Corporation, North Brunswick, NJ; [2]Optivia Biotechnology, Menlo Park, CA.*
KEYWORDS: Reactive Intermediate; Biotransformation and Toxicokinetics; Biliary Excretion

ABSTRACT BODY: Drug-induced interference with normal bile flow can range from relatively mild cholestasis to much more severe hepatocellular injury, leading to drug-induced liver injury (DILI). Identifying drug candidates with the potential to cause cholestatic DILI as early as possible in the development pathway is of paramount importance, and having tools available to conduct early studies to retain beneficial pharmaceutical properties while minimizing DILI risk of those candidates could prove invaluable. Here, we have developed an integrated approach for assessing the DILI potential of drugs by combining two assay systems: a recombinant cell based system expressing key transporters involved in drug uptake and bile flow, and a holistic primary hepatocyte co-culture model which has been shown to more accurately predict metabolite generation, and hepatotoxicity potential of drugs. Using the first system, a panel of drugs known to have hepatic liabilities was tested against MDCK-II cells co-transfected with OATP1B1, NTCP, and BSEP transporters. Several of these drugs associated with cholestasis, but not necessarily hepatotoxic, were shown to inhibit bile salt uptake transporters (NTCP, OATP1B1), which would result in decreased bile flow. Drugs like rifampicin, which are known to be hepatotoxic, inhibited the bile salt efflux transporter (BSEP) but not the uptake transporter (NTCP), resulting in high cellular retention of bile acids, which could lead to cell death due to their detergent-like properties. Using the second system, a selection of drugs was tested in the Hurel hepatocyte model to assess toxicity in the absence and in the presence of a mixture of bile acids. Drugs such as ritonavir and cyclosporine were found to increase hepatotoxicity in the presence of bile acids. Drugs which were found to not increase cellular retention of bile acids in the first system, like erythromycin, showed no increase in hepatotoxicity in the presence of bile acids in the second system. Unlike transfected cell lines, the primary hepatocytes are capable of addressing potential toxicity due to drug metabolites and their longer term effects on bile acid accumulation. If bile acid enhanced toxicity arises due to a metabolite, the mechanism of that toxicity can then be further investigated using the first system. Combining these two assay systems enables a novel and powerful approach to assess the DILI potential of drugs and their metabolites.

ABSTRACT FINAL ID: 3891 Poster Board: P583
TITLE: Individual Variations of MDR3 and BSEP Activity in Human Hepatocytes

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KEYWORDS: Hepatocytes; Liver; Biliary Excretion; BSEP, MDR3

ABSTRACT BODY: Hepatic multidrug resistance protein 3 (MDR3, ABCB4) and bile salt export pump protein (BSEP, ABCB11) are primarily responsible for biliary secretion of phosphatidylcholine and bile salts, respectively. Loss-of-function mutations of either BSEP or MDR3 cause severe liver injury, leading to hepatic failure and in some cases death. Accumulated evidences in the past years indicate that inhibition of MDR3 and BSEP activities is associated with DILI. Further, abnormal function of these transporters may predispose individuals to drug-induced liver injuries (DILI). As of now, individual variation of MDR3 and BSEP activities in human hepatocytes from different donors has not been reported. In this study, we evaluated individual variations in MDR3 and BSEP activities in primary human hepatocytes, the "gold standard" for *in vitro* hepatotoxicity studies. MDR3 and BSEP activities were measured using novel physiologically relevant assays developed in our laboratory. The MDR3 assay involves *in situ* biosynthesis and specific LC-MS/MS determination of deuterium labeled phosphatidylcholine, while the BSEP assay involves *in situ* biosynthesis of bile salts from precursor bile acids coupled with specific LC-MS/MS determination of transported bile salts. Individual variability of MDR3 and BSEP activities in hepatocytes was evaluated from >12 individual human donors. The activity of MDR3 or BSEP was found to vary by approximately 10-fold among these individuals. The hepatocytes from some donors showed substantially lower activity for either MDR3 or BSEP, suggesting that they may be predisposed to DILI. No apparent correlation between MDR3 and BSEP activity was observed. Gene expression of MDR3 and BSEP is being investigated to understand if the functional variation is due to gene expression.

ABSTRACT FINAL ID: 3892 Poster Board: P584
TITLE: Evaluation of BDE-47 to Potentiate Hepatic Steatosis in Combination with Lipid Challenge

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KEYWORDS: Undergraduate Student; Lipids; Polychlorinated Biphenyls; Liver; BDE-47

ABSTRACT BODY: Flame retardants, such as polybrominated diphenyl ethers (PBDEs), are chemical compounds added to materials such as, textiles, plastics, wire insulation, and automobile to delay the onset of fire. They are widely used for industrial purposes and household materials. PBDEs have become prevalent environmental pollutants, with bioaccumulative properties. In studies using human cadaver tissue, liver is one of the organs in which PBDEs concentrate. In this preliminary study, the purpose was to investigate whether a ubiquitous PBDE, 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) could induce hepatic lipid accumulation in HepG2 cells, as well as, in mice fed a normal or moderately high fat diet. HepG2s were treated with vehicle (0.01% DMSO) or BDE-47 (0.1 nm-25 µm) alone or in combination with oleic acid for 48 hours in a low glucose phenol-red free media. Triglycerides were quantified from the lipid extracts and Oil Red O stains were performed. BDE-47 (0.1 nm) approximately doubled total cellular lipid and triglyceride content, but did not affect OA-induced cellular lipid accumulation. Data will be presented from an ongoing study in which adult male C57BL/6 mice have been fed BDE-47 (0.003% in 10% kcal or 45% kcal high fat diets) for approximately 8 weeks. Markers of adiposity, insulin resistance, and steatosis are currently being evaluated. Thus far, our preliminary data suggest that BDE-47 possesses a lipid modulating effect in HepG2 cells. In addition, data evaluating this effect in an *in vivo* model of BDE-47 exposure on markers of NAFLD will be presented.

ABSTRACT FINAL ID: 3893 Poster Board: P585
TITLE: Evaluation of Bone Healing, Biocompatibility, and Safety with a Photodynamic Bone Stabilization System in Rabbits

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KEYWORDS: Bone

ABSTRACT BODY: Bone healing, biocompatibility, and safety employing a photodynamic bone stabilization system (PBSS), comprised of an inflatable balloon filled with photopolymerizable liquid monomer, was evaluated. Radiologic and histopathologic assessment showed abundant bone healing and progressive callus remodeling over 6 months with PBSS implants in fenestrated femoral cortices of New Zealand white rabbits. In additional rabbits, PBSS implants in brushed and saline-aspirated femoral intramedullary spaces elicited no adverse, local or systemic responses and displayed similar biocompatibility to K-wires in contralateral femurs up to 1 year post-implant. Simulated clinical failures up to 1 year were performed in other rabbits with intramedullary space exposure to PBSS components: liquid monomer or light hardened polymer. PBSS monomer leaked into intramedullary spaces were remediated or negated by light polymerization, resulting in histopathology indistinguishable from sham procedures. PBSS polymerized material displayed cortical bone and vasculature effects comparable to mechanical disruption of the endosteum. In a clinically unlikely scenario with no remediation or polymerization, a high dose monomer injection resulted in marked necrosis of cortical bone, as well as associated vasculature, endosteum, and bone marrow. Overall, when polymerized and hardened within bone intramedullary spaces, this light curable monomer system may provide a safe and effective method for fracture stabilization.

ABSTRACT FINAL ID: 3894 Poster Board: P586
TITLE: Risk Assessment of Elastomeric Infusion Pumps

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KEYWORDS: Risk Assessment; Exposure Assessment; Safety Evaluation

ABSTRACT BODY: The human health risk assessment of an elastomeric infusion pump is described herein. In accordance with ISO 10993 Guidelines for Biological Evaluation of Medical Devices and the intended use, the infusion pump is categorized as an externally communicating device with indirect blood contact or tissue/bone/dentin contact for prolonged patient contact duration of up to 30 days. Biocompatibility and material characterization studies were conducted on the infusion pump. Gas Chromatography/Mass Spectrometry (GC/MS) was utilized to test for volatiles and Gel Permeation Chromatography (GPC) for molecular weight distribution. Extractables were isolated by exhaustive extraction with purified water, isopropyl alcohol, and hexane. Non-Volatile Residue (NVR), Infrared analysis (FTIR), GC/MS, High Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS), Inductively Coupled Plasma Spectroscopy (ICP), and GPC were used to characterize the extracted residue and the highest level of non-volatile residue was determined. The study isolated 71 unique compounds from the infusion pump. Of these, 60 were identified in the initial material characterization study. The material characterization study was repeated on concentrated extracts using a more sensitive high resolution LC/MS/MS with two ionization sources and an LC/FTIR which provided enough information to identify 10 of the 11 remaining compounds. A detailed risk assessment was then conducted to substantiate the safety of the identified and unidentified compounds using the threshold of toxicological concern (TTC) approach. In biocompatibility studies, the infusion pump was shown to be non-cytotoxic, non-sensitizing, non-irritating, systemically non-toxic, non-pyrogenic, non-genotoxic, and non-hemolytic. Based on results of the biocompatibility and material characterization studies, and the intended use of the device, the toxicological risk of the elastomeric infusion pump is negligible and deemed acceptable for use.

ABSTRACT FINAL ID: 3895 Poster Board: P587
TITLE: Development, Optimization and Validation of an *In Vitro* Skin Irritation Test for Medical Devices Using the Reconstructed Human Tissue Model Epiderm

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KEYWORDS: Alternatives to Animal Testing; Safety Evaluation; Risk Assessment; Medical Devices

ABSTRACT BODY: Assessment of dermal irritation is an essential component of the safety evaluation of medical devices. Reconstructed human epidermis (RhE) models have replaced rabbit skin irritation testing for neat chemicals (OECD TG 439). However, medical device extracts are dilute solutions with low irritation potential, therefore validated RhE-methods needed to be modified to reflect the needs of ISO 10993. A protocol employing RhE EpiDerm was optimized in 2013 using known irritants and spiked polymers extracts (Casas et al., TIV, 2013). In 2014 a second laboratory assessed the transferability of the assay. Two additional exposure times were tested along with other medical device materials. After the successful transfer and standardization of the protocol, nine EU and USA laboratories were trained in the use of the protocol in preparation for a validation study. All laboratories produced data that was nearly 100% in agreement with predictions for the selected references. Two of the laboratories performed additional tests with heat-pressed PVC sheets spiked with Genapol X-080 (Y-4 polymer), Vicryl suture, and polymers spiked with heptanoic acid and sodium dodecyl sulfate. All materials were extracted for 24 or 72 hours in both saline and sesame oil at 37°C. Significant irritation responses were detected for Y-4 under all conditions. These results were consistent with those reported by other research groups involved in the upcoming validation study. Vicryl suture was negative and spiked polymers were either positive or negative depending on the extraction solvent. Therefore we conclude that a modified RhE skin irritation assay has the ability to address the skin irritation potential of medical devices, however, standardization and focus on technical issues is essential for accurate prediction. A round robin validation study of *in vitro* skin irritation testing for the assessment of medical devices extracts is beginning in March 2016.

ABSTRACT FINAL ID: 3896 Poster Board: P588
TITLE: Evaluation of Immune Stimulation following Exposure to Metal Particles and Ions Using the Mouse Popliteal Lymph Node Assay

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: B. Tvermoes[1], K. Unice[2], B. Winans[3], M. Kovochich[4], W. Christian[2], E. Donovan[3], B. Finley[3], I. Kimber[5], D. Paustenbach[3]. *[1]Cardno ChemRisk, Boulder, CO; [2]Cardno ChemRisk, Pittsburgh, PA; [3]Cardno ChemRisk, San Francisco, CA; [4]Cardno ChemRisk, Aliso Viejo, CA; [5]University of Manchester, Manchester, United Kingdom.*
KEYWORDS: Metals; Particles, Hypersensitivity; Lymph Node Cells Activation

ABSTRACT BODY: Cobalt-chromium (CoCr) containing hip implants have been used since the 1930s. It has been hypothesized that wear debris released from CoCr hip implants may lead to a localized metal sensitization response, and subsequent revisions, in some metal-on-metal (MoM) hip implant patients. The objective of this preliminary study was to evaluate the threshold for immune stimulation following exposure to metal particles and ions that are representative of low-wear, well-functioning CoCr MoM hip implants. For well-functioning MoM hip implants the majority of the wear debris is composed of oxidized Cr nanoparticles. The popliteal lymph node assay (PLNA) was used to assess immune responses in BALB/c mice following treatment with chromium-oxide (Cr_2O_3) particles, metal salts ($CoCl_2$, $CrCl_3$, and $NiCl_2$), or Cr_2O_3 particles together with metal salts at exposure doses representing approximately 10 days, 20 years, and 40 years of normal implant wear. The mice were injected subcutaneously (50 µL) into the right hind foot with the test article or the vehicle control. The proliferative response of the primary draining lymph node cells was measured 4 days after treatment and stimulation indices (SI) were derived relative to vehicle controls. The PLNA was negative ($SI < 3$) for all Cr_2O_3 particle doses and was also negative at the lowest dose (10 days of normal implant wear) of the metal salt mixture and Cr_2O_3 particles with metal salts. The PLNA was positive ($SI \geq 3$) at the highest two doses (20 and 40 years of normal implant wear) of the metal salt mixture and the Cr_2O_3 particles with metal salts, and the relative percentage of B220+ B cells was also increased in these groups. Taken together, particles representative of low wear, well-functioning hip implants did not evoke an immune response when administered alone in the PLNA. Further, no immune activation was observed at doses of the metal salt mixture and the Cr_2O_3 particles plus metal salts representing 10 days of normal wear. At doses equivalent to approximately 20 and 40 years of normal implant wear administered in a single dose, there was evidence of immune activation that may or may not have been related to allergic sensitization. Additional work involving repeated doses is needed to confirm these observations.

ABSTRACT FINAL ID: 3897 Poster Board: P589
TITLE: *in Vitro* Permeation of Latanoprost Eye Drop Formulations in the 3-Dimensional Normal Human Corneal Tissue Model

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KEYWORDS: *In Vitro* and Alternatives; Alternatives to Animal Testing; Cell Culture; Ocular Drug Absorption

ABSTRACT BODY: Permeation of topically applied ocular drugs occurs predominantly through the cornea and therefore absorption studies using corneal tissues play a critical role in ocular drug formulation. The purpose of the study was to evaluate the permeation of Latanoprost eye drops through *in vitro* reconstructed normal human corneal tissue model. Seven different formulations of Latanoprost eye drops were tested for ocular permeation using a reconstructed corneal tissue model (EpiCorneal). 50-100 µl of eye drop formulations were applied topically onto the EpiCorneal tissue surface and incubated at standard cell culture conditions (SCC, 37°C, 5% CO2). At permeation times of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0 and 12.0 hrs, the tissues were moved into new wells filled with receptor medium (Krebs Ringer Buffer, pH 7.4) and returned into SCC. The samples were collected and analyzed with a fully-validated HPLC-PDA method for Latanoprost acid determination. Tissue integrity (Lucifer Yellow leakage assay) and tissue viability (MTT assay) were determined after the permeation study. For each formulation, plots of the cumulative % of Latanoprost acid that permeated through the tissue versus time were constructed. From the steady state flux region of the plot (<4 hrs for most formulations) the Papp was calculated. Xalatan eye drops (containing 0.02% BAC solution) had the fastest permeation rate (Papp=8.81 cm·s-1) while Monoprost (preservative-free) had the lowest permeation rate (Papp=1.15 cm·s-1). Formulations containing Poloxamer 407 had higher Papp (6.05 and 6.27) when compared to formulations without surfactants (1.69 to 2.57). Tissue integrity and viability were maintained in all experiments as evidenced by Lucifer Yellow and MTT results. The EpiCorneal tissue demonstrated very high reproducibility and presented a permeation profile of different formulations similar to *in vivo*.

ABSTRACT FINAL ID: 3898 Poster Board: P590
TITLE: Toxicity Evaluation of Benzo[a]pyrene and Naphthalene Metabolites in HepG2 Cells

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: S. Muthusamy[1,2], C. Peng[1,2], J. Ng[1,2]. [1]*ENTOX, The University of Queensland, Brisbane, Australia;* [2]*CRC CARE, Newcastle University, Callaghan, Australia.*
KEYWORDS: Polycyclic Aromatic Hydrocarbons; Metabolic Activation; Genetic Toxicology; Oxidative Stress, *In Vitro* Bioassays

ABSTRACT BODY: Poly aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants. PAHs like benzo[a]pyrene (B[a]P) and naphthalene require metabolic activation to elicit their toxicity. Reactive metabolites of these PAHs play an important role in toxic effects. In this study, we have characterized the toxicity of four B[a]P metabolites namely B[a]P-4,5-diol, B[a]P-7,8-diol, B[a]P-7,8-dione and benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BDPE) and two naphthalene metabolites, 1-hydroxy- and 2-hydroxy-naphthalene respectively. These metabolites were evaluated for cytotoxicity (MTS assay), oxidative stress response (Nrf2 induction in ARE (antioxidant response element)-reporter HepG2 cells) and genotoxicity (flow cytometry based micronucleus test) in HepG2 cells. The metabolites of naphthalene were found to be more toxic (cytotoxicity) to HepG2 cells than the B[a]P metabolites. All six metabolites of B[a]P and Nap activated Nrf2 antioxidant pathway in ARE reporter cells indicates their potential to induce oxidative stress adaptive response. Among them, B[a]P-4,5-diol was found to be the most potent inducer of oxidative stress adaptive response than other metabolites. BDPE showed positive response in micronucleus test and other metabolites did not induce MN formation. The results suggest that toxicity response to these selected PAH metabolites varies depending on specific metabolite and/or biological end point is being evaluated. Data for metabolite toxicity may be utilize to inform risk assessment of these PAHs.

ABSTRACT FINAL ID: 3899 Poster Board: P591
TITLE: Modeling Chemical Effects on Estrogen Receptor Driven Breast Cancer Progression in a Microphysiological System

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KEYWORDS: Predictive Toxicology; Endocrine; Estrogens; Alternatives to Animal Testing

ABSTRACT BODY: Environmental factors, including exposure to natural products and industrial chemicals, is considered to be the major preventable risk factor that contributes to breast cancer progression. Estrogen receptor positive (ER+) breast cancer accounts for the majority of breast cancer cases yet most chemicals have not been evaluated for their effects on disease progression. Current testing strategies rely on animal models which are expensive, time consuming, have ethical issues and are often difficult to extrapolate to humans. While there are available *in vitro* models, they're often overly simplified and lack multiple cell types, cell:cell contact and cellular communication that could be targeted by toxicants. To address this issue, we have developed an organotypic system that can be used to aid in identifying the effects that chemicals have on breast cancer progression. We have engineered a microscale model that incorporates 3D ductal structures lined with ER+ MCF7 cells cultured near stromal cells embedded in collagen. Ductal structure and confluency were validated by staining for F-actin and e-cadherin. We conducted a live/dead assay after cells were in culture for seven days and observed approximately 90% cell viability. We stained for golgi and laminin-5 and found cells in the ductal structures adopt apical basal polarity. We have evaluated estrogen responsiveness in our model by exposing our system to estradiol, Fulvestrant, and diethylstilbestrol (DES) and observed an increase in estrogen response element activity when exposed to estradiol ($p<0.0005$) and DES ($p<0.0005$) and a decrease in response to Fulvestrant ($p<0.05$). We have investigated additional estradiol induced effects and observed an approximately two fold increase in both proliferation ($p<0.05$) and oxidative stress ($p<0.05$). We conclude that our organotypic system is capable of evaluating estrogen receptor driven effects on breast cancer progression. Once we have further developed our platform, we will screen the ToxCast library of chemical compounds to identify individual and classes of toxicants that speed breast cancer progression.

ABSTRACT FINAL ID: 3900 Poster Board: P592
TITLE: Molecular Mechanism of Ocular Surface Damage: Applications to Dry Eye and Wound Healing Models on *In Vitro* Reconstructed Human Corneal Tissues

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KEYWORDS: *In Vitro* and Alternatives; Alternatives To Animal Testing; Ocular Toxicity; Dry Eye Disease

ABSTRACT BODY: Current methods used to investigate mechanisms of corneal wound healing (CWH) and pathogenesis of dry eye disease (DED) utilize monolayer cell cultures or animals; hence, there is a need for more physiologically relevant, human-based *in vitro* models for ocular surface research. This study utilized EpiCorneal tissue model comprised of normal human corneal epithelial cells that are cultured at the air-liquid interface. Corneal wounds were introduced by abrasion or chemicals (1N NaOH). Wounded tissues were cultured in the presence or absence of human corneal keratocytes (HCK) or EGFR inhibitor (erlotinib, 10 uM). A DED model was generated by placing EpiCorneal tissues under desiccating stress conditions (DSC, 40% RH, 40°C, and 5% CO2) that stimulate morphological, cellular, and molecular changes relevant to dry eye. CWH was analyzed by transepithelial electrical resistance (TEER), histology, confocal microscopy, and gene expression. TEER recovered to 933.7/502.4 Ω·cm2 in the presence/or absence of HCK in 4 days post-wounded cultures. mRNA expression was analyzed using a 96-gene wound healing microarray. 13 genes (including collagen, integrin, chemokine, and protein kinase families) were up-regulated in the EpiCorneal tissues 24h post-abrasion in the absence of HCK and 16 genes (including WNT, FGF, small GTPases, chemokine, and integrin families) were up-regulated in the presence of HCK, but not in control cultures. DED was analyzed by TEER, histology, tissue viability, mucins and tight junction (TJ) protein expression. Dramatic reduction in tissue thickness was observed after 48h in DSC that coincided with decreased expression of mucins, increased TEER and atypical expression of TJ proteins. Topical application (25 µl/tissue) of lubricant gel drops (GenTeal, Alcon) improved tissue morphology and barrier function. The results demonstrate that the *in vitro* organotypic human corneal tissue structurally and functionally reproduces CWH and DED. The model will avoid species extrapolation, be more cost effective and more reproducible than animal methods, and will facilitate drug discovery by allowing screening and optimization of active pharmaceuticals prior to clinical studies.

ABSTRACT FINAL ID: 3902 Poster Board: P594
TITLE: Application of SV40 T-transformed Human Corneal Epithelial Cells to Evaluate Potential Irritant Chemicals for *In Vitro* Alternative Eye Toxicity

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KEYWORDS: Alternatives to Animal Testing; Cell Lines, Transfected

ABSTRACT BODY: Assessment of eye irritation potential is important to human safety, and it is necessary for various cosmetics and chemicals that may contact the human eye. Until recently, the Draize test was considered the standard method for estimating eye irritation, despite its disadvantages such as the need to sacrifice many rabbits for subjective scoring. Thus, we investigated the cytotoxicity and inflammatory response to standard eye irritants using SV40 T-transformed human corneal epithelial (SHCE) cells as a step toward development of an animal-free alternative eye irritation test. MTT and NRU assays of cell viability were performed to investigate the optimal experimental conditions for SHCE cell viability when cells were exposed to sodium dodecyl sulfate (SDS) as a standard eye irritant at 6.25×10-3 to 1×10-1%. Additionally, cell viability of SHCE cells was examined in response to six potential eye irritants, benzalkonium chloride, dimethyl sulfoxide, isopropanol, SDS, Triton X-100 and Tween 20 at 5×10-3 to 1×10-1%. Finally, we estimated the secretion level of cytokines in response to stimulation by eye irritants in SHCE cells. SHCE cells showed a good response to potential eye irritants when the cells were exposed to potential irritants for 10 min at room temperature (RT), and cytokine production increased in a concentration-dependent manner, indicating that cytotoxicity and cytokine secretion from SHCE cells may be well correlated with the concentrations of irritants. Taken together, these results suggest that SHCE cells could be an excellent alternative *in vitro* model to replace *in vivo* animal models for eye irritation tests.

ABSTRACT FINAL ID: 3903 Poster Board: P595
TITLE: Induction of Skin Cytochrome P450 by Xenobiotics: How to Deal with Risk Assessment of Topically Applied Products

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KEYWORDS: Biotransformation And Toxicokinetics; Cytochrome P450; Alternatives to Animal Testing

ABSTRACT BODY: Risk assessment for personal care products requires data on exposure to manage and mitigate the risk of developing dermal side effects. It is becoming increasingly accepted that skin metabolism may play a relevant role in biotransformation of molecules able to cross the stratum corneum. Even if basal activity is very low, often close to detection limits, some enzymes may be induced by various compounds. In this work, we have studied the effect of three known inducers (3-Methylcholanthrene, β- Naphthoflavone, Phenobarbital) on CYP 1A1, CYP1A2 and CYP 3A4 in skin models. Incubation time was 24 hours. Using a real-time quantitative PCR method, we show a biologically significant increase in the amount of mRNA of CYP 1A1 and 1A2 (about 200-fold) in treated cultures of human primary keratinocytes when using 3-Methylcholanthrene or β-Naphthoflavone. Phenobarbital did not lead to any induction. In parallel, in order to check the metabolic capacity of the induced CYPs, paraxanthine, resulting from caffeine metabolisation, was identified. This was achieved using a LC/MS/MS method that allows the identification of the cutaneously synthetised metabolites from caffeine, which is a well-known substrate of CYP 1A2. However, no changes have been observed on CYP 3A4 expression whatever the inducer. Basal activity was too low, or conditions of induction selected were not optimal for this CYP. Phenobarbital is known to induce liver CYPs, particularly in rat. In this experiment, we had no effect on the molecule regarding induction on CYP 1A1, CYP1A2 or CYP 3A4. This finding can be explained by a too short incubation time. The induction phenomena evidenced in this work needs to be taken into consideration when applying mixtures of molecules by the dermal route, particularly on a long term basis, as induction phenomena may be triggered and can in consequence, modify exposure resulting in a potential change of risk.

ABSTRACT FINAL ID: 3904 Poster Board: P596
TITLE: Update on the Society of Toxicology – Colgate Palmolive Grant for Alternative Research: *In Vitro* Co-Culture Assay for Identification of Dermal Sensitizers

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KEYWORDS: *In Vitro* and Alternatives; Chemical Allergy; Hypersensitivity

ABSTRACT BODY: This research project combined a 3D reconstructed human epidermal (RHE) tissue that is co-cultured with human plasmacytoid Dendritic Cells (pDCs) for use as an *in vitro* CoCulture dermal sensitization assay. In this assay, RHE tissues were placed at the air-liquid interface above a media suspension of pDC. The tissues are then exposed to test materials, and after 4 hours of incubation together, the RHE tissues and pDC were separately cultured for an additional 20 hours. The RHE media was analyzed for IL-18 release by ELISA, and the pDC were analyzed for changes in CD86 surface expression by flow cytometry. Two non-sensitizing irritants (Lactic Acid and Phenol), along with two weak/moderate sensitizers: Eugenol and Hexylcinnamaldehyde, and two strong sensitizers: 1-Chloro-2,4-Dinitrobenzene and 4-Nitrobenzyl Bromide were assayed. A positive response from the RHE tissues was determined to be a 2-fold increase in IL-18 secretion, and a 1.5 fold increase in CD86 expression on pDC. Tissue viability was measured using the MTT assay. The responses we obtained in both the RHE tissue versus pDC were very consistent. Increases in both secretion of IL-18 and expression of CD86 were detected after exposure to dermal sensitizers. A prediction model was developed in which a sensitizer result for a chemical is defined as either a positive result in the RHE tissue (IL-18) or a positive result in pDCs (CD86). From three individual experiments, and using a 2x2 contingency table to determine Cooper statistics, we obtained an Accuracy of 100%, 83%, and 83% (89% mean Accuracy). All four of four sensitizers were positively predicted in each experiment (100% Sensitivity). This research was funded by the Society of Toxicology Grant for Alternatives Research (sponsored by Colgate-Palmolive).

ABSTRACT FINAL ID: 3905 Poster Board: P597
TITLE: An *In Vitro* Test Method for Screening Potential Androgenic Agonists and Antagonists in MDA-kb2 Cells

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KEYWORDS: *In Vitro* and Alternatives; Endocrine Toxicology; Endocrine; Androgens; MDA-kb2; Androgen Agonists Antagonists

ABSTRACT BODY: We report that an MDA-kb2 human cell line that has both endogenous androgen (AR) and glucocorticoid (GR) receptors and a stably transfected MMTV-luciferase (Luc) can be reliably used to screen for substances that may induce or inhibit gene activation via AR-or GR-mediated pathways. Confirmation assays are essential components of our protocols to verify that induction or inhibition of luciferase expression occurs through AR receptor activation or inactivation. A pure AR antagonist (nilutimide: NIL) is used for agonist confirmation and a pure AR agonist (R1881 or dihydrotestosterone: DHT) is used for antagonist confirmation. DHT and R1881 have EC50's of 1.5x10-10M and 8x10-11M, respectively, and NIL an IC50 of 1x10-7M. We use progesterone and cycloheximide as the negative controls in AR agonist confirmation and antagonist confirmation assays, respectively. Although progesterone is commonly reported as an AR agonist in the literature, our use of a confirmation assay shows that progesterone is a false positive that makes a good negative control. Previously published androgen antagonist assays lacking a confirmation assay have increased false positives due to cytotoxicity, protein synthesis inhibition, or other cell cycle disruption. While earlier studies have managed to control for cytotoxicity, using cycloheximide as the negative antagonist control eliminates false positives due to other factors. We tested 30 coded chemicals supplied by NICEATM using protocols that included confirmation assays, and found no false positives or negatives compared to NICEATM/ICCVAM meta-analyses. The assay protocols using DHT as the positive control are currently undergoing a single lab validation study.

ABSTRACT FINAL ID: 3906 Poster Board: P598
TITLE: Developing an *In Vivo* Screen to Test the Efficacy and Safety of Curcumin Against Mcf-7 Breast Cancer Cells

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KEYWORDS: Cell Lines, Transfected; Natural Products; Curcumin

ABSTRACT BODY: Curcumin is a polyphenol derived from the rhizome of turmeric (Curcuma longa) that has been reported to exhibit anti-cancer effects in multiple cell types *in vitro* and *in vivo*. Studies suggest that curcumin has lower toxicity and even chemopreventive and chemoprotective properties, as compared to conventional chemotherapeutic compounds. In the current study, we examined the cytostatic and cytotoxic effects of curcumin and pravastatin, along with negative (tamoxifen) and positive (gemcitabine) controls. *In vitro*, compounds were examined in estrogen receptor positive MCF-7 cells, and a derivative cell line stably transfected with a red fluorescence protein plasmid, MCF-7-RFP. The derivative cell line was created to enable the simultaneous monitoring of toxicity and anti-cancer efficacy of compounds in zebrafish following xenotransplantation. Cells were exposed for 72 hr to 5-6 log doses of the compounds (up to 100 µM), and cell viability was determined using MTS assay. Tamoxifen had no effect in either cell line. With the other compounds, the RFP transfected cell line was consistently more sensitive to anti-proliferative effects with IC50s three to four-fold lower than the parental cells. Both pravastatin and gemcitabine were cytotoxic (IC50's were 10-30 µM and 10-44 µM, respectively). Curcumin demonstrated a cytostatic anti-proliferative profile. Specifically, the cells plateaued at ~40% of control cell growth at the higher doses versus 0-10% with the other compounds. The IC50 to reach this static effect was 4-25 µM. We are currently examining the safety and efficacy of curcumin *in vivo*. While the mouse is commonly used *in vivo* cancer studies, it has significant drawbacks, including the high cost of animal maintenance. In comparison, larval zebrafish are less costly, amenable to xenotransplantation, and easy to image, particularly in the transgenic casper/fli line that our laboratory has bred. Xenotransplanting MCF-7-RFP cells in transparent (casper) zebrafish embryos with GFP-labeled vasculature (fli) to screen natural products such as curcumin will provide a unique animal model to study the *in vivo* safety and efficacy of these compounds.

ABSTRACT FINAL ID: 3907 Poster Board: P599
TITLE: Repeatability of Transcriptomic Responses Induced by a Benchmark DIVI Compound

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KEYWORDS: Predictive Toxicology; Cardiovascular System; Safety Pharmacology

ABSTRACT BODY: Drug-induced vascular injury (DIVI) findings in animal studies can cause the delay or termination of promising drugs in development. There is a lack of robust *in vitro* models to predict DIVI and investigate its molecular mechanisms. This study was designed to assess the repeatability of the transcriptomic response of an *in vitro* rat vascular system to a benchmark compound (BM) (PF-04950834, a rho kinase inhibitor) that causes DIVI in the rat mesentery. Hemodynamic waveforms were calculated from *in vivo* blood velocity measurements of the rat mesenteric circulation. Primary rat endothelial (EC) and smooth muscle cells (SMC) from multiple donors were co-cultured and ECs exposed to DIVI-induced hemodynamic waveforms by a cone-and-plate viscometer technology. BM compound was added to 24 hr hemodynamic EC:SMC co-cultures at 2 concentrations for an additional 72 hrs. Measured in the cell culture media, BM concentrations were ~40% and 200% of a C_{max} concentration that consistently causes DIVI in the rat mesentery. At 96 hrs, RNA was isolated and RNAseq-based mRNA abundance in ECs and SMCs was measured. Samples exposed to DIVI-induced hemodynamics and BM compound were compared to samples exposed to baseline hemodynamics and compound vehicle. The experiment was repeated 5 times to assess repeatability. Three metrics of repeatability were established *a priori*: Fold-change correlation; enriched agreement of signed test statistics; and enriched rank-overlap of gene significance. By these criteria, all 5 experiments repeated at the high BM concentration in ECs and SMCs: median fold-change correlation 65%; median test statistic enrichment 1e-370; median rank-overlap enrichment 1e-73. At the low concentration, the majority of SMC responses repeated, while ~1/2 of the EC responses repeated. In conclusion, an *in vitro* model composed of primary rat vascular cells in hemodynamic co-culture met established repeatability criteria at relevant *in vivo* concentrations, adding confidence in the future predictive power of the system.

ABSTRACT FINAL ID: 3908 Poster Board: P600
TITLE: Evaluation of New Solvents for the Use in the Multi-Dose Reconstructed Human EpiDermis (RhE) Phototoxicity Assay

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KEYWORDS: Alternatives to Animal Testing; Cytotoxicity; Reconstructed Human Epidermis; Chlorpromazine

ABSTRACT BODY: The phototoxic potential of test materials after exposure to UVA/visible light is evaluated by the 3T3 Neutral Red Uptake Phototoxicity Assay using Balb/c 3T3 mouse fibroblasts (OECD TG 432). To address challenges related to testing of finished products or materials that are not completely soluble, the Reconstructed Human EpiDermis (RhE) Phototoxicity Assay (In Vittox Protocol 121) can be used as a stand-alone or in a tiered approach. Water and sesame oil were recommend solvents for the RhE Assay, however additional solvents may be investigated on a case-by-case basis to accommodate a wider variety of test materials. Alternate solvents should first be assessed to ensure that they: 1) do not cause cytotoxicity; 2) do not diminish and/or inhibit phototoxic reactions; and 3) do not interfere with the UVA exposure. We investigated 5% DMSO in Hanks' Balanced Salt Solution (HBSS), 5% acetone in HBSS, and polyethylene glycol (PEG) and their influence on the prediction of phototoxicity of chlorpromazine, a known phototoxicant. Duplicate tissues (EpiDerm™ from MatTek Corporation, Ashland, MA) were treated with each group for 24 hours, followed by a UVA (~6 J/cm^2) or dark exposure, and then a 21 hour post-exposure period before viability assessment using MTT dye. The assay positive control, 0.02% chlorpromazine in HBSS containing 1% DMSO, was tested concurrently. A test material was considered to have phototoxic potential if it induced a ≥30% difference in viability between tissues exposed to UVA as compared to the dark-exposed tissues. Our data showed that the solvents performed in a comparable manner to the assay negative control (HBSS) and they did not induce significant toxicity when the viability of the UVA-exposed and dark-exposed tissues was compared: 92.9% and 104.8% (5% DMSO), 94.0% and 97.5% (5% acetone), and 81.3% and 90.0% (PEG), respectively. The difference between the viability of the UVA-exposed or dark-exposed tissues after treatment with 0.02% chlorpromazine dissolved in 1% DMSO, in 5% DMSO, and in PEG was 60.0%, 59.7%, and 72.5%, respectively. These data indicate that the new solvents we investigated were suitable for use in the detection of phototoxicity. The evaluation of 0.02% chlorpromazine in 5% acetone, as well as additional solvents (e.g. ethanol), is currently ongoing. Our future investigations will concentrate on the assessment of different solvents that can accommodate the phototoxicity testing of novel chemistries.

ABSTRACT FINAL ID: 3909 Poster Board: P601
TITLE: A Case Study Analysis of the Difference Between BCOP and *In Vivo* Tests Used to Predict Eye Hazard in Cleaning Products

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KEYWORDS: Alternatives to Animal Testing; BCOP

ABSTRACT BODY: The bovine corneal opacity and permeability (BCOP) assay has become an integral part of screening formulas for eye hazard with respect to product stewardship, as it reduces animal use and is looked upon favorably by regulatory agencies. A recent screening of a liquid cleaning product was performed to understand the eye irritation potential. In total, five similar experimental formulas (all containing hydrogen peroxide and surfactant[s]) were screened with the BCOP along with a tandem chorioallantoic membrane vascular assay (CAMVA) in order to approximate eye hazard. An additional commercialized product with a formulation similar to the experimental formulas and having known *in vivo* eye testing results was also screened with the BCOP and CAMVA. The five screened formulas had corneal opacity scores from 20.5-35.7, permeability scores from 0.648-1.202, and *in vitro* irritation scores (IVIS) from 33.52-52.83. These results all suggest moderate bordering on severe eye irritation. The CAMVA conducted with the same samples had results indicating non-irritation for four samples and an indeterminate result for the other. The commercialized product characterized as a mild irritant based on *in vivo* testing produced an IVIS within the moderate irritation range (25.1-55 IVIS), with the value bordering on severe irritation, and had a CAMVA score indicative of non-irritation. Due to the difficulties in interpreting the data, a single formula was tested in rabbits to confirm its eye hazard. Corneal opacity and iritis were not observed at any endpoint. Conjunctival irritation was observed in 3/3 rabbits at 1-hr but cleared by 48 hours. This result is considered mildly irritating, not rising to the level of classification under the Globally Harmonized System (GHS) and the EPA pesticide classification guidelines. Overall, the results of the *in vitro* assays performed indicate that in certain instances, BCOP can significantly over-predict the eye hazard and is not representative of *in vivo* testing results. This study, though limited in scope, suggests that for some chemistry types, the BCOP may not be the most suitable screening assay for evaluating eye irritation potential.

ABSTRACT FINAL ID: 3910 Poster Board: P602
TITLE: Zebrafish: A Novel Tool for Toxicology Studies on the Chemical Warfare Nerve Agent Cyclosarin (gf)

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KEYWORDS: Organophosphates; Toxicity; Acute; Aquatic Toxicology; Cyclosarin

ABSTRACT BODY: Zebrafish (*Danio rerio*) is used in many areas of toxicology for predicting human risk and for drug discovery. It is an inexpensive and high throughput animal model to study the toxic effects of organophosphorus nerve agent (NA) and the effectiveness of oximes to reactivate NA-inhibited acetylcholinesterase (AChE). Another advantage of utilizing zebrafish is that it only expresses AChE. The current study determined the median lethal concentration (LC50) of cyclosarin (GF) at four exposure times and evaluated the efficacy of MMB-4, MINA and 2-PAM in reactivating GF-inhibited AChE. Larval zebrafish at 6 days post-fertilization (dpf) were exposed to various concentrations of GF ranging from 0.5 to 1000 µM for 0.5, 1, 1.5 and 2 hours. Rapid and erratic swimming activities were observed as the initial clinical signs of GF intoxication, followed by a complete lack of locomotion. Mortality at 24 hours was determined by the cessation of a heartbeat. A probit analysis estimated the LC50 of GF for 0.5, 1, 1.5 and 2 hours of exposure to be 31.3 µM, 21.0 µM, 14.5 µM, and 6.6 µM, respectively. Survivors of exposure exhibited loss of equilibrium, bilateral exophthalmia, and ascites during clinical observations. To evaluate the effectiveness of MMB-4, MINA or 2-PAM to reactivate cyclosarin-inhibited AChE, 6 dpf larvae were exposed to 5 µM of GF, and their AChE activity was measured by the Ellman assay. When the larvae were completely immobile after 1 min of exposure, they were treated with 0, 25, 50, 100, 200 or 400 µM of MMB-4, MINA or 2-PAM. The percentage of AChE inhibition was 99.7% when the larvae lacked locomotion at 1 min. With MMB-4, MINA or 2-PAM treatment, the AChE percentage inhibition was decreased from 99.7% to 18%. The results suggest that 1) zebrafish larvae are sensitive to the toxic effects of GF in a concentration- and time-dependent manner and 2) zebrafish larvae AChE inhibited by GF can be reactivated with MMB-4, MINA or 2-PAM, oxime reactivators currently used as standard treatment for CWNAs in the military. These observations provide evidence that the zebrafish is a suitable animal model system for *in vivo* evaluation of NA toxicity and novel oxime reactivators.

ABSTRACT FINAL ID: 3911 Poster Board: P603
TITLE: Development of a 3D Dog Liver Microtissue Model

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KEYWORDS: Hepatocytes; *In Vitro* and Alternatives; Predictive Toxicology; Microtissues

ABSTRACT BODY: Currently, there are no commercially available 3-dimensional (3D) tissue models of the dog liver. Advantages of 3D microtissue (MT) models include longer culture time, the ability to co-culture with non-parenchymal cells (NPC) and creation of a homotypic environment which mimics the *in vivo* setting. Our aim was to generate normal liver MTs from cryopreserved primary dog hepatocytes with or without NPCs, determine their viability in culture and response to stimuli. H&E-staining of dLiMT revealed dense cellular contacts and presence of healthy hepatocytes, with viability preserved over 21 days in culture. Induction of cytochrome P450 CYP3A12 activity was measured after addition of phenobarbital or rifampicin, resulting in 2- and 4-fold induction, respectively, demonstrating metabolic competency. To test as a model of liver toxicity, dLiMTs were cultured with or without 10 µg/ml LPS and dexamethasone (DEX). A dog-specific cytokine ELISA was used to measure inflammatory markers from cultured media. Cytokines were increased with LPS treatment and suppressed by the presence of DEX at 48h, as expected. When using the hepatocyte+NPC MTs, maximal LPS-stimulated cytokine responses occurred at 72h: IL-6 was 98.7±48.0 pg/ml compared with no response by non-LPS controls MTs, and IL-8 was 8694.2±2183.0 pg/ml, 18.5-fold greater than hep-only MTs (468.9 203.4 pg/ml), or 3.7-fold greater than non-LPS controls (125.1±59.0 pg/ml), ($p<0.01$). The stimulation of cytokine release by LPS indicates the presence of Kupffer cells within the NPCs; providing an inflammation-mediated model of the dog liver. The known hepatotoxic compounds bromfenac, acetaminophen, aflatoxin and troglitazone (TRG) revealed toxicity after 5 days exposure, but negative control compounds buspirone, isoniazid and zileuton showed no toxic effect on dLiMTs. Specifically, TRG IC50 decreased over time; 87.4 µM, 24 h; 47.96 µM, 72 h; and 25.00 µM, 144 h, indicating the utility of chronic toxicity testing. Here we demonstrate a viable, cytokine-responsive dLiMT model which can be cultured for at least seven days, enabling longer-term studies than 2D sandwich hepatocyte cultures. Additional characterization and optimization of this model has the potential to serve as an *in vitro* approach to de-risking dog-specific liver findings.
