Dissertation

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Presented by: Diploma biologist Jan Wölz
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Impact of contaminants on aquatic systems and inundated sites with respect to flood events

In vitro biotests, chemical target analysis and fractionation methods

Referees: Prof. Dr. Thomas Braunbeck
Heidelberg Institute of Zoology, University of Heidelberg

Prof. Dr. Heinz Karrasch
Geographical Institute, University of Heidelberg

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Contents

Abstract

Scope of the present study is the development and application of aquatic *in vitro* bioassays and methods of effect-directed analysis (EDA). It aims at investigating contamination of suspended particulate matter (SPM) and pollution of inundated sites and riparian aquifer, respectively. In the first part of this study, SPM was sampled during flood events and toxicological activities were determined. The second part of the study dealt with possible conflict of interests between flood management (operation of retention basins) and drinking water supply (sustainment of water protection areas).

Cytotoxic potencies were determined with the Neutral Red retention assay and dioxin-like and aryl hydrocarbon receptor mediated activities with the 7-ethoxyresorufin-*o*-deethylase (EROD) assay, both using RTL-W1 cells derived from rainbow trout (*Oncorhynchus mykiss*). Both bioassays indicated elevated potencies associated with SPM sampled during flood events. Highly active samples were fractionated in order to determine effective compounds. Strongly persistent compounds had an only minor contribution to total biological effects, whereas less persistent substances caused the bulk of biological activity. Chemical analysis showed that compounds analyzed with priority are not capable of adequately explaining the biological effects measured. Non-priority and *a priori* unknown compounds were mainly effective.

The second part of the study aimed to investigate impacts of river contaminants to inundated sites and aquifer in flood events. For this end, the biotest battery was extended with the Ames Fluctuation assay and the bacterial tester strains TA98 and TA100 (*Salmonella typhimurium*) to detect mutagenic activity, as well as the Yeast Estrogen Screen (YES) assay with bakery yeast (*Saccharomyces cerevisiae*) to determine endocrine activity. Further, a recently developed method of effect-directed analysis (EDA) was used to separate more polar compounds in SPM and soil. Less persistent compounds were shown to be highly active. However, more polar compounds caused the highest effects. In accordance to findings of the first part of the study, chemical analysis showed that priority compounds only made a minor contribution to biological effects.

River contaminant infiltration in the aquifer was assessed following a flood event with a recurrence interval of ten years by measurement of a tracer compound and hormonal activity. Both parameters indicated contamination of the aquifer following the flood. Water that was sampled in the hinterland showed delayed effects and, thus, indicated mass transport in groundwater layers over elevated distances.

The findings of this study document high contamination of flood SPM that may be deposited at inundated sites. In particular, increased biological effects and chemical loads of more polar compounds indicate an increased impact of contaminant transfer through soil and aquifer contamination. Furthermore, infiltration and increased toxicological effects indicate a general risk of groundwater contamination in consequence of flood events.

The results of the present study directly contribute to a manual assisting stakeholders and operators of retention basins and waterworks to *a priori* avoid potential conflict of interests and, thus, could directly be implemented in practical work.

Zusammenfassung

Die vorliegende Arbeit befasst sich mit der Entwicklung und Anwendung von *In vitro*-Biotests und Methoden der Effekt-dirigierten Analyse (EDA). Ziel ist die Untersuchung der Belastung von Schwebstoff (SPM) sowie des Schadstoffeintrags auf Retentionsflächen und in den flussnahen Aquifer. Dazu wurden im ersten Teil der Studie SPM aus Hochwasser entnommen und hinsichtlich toxischer Wirksamkeiten untersucht. Im zweiten Teil wurde an einem Modellstandort untersucht, ob es zu einem Interessenkonflikt zwischen Hochwassermanagement (Betrieb von Retentionsräumen) und Trinkwasserversorgung (Erhaltung von Wasserschutzgebieten) kommen kann.

Zytotoxische Schädigungspotentiale wurden im Neutralrot-Test und Dioxin-ähnliche und Arylhydrocarbonrezeptor-vermittelte Wirksamkeit im 7-ethoxyresorufin-*o*-deethylase (EROD)-Assay mit RTL-W1-Zellen der Regenbogenforelle (*Oncorhynchus mykiss*) untersucht. In beiden Biotests wurden erhöhte Wirksamkeiten durch SPM aus Hochwasser ermittelt. Zur Bestimmung der Effekt-verursachenden Substanzklassen wurden hoch wirksame Proben fraktioniert. Während Fraktionen mit sehr persistenten Schadstoffen nur geringe Effekte bewirkten, wurde der Großteil der biologischen Wirksamkeit durch mäßig persistente Verbindungen verursacht. Mittels chemischer Analytik konnte gezeigt werden, dass prioritär untersuchte Kontaminanten die ermittelten Effekte nicht hinreichend erklären konnten. Nicht-prioritäre und *a priori* unbekannte Substanzen wiesen folglich die größten Schädigungspotentiale auf.

Der zweite Teil der Studie befasste sich mit der Fragestellung, ob Kontaminanten aus dem Fluss im Hochwasserfall zu einer Belastung von Überflutungsflächen sowie des Aquifers führen können. Zur Bearbeitung dieser komplexen Fragestellung wurde die eingesetzte Biotestbatterie erweitert und zusätzlich mutagene Potentiale im Ames-Fluktuationstest mit den Bakterienstämmen TA98 und TA100 (*Salmonella typhimurium*) sowie hormonelle Aktivität im Yeast Estrogen Screen (YES)-Assay mit Bäckerhefe (*Saccharomyces cerevisiae*) gemessen. Weiterhin wurde eine neuartige, kürzlich entwickelte Methode der Effekt-dirigierten Analyse (EDA) zur Anwendung gebracht, mittels derer erstmals auch polarere Substanzen in SPM und Bodenproben für die Biotestung aufgetrennt wurden. Hohe Wirksamkeiten wurden für mäßig persistente Schadstoffe ermittelt. Die größten Wirksamkeiten wiesen jedoch polare Verbindungen auf. Mittels erweiterter chemischer Analytik konnte gezeigt werden, dass, in Übereinstimmung mit den Ergebnissen aus dem ersten Teil der Studie, die prioritären Schadstoffe nur zu einem sehr geringen Anteil zur biologischen Gesamtwirksamkeit beitrugen.

Weiterhin wurden die Auswirkungen der Infiltration von Schadstoffen aus Flüssen in den Aquifer anhand eines Hochwassers mit einem Wiederkehrintervall von zehn Jahren untersucht. Zu diesem Zweck wurde das Vordringen von Flusswasser durch den Nachweis einer Leitsubstanz sowie die Veränderung hormoneller Wirksamkeiten infolge des Hochwassers erfasst. Beide Parameter wiesen auf eine direkte Beeinflussung des Aquifers durch das Hochwasser hin. Effekte von Proben die mit größerem Abstand zum Fluss entnommen worden waren wiesen eine zeitliche Verzögerung auf, die auf Stoffinfiltration in den Grundwasserleiter hinweist.

Die Ergebnisse dieser Dissertation belegen eine hohe Schadstoffbelastung von Schwebstoffen aus Hochwasser, die auf Überflutungsflächen abgelagert werden können. Insbesondere die hohen Belastungen mit eher polaren Verbindungen machen einen Stoffeintrag in den Aquifer über längere Zeiträume wahrscheinlich. Weiterhin weisen die Befunde der Grundwasseruntersuchungen nach dem Hochwasser auf das generelle Risiko von Schadstoffinfiltration auch ohne den Betrieb von Retentionsbecken hin.

Die Resultate dieser Dissertation fließen unmittelbar in ein Handbuch ein, das Entscheidungsträger und Betreiber von Rückhaltebecken und Wasserwerken dabei unterstützen soll mögliche Interessenkonflikte von vornherein zu vermeiden. Diese Arbeit konnte somit direkt in die praktische Anwendung eingebunden werden.

Chapter 1

Introduction

1.1 Chemicals in the (aquatic) environment and legal handling

Water is the most fundamental substance for life, and fresh water is, in particular, most important for organisms and subject to multiple use by humans as drinking water, agriculture and industrial processes (Baron et al. 2002, Gleick et al. 2008). Although pure water is absolutely essential for human beings, anthropogenic activities have resulted in significant impairment of the aquatic environment. Due to dynamics of riverine systems, rivers are, e.g., used as 'solvent' and means of transportation for human waste (Oki & Kanae 2006, Schwarzenbach et al. 2006).

Chemical pollution has profound impacts on aquatic ecosystems, and hazardous compounds are released intendedly (application products) or accidentally into the aquatic environment (Bendz et al. 2005, Vassiliadou et al. 2009). Chemicals are used to make virtually every man-made product and play an important role in everyday life of people around the world. On the one hand, chemical products provide protection for crops and increase yields, prevent and cure diseases, provide insulation to reduce energy use and so forth. On the other hand, chemicals can also negatively impact human and environmental health, leaving a considerable footprint, when their production and use are not managed responsibly (Helland et al. 2007, Weber et al. 2008b).

Over the entire life cycle of a chemical product – from “cradle to grave” – there is a potential for adverse effects on man and environment. Chemical risk reduction requires a management that involves continuous review of each compound from conceptual design in the laboratory over development up to distribution, marketing and a handling guide for degradation and/or waste disposal (Norgate et al. 2007, Schiefer et al. 1997). Today, about 100,000 compounds are on the market in the EU and worldwide, 30,000 to 70,000 out of which are in daily use (Schwarzenbach et al. 2006). For thousands of chemicals that are sold or used in products today, incomplete information exists on the volumes released to the environment, the targets of exposure and the toxic properties. This means that the risk of many chemicals has neither been thoroughly evaluated nor have they been adequately managed, because the necessary information to do so is not available (Hofer et al. 2004, Petry et al. 2006).

Almost every country has chemicals industries; yet, almost 80% of the world’s total output is currently being produced by only 16 countries: USA, Japan, Germany, China, France, UK, Italy, Korea, Brazil, Belgium, Luxembourg, Spain, The Netherlands, Taiwan, Switzerland and Russia. Usage and consumption of chemicals is far higher in OECD countries than in non-OECD countries. It is assumed that OECD countries will remain both the largest chemical producers and consumers until at least 2020, while production and consumption will grow much faster in non-OECD countries. Therefore, it was necessary to develop chemical management at a world-wide level (OECD 2001).

Overall, the chemicals industry in OECD countries has made significant progress in reducing releases of pollutants to the environment from manufacturing processes. Although there are no consolidated data on emissions of known hazardous substances across OECD countries, it is probable that, overall, such releases from chemical industries in these countries are declining. Nevertheless, releases of hazardous substances per unit of output still rank high compared to other industries (OECD 2001). Further, direct discharges *via* municipal treatment plants represent an important source of contaminants to the aquatic environment. This holds even more true, since specifically acting substances such as drugs are usually released by households *via* waste water and are not (completely) degraded (Heidler & Halden 2007, Zuccato et al. 2006).

Over the years, policies have been designed to protect man and the environment from both the hazardous emissions released during the production of chemicals and the risks posed by chemicals which are contained in consumer products (Kocha & Ashford 2006). Industries are also subject to regulations aimed at managing risks posed by the chemicals themselves, e.g. collection and assessment of data on hazard and exposure, material safety data sheets, labeling, marketing and use restrictions (Foth & Hayes 2008).

Historically, most of the management approaches used for controlling emissions during production have dealt with “end-of-pipe” solutions (Lee & Rheeb 2005, Sarkis & Cordeiro 2001). Recently, governments and industries in the European Union (EU) implemented more holistic approaches to minimize impacts on health and the environment throughout the lifecycle of a product – from raw material use to final disposal – by designing more environmentally benign chemicals and adopting integrated product policies, including extended producer responsibility (Clift & France 2008, EU 2006).

Given increasing trade volumes of chemical products and the growing awareness of pollutant transportation across national borders, the last three decades have seen a significant increase in international efforts by governments to co-ordinate the management of chemicals. Overall direction for this work was provided by the 1992 United Nations Conference on Environment and Development (UNCED) held in Rio de Janeiro when it adopted Chapter 19 of Agenda 21 (UN 2001). Among other things, this chapter calls for accelerating international work on the assessment of chemical risks, harmonization of classification and labeling of chemicals, establishing risk reduction programs and strengthening national capacities for managing chemicals. The REACH guideline of the European Union (EU), enacted in June 2007, aims at harmonizing such regulations and at minimizing risks of chemicals at least within the European Union markets (European Union 2006).

1.1.1 Excursus 1: REACH guideline of the European Union for regulation of chemical

Industrial chemicals have been used for many decades, and new products are regularly introduced to the market. However, there was a confusing patchwork of current legislations in the EU used for regulation. Thus, the old chemical regulation is currently replaced by the new regulations on industrial chemicals control, the EU guideline REACH (EC 1907/2006). This guideline deals with Regulation, Evaluation, Authorization and Restriction of Chemical substances and eventually came into force on June 1, 2007. It aims at overcoming limitations in testing requirements of former regulations on industrial chemicals in order to enhance competitiveness and innovation with regard to the manufacture of safer substances and, furthermore, at promoting the development of alternative testing methods (Ahlers et al. 2008, Foth & Hayes 2008).

Without registration, commercialization of chemicals is no longer possible, while authorization is limited to 'hazardous' substances. Information on properties and possible impacts of chemicals are registered in a central database which is run by the European Chemicals Agency of the EU (ECHA) in Helsinki, Finland (ECHA 2008, Kemmlein et al. 2009).

The purpose of REACH is to guarantee that substances produced, put onto the market and used are not hazardous to human and environmental health. Therefore, this regulation is based on the principle that confers responsibilities of product safety to producing industries and downstream users. Furthermore, REACH intends to increase transparency and to extend information publications about applied chemicals (Hansen et al. 2007, Homa et al. 2009).

Notably, hazardous compounds with carcinogenic, and mutagenic properties as well as reprotoxic (CMR), along with those substances with persistent, bioaccumulative and toxic (PBT) properties, as well as very persistent and very bioaccumulative compounds (vPvB) have to be identified and authorized by ECHA, regardless of the production volume (Hansson & Ruden 2006, Pouillot et al. 2009).

Producers and importers of chemicals with production volumes of > 1 ton per annum have to accomplish registration including a technical document. Existing products will have to be registered until 2018 (Black 2008). Compounds of volumes > 10 tons per annum need an additional 'Chemical Safety Report'. Compounds with volumes > 100 tons have to be registered until 2013; such with > 1000 tons until 2010. Substances with production volumes < 1 tons are excluded from registration (Edser 2008, Gubbels-van Hal 2007).

REACH has been estimated to affect about 30,000 compounds (> 1 tons/a) out of a total of 100,000 old chemicals on the market as listed in the European EINECS index (Loewenberg 2006, Wolf & Delgado 2003). To manage these substances, a base set of data is required containing information on identity, classification and labeling, as well as exposure assessments. Further on, chemical safety reports have to be provided containing more specified information as well as management strategies to minimize risks (Laamanen et al. 2008, Petry et al. 2006,).

Since estimations of potential exposure and effects of each chemical have to be provided, an effect-assessment is carried out using biotests with at least a subset of species and exposure scenarios to determine either biological effects or non-effect levels. In general, depending on the production volume, all trophic levels need to be considered, and effects are determined with at least destruents (bacteria), producers (algae) and consumers (daphnids, fish) providing a base set of information (Wei et al. 2006).

1.2 Chemicals related to suspended particulate matter

In this study, suspended particulate matter (SPM) is defined as the particles that, given at any time, are maintained in suspension by turbulent currents in a river. With decreasing flow, SPM tend to settle down on the ground and, thus, becomes part of sediment. However, with increasing discharge in flood events, sediments are eroded and re-contribute to SPM. This definition is close to that of the Environmental Specimen Bank of the Federal Republic of Germany as given by Schulze et al. (2007).

SPM is a heterogeneous mixture of compounds of various origin, size, shape, density and surface structure. In detail these components may be topsoil, sand, carbonates, clay minerals and organic matter in various stages of composition (Cornelissen et al. 2005, Doxarana et al. 2002). These solid matters originate from biogenic or geogenic erosion processes in catchment areas and cover sizes of about 0.02 - 2 mm. Translocation and downstream deposition in direction to the coast is mainly given for particles with grain sizes < 2 mm. Transportation distances of greater particles are reduced, and deposition takes place close to the sources, except in the case of mountain catchments or during flood events with increased sheer-stress and flow intensities (SedNet 2004, Slattery & Burt 1997,).

SPM components provide more or less numerous binding sites for substances and, thus, also for contaminants. Whereas sand provides less sportive surfaces, clay and organic matter are minor sized and provide a higher surface-to-volume ratio and manifold binding sites for compounds such as organic contaminants. In particular, humic substances which, e.g., bear polymerized organic complexes of nitrogen and oxygen represent attractive binding sites for organic pollutants (Gagne et al. 1999, Cornelissen et al. 2005).

SPM are an integral part of aquatic ecosystems and the raw material of habitats for numerous benthic species (Heise & Apitz 2007). They are fundamental in the cycle of inorganic and organic matter in aquatic systems (Netzband 2007), and, thus, adsorbed pollutants can have a negative influence on ecosystem functions. Since SPM play a major role in aquatic ecosystems, they have to be integral parts of characterization and environmental evaluation (Hakanson 2006).

Furthermore, SPM are most relevant with respect to safety of waterway transportation. Introduction and transportation of SPM causes sedimentation filling waterways and in particular harbors. Ports have many still-water zones where fine-grained material accumulates (Aria et al. 2009, Koethe 2003). Regular dredging is necessary to ensure that ports remain fully functional. However, materials mainly consist of sediments and contaminants from the whole course of the river. At the Hamburg Port, every year 3 to 5 Mio. m^3 of dredged materials are removed. For that portion of the materials that is too highly contaminated for relocation in the North Sea, storage at land is necessary which causes expenditures of 35 Mio. € per year (Netzband 2007).

Certain pollutants have always been of concern in aquatic systems, including the 16 EPA-PAHs as defined to be of superior relevance by the United States Environmental Protection Agency (US-EPA). Further on, Persistent Organic Pollutants (POPs) including toxic, persistent and accumulating compounds as defined and restricted by the Stockholm Convention in 1995, are prioritized. These are further characterized by the so-called 'grasshopper effect' which means that they can travel long distances in the environment by the repeated processes of evaporation and deposition (Mackay & Wania 1995, Gouin et al. 2004). Thereby, POPs are found anywhere in the environment as well as in human and animal tissues all over the world (Weber et al. 2008a, b). At present, dioxins, furans, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and some other hazardous compounds, pose a threat to the environment. Worldwide, the number of (highly) polluted sites continuously increases, causing a considerable problem with a need for regulation and management (Apitz & White 2003, Bridges et al. 2006, Weber et al. 2008b).

Pollutants can be introduced into rivers through various pathways which include the atmosphere (emissions, aeolian transportation), effluents (wastewater treatment plants) or surface runoff (precipitation, irrigation; Boxall & Maltby 1995, Brown & Peake 2006, Kay et al. 2006). Furthermore, a distinction can be made between rural areas with soil and channel bank erosion as well as atmospheric deposition on soils, urban areas with leaching (sewer drainage) and direct inputs from industries and shipping (Vink 2001).

Sources of contaminants differ between point and diffuse sources (see also fig. 1): Point sources are identified sources of steady inflow over the scale of years, and the magnitude of pollution is not influenced by meteorological conditions. Thus, point sources include municipal and industrial wastewater effluents (Förstner et al. 2004, Stronkhorst & van Hattum 2003,).

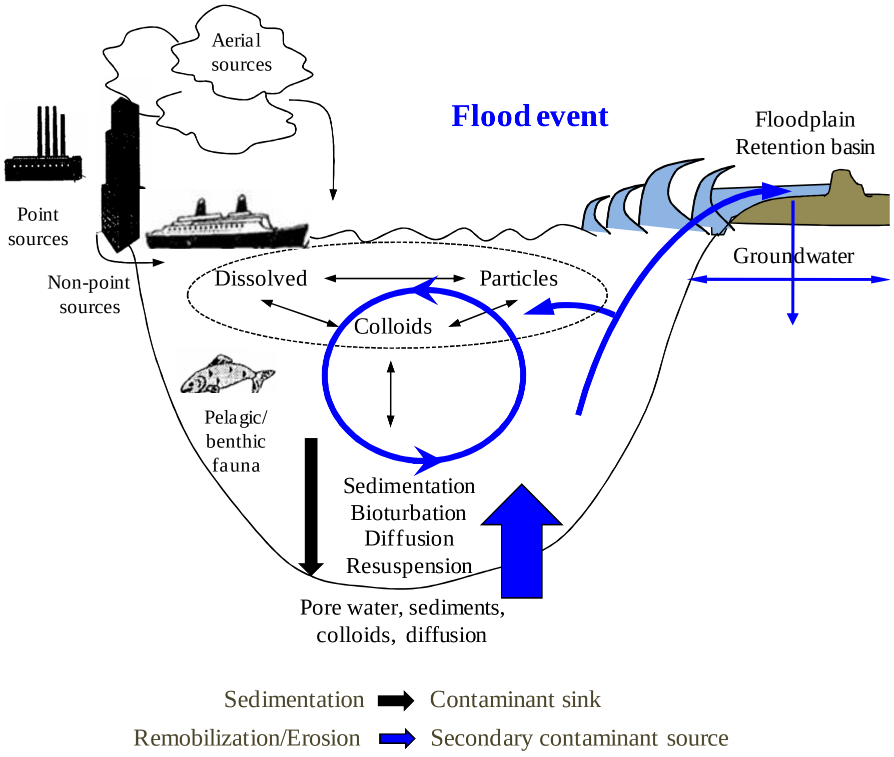

Fig. 1 Pathways of contaminant introduction into the aquatic environment and fate in the river system, with emphasize on the impacts through flood events (modified; Power & Chapman 1992).

Diffuse sources are highly dynamic and widely spread pollution sources with a close link to meteorological factors such as precipitation. These sources include loads of surface runoff as of cultivated fields, erosion and paved urban areas with traffic and atmospheric deposition (Ferreira et al. 2003).

Whereas dissolved compounds remain easily available in the free water column for aquatic organisms, less solvable hydrophilic compounds as, e.g., PAHs tend to adsorb to non-polar surfaces of inorganic and organic particles (Knezovich et al. 2004, Tusseau-Vuillemin et al.

2007). Thus, they become less available for organisms. With currents and turbulences below certain thresholds, SPM particles deposit on the river bed, thus acting as a sink of pollutants and reducing their availability in the water column (Schneider & Reincke 2006, von der Heyden & New 2004). Therefore, only bottom feeders and benthic fauna may still be exposed to these particle-bound substances, whereas particles remaining in the water column (SPM) have an impact on pelagic organisms (Caldas et al. 1999). Deposited particle-bound pollutants may further consolidate with sediment and become part of new sediment layers. SPM act as a buffer with respect to, e.g., nutrients, but also to persistent organic pollutants and act as sinks and important secondary sources of contaminants due to absorption of settling particulate matter (Ahlf et al. 2002b, Westrich & Foerstner 2005). As a consequence, in densely populated and industrialized regions, a great variety of man-made hazardous compounds are typically associated with aquatic SPM and sediments. Thus, contamination is a serious problem in areas of rivers with intensive sedimentation, as is given in naturally (floodplain) or artificial (retention areas) inundated areas along the rivers and, in particular, in deltas and estuaries that act as large 'sediment traps' (Santschi et al. 2001, Vigano et al. 2003, Yang et al. 2008).

Previously deposited and consolidated SPM can be remobilized following sediment dredging (Koethe 2003), with bioturbation being a major post-sedimentation process with normal discharge (Butcher & Garvey 2004) and flood events (Hollert et al. 2003b, Wölz et al. 2008). In fluvial systems, cycling of pollutants is dominated by processes of resuspension, settling and burial of particulate matter (Heise et al. 2004).

Particle-bound contaminants are most relevant when assessing the pollution of aquatic systems, in particular since they act as sinks and sources for contaminants. However, they were not considered in the EU Water Framework Directive (EU-WFD 2000/60/EC), with dissolved compounds being prioritized (EU 2000). Although, in a daughter directive to the EU-WFD, the evaluation of water pollution is proposed including the assessment or priority compound concentrations in sediments, the major relevance of sediments as secondary source of pollution is not accounted for (Bergmann & Maass 2007, Hollert et al. 2007b). Further, the implementation of sediments in the WFD underlines the particular need to focus on particle-bound contaminants. Since sediments are often highly polluted, certain contaminants may cause a failure to reach the quality goals in the WFD and may require additional measures for its control (Heise & Foerstner 2006, Hollert 2007).

At the river Rhine, hexachlorobenzene (HCB) is such a candidate, since it has a significant effect on the quality of sediments downstream and, e.g., on the dredged materials from Rotterdam harbor. Therefore, HCB is assumed to be categorized as Category 1 contaminant according to Article 16 Source/Pathway p. 11.1 in the WFD (Heise & Foerstner 2006). HCB accumulates with depositing particulate matters in particular at the barrages along the Upper Rhine; they can be remobilized during floods. Impacted downstream areas are highly polluted and may become areas of important challenges for sediment management. Hamburg Port Authorities are anxious that HCB may cause exceeded contamination levels that would prevent relocation of sediments at sea. The relocation at sea is the least expensive option (Netzband 2007). HCB loads in flood SPM were determined with up to 50 µg/kg in floods with recurrence intervals of 1 year and increasing concentrations up to 350 µg/kg in floods with recurrence intervals of 20 years. Thus, most HCB concentrations would exceed thresholds for quality goals by Ahlf et al. (2002a) that would be reached with < 5 µg/kg. Using the Chemical Toxicity Test (CTT) approach as well as the Uniform Content Test (UCT) action levels (20 µg/kg) would also clearly be exceeded (Stronkhorst & van Hattum 2003).

1.2.1 Excursus 2: EU Water Framework Directive (WFD) − Integrated river basin management for Europe

Since the end of the year 2000, a novel legislative approach has guided European water protection policy: The Water Framework Directive (WFD; 2000/60/EC). The new concept includes replacing, merging and renewing of the previous protection policies from the 1970s and provides a more consistent, transparent and comprehensive conception. The WFD aims at a holistic and integrated water protection and sets ambitious high-quality goals to achieve a 'good status' for European lakes and rivers until 2015 (Bald et al. 2005, Wilby et al. 2006). Thereby, ecological terms are of superior relevance, essential processes are detailed, as well as instruments to reach the set aims, and, finally, there is a strict time schedule to be followed (Anderson et al. 2006).

The WFD European Union legislation is double-tracked. On the one hand, community-wide, substances of concern were selected (European Commission 2000), and, on the other hand, each member state has to take measures at river basin level to manage prioritized pollutants (Westrich & Foerstner 2005). Policies concerning hazardous substances in European waters were introduced in a previous 'old' Framework Directive (76/464/EEC). In so-called daughter directives of the 1980s, certain substances were regulated defining emission limits and quality objectives in the surface and coastal waters valid community-wide. Until 2013, the 'old' directive will be in force and subsequently replaced by the 'new' Water Framework Directive (2000/60/EC) that integrates the ' old' directive (as codified under 2006/11/EC).

Identification of substances of concern for surface waters (significant risk for or *via* the aquatic environment) and development of control measures are set out in Article 16 of WFD, and priority compounds are listed and were adopted in Decision No. 2455/2001/EC (EU 2001).

To reach the aim set, the European Commission adopted another directive, setting environmental quality standards for the priority substances in July 2006, which each member state will have to achieve by 2015, to ensure a 'good chemical surface water status'. Furthermore, within 20 years, emissions as well as losses and discharge of priority substances will have to be reduced (Crane 2003).

The WFD implements the assessment of sediment contamination, e.g., in Article 16: “The Commission shall submit proposals for quality standards applicable to the concentrations of the priority substances in surface water, sediments or biota” (EU 2001). If quality criteria were to be defined for sediment, then monitoring would be required to establish compliance with such criteria. Certain compounds were selected and categorized as priority, priority substances subject to review to priority hazardous substances and priority hazardous (see tab. 2). According to the WFD, good chemical status for a water body is obtained when the concentrations of the priority substances in water, sediment or biota are below the Environmental Quality Standards (EQSs): this is expressed as ‘‘compliance checking’’ (Coquery et al. 2005, Lepom et al. 2009).

The member states shall also set quality standards for river basin-specific pollutants and take action to meet the WFD standards at latest by 2015 as part of the ecological status (Article 4, 11 and Annex V, WFD). Measures shall be in place by 2009 and become operational by 2012, replacing Directive 76/464/EEC (Mostert 2003).

Table 2 Priority Substances List (according to EU 2008)

Priority substances	Priority substances subject to review to priority hazardous substances	Priority hazardous substances
Alachlor	Anthracene	Pentabromobiphenylether
Benzene	Atrazine	Cadmium and its compounds
Chlorfenvinphos	Chlorpyrifos	C10 - 13-chloroalkanes
1,2-Dichloroethane	Di(2-ethylhexyl)phthalate	Hexachlorobenzene
Dichloromethane	Diuron	Hexachlorobutadiene
Fluoroanthene	Endosulfan	Hexachlorocyclohexane
Nickel and its compounds	Isoproturon	Mercury and its compounds
Trichloromethane	Lead and its compounds	Nonylphenols
	Naphthalene	Pentachlorobenzene
	Octylphenols	Polyaromatic hydrocarbons Tributyltin compounds
	Pentachlorophenol	
	Simazine	
	Trichlorobenzenes	
	Trifluralin	

1.3 Flood events − Impact on flood plains

Next to the more general introduction of the previous chapter, here the focus is on processes in times of flood events. First of all, floods are characterized by modified hydrological conditions and may occur when snow melts in spring or in consequence of (extreme) meteorological events such as intense rain or storm (Barnett et al. 2005, Dankers & Feyen 2008). Precipitation volumes may no longer be retained by the soil and later be evaporated by vegetation, respectively. Water level rises and river water inundates the surrounding floodplains, often causing considerable socio-economic damage. At least when flooded areas are agriculturally used or in particular when populated areas, industries and cities are affected. In the past, many parts of European river catchments have severely been flooded (Becker & Grunewald 2003, Frei et al. 2000, Hilscherova et al. 2007). Thus, rivers have been straightened, dikes were erected and meanwhile housing at river sites was hindered or prohibited, at least as long as dikes often failed to protect man and buildings (Faeh 2007, Merz & Didszun 2005).

Next to socio-economic aspects, flood events have to be evaluated in the context of sediment erosion, sediment contamination and sediment deposition on inundated areas with different, but nevertheless significant consequences. Flood events caused by intense rain come along with extensive surface run-off at first (streets, farmland) introducing especially PAHs and pesticides to the river and causing considerable toxicological impact (Boxall & Maltby 1997, Brown & Peake 2006, Donald et al. 2005, Maltby et al. 1995a, b, Rocha et al. 2007). With increasing discharge, critical sheer stress thresholds are exceeded and sediment erosion becomes the dominant source of contaminant remobilization (Gerbersdorf et al. 2007a, Haag et al. 2001, Hollert et al. 2003b, Lick & McNeil 2001, Ulrich et al. 2002,). Whereas less intensive and more frequent flood events cause minor sediment erosion, more extreme floods can cause deeper incisions remobilizing older and possibly more highly contaminated sediments. These are transported downstream and deposited later on the surface sediments. Thus, even older sediments can easily be (re-)eroded during subsequent floods and may constitute significant sources of contaminants (Alekseevskiy et al. 2008, Hollert et al. 2003a).

Contaminant introduction and deposition on catchments may require regulation and management of land use. At the river Rhine, serious flooding can leave up to 10 cm of sediments along its banks. A few millimeters per flood cycle is deposited on the remaining flood plain. Thus, over the centuries, a layer of sedimentary clay more than a meter thick has been built up at various sites (Knepper 2006). Downstream transportation and deposition through flood events may cause the removal of contaminated sediments and, thus, an improvement of the water quality (Müller et al. 2002).

SPM begins to deposit with reduction of the flow following the flood peak (Jacoub & Westrich 2006). With respect to inundated sites, the bulk of sedimentation takes place above flat areas on floodplains. Furthermore, introduced sediments and adsorbed contaminants do not necessarily remain on inundated sites. Subsequent flood events and after reflow of water, dry sediments can be drifted to other areas, causing considerable pollutions elsewhere (Asselman & Middelkoop 1995, Coulthard & Macklin 2003).

On inundated floodplains, introduced SPM preferentially deposit and accumulate in depression zones from which aeolian export is minor. Thus, these geomorphologic structures act as SPM sinks and tend to be highly polluted with particle-bound contaminants. Further on, they are closer to the aquifer layer, are loaded with elevated contaminant concentrations and increase the probability of passage through the unsaturated soil zone into the aquifer (Hallfrisch 2008).

Past flood events take an impact on adjacent areas and cause tremendous damage in times with extreme flooding (hundred-year-floods). As a result of extreme precipitation in August 2002, e.g., major flooding occurred in the catchment area of the rivers Elbe, Vltava (Moldau) and Mulde (Navratil et al. 2008, Stachel et al. 2004). Pollutants from industrial sites and municipal sewage treatment works entered the Elbe and led to serious pollution problems in the river. At the Mulde river, the Spittelwasser-Schachtgraben and communities north of Bitterfeld, a region with numerous and highly contaminated soils and sediments, was inundated. Residential areas and farmland was contaminated through deposition of polluted sediments (Brack et al. 2002a, Götz et al. 1998, Grote et al. 2005). Contaminated sediments were remobilized and flood plains were considerably impacted. This resulted in contamination of milk from cows grazing on the flood plains, and from two farms the milk had to be destroyed, since the toxicity equivalency concentration (TEQ) values were above the thresholds given by European regulation (Stachel et al. 2006, Umlauf et al. 2005).

Alluvially deposited matter of the Mulde River in the region of Bitterfeld was assessed by Brack et al. (2002a, 2003b). The load of heavy metals and organic contaminants was shown to exceed thresholds for sewage sludge. Ecotoxicological assessments by Heise et al. (2003) with sediments sampled close to Brunsbuettel just before and up to 1.5 months after the Elbe flood indicated a significantly increased toxicity after the flood event using standard luminous bacteria (*Vibrio fischeri*), algae (*Pseudokirchnella subcapitata*), bacterial (*Bacillus cereus*) and nematode assays (*Caenorhabditis elegans*).

1.4 Contaminants in the groundwater and aquifers

Organic contaminants that were introduced into the aquatic environment are of high diversity with respect to their physico-chemical properties and molecular structures, as well as to environmental compartments, e.g., for transformation and transportation processes. Thus, fate and distribution of compounds are complex and require differentiated evaluation (Reichenberg & Mayer 2006).

In this context, groundwater pollution has been regulated in the 1976 Dangerous Substances Directive, which was later replaced by the Groundwater Directive 80/86/EEC (Lanz & Scheurer 2001). With this new legislation, contaminants were divided into two categories: (a) substances that must be prevented from entering groundwater ('black list') and (b) substances the introduction into the groundwater of which must be limited ('grey list'). The Groundwater Directive is of limited value and will expire and be repealed by the WFD by the end of 2013 (Lanz & Scheurer 2001, Mostert 2003).

Contaminants may enter the riverine groundwater through pressure of river flow towards the bank, at least when landside groundwater flow and ground gradient in direction to the river is plain. Besides, contaminants can pass the unsaturated zone and enter the aquifer through the soil, in particular at frequently inundated sites, e.g. floodplains and retention areas (Boulding & Ginn 2004). Compounds are either hydrophilic or hydrophobic and, thus, accumulate in the water phase or on the surface of suspended matters. Vertical flux in the water-unsaturated zone as well as horizontal flux in the saturated zone has to be expected; an associated transport of dissolved and particle-bound contaminants can be observed (Schwarzbauer 2006). In particular, the heterogeneous soil composition caused by many flood dependent shifting is of major importance for the evaluation of the risk of contaminant transportation through the soil (McKee et al. 1967).

Many studies worked on groundwater contamination and focused on inorganic contaminants such as arsenic, lead and copper pollution (Black & Williams 2001, Fernandez-Galvez et al. 2007, Pasternack & Brown 2006). In contrast, groundwater and aquifer water has usually been found contaminated with a variety of personal care products, pharmaceuticals, herbicides and others more (Scheytt et al. 2007, Zhang et al. 2008a). In contrast, to date, organic contaminant introduction from inundated floodplains and retention areas into the groundwater has hardly been assessed.

One of the rare studies (Rudis et al. 2009) analyzed the deposition of zinc-polluted sediments which were transferred by a catastrophic flood; this was related to changes in groundwater quality. Modeling the fate of pollution leached from settled sediments to groundwater could be show that zinc can leach to the groundwater aquifer both from bottom sediments of a pond into the saturated zone and from the flood-pool sediments into the unsaturated zone and thereafter into the saturated zone.

A more holistic approach was applied in the AquaTerra project that worked on a better understanding of river-sediment-soil-groundwater systems (Gerzabek et al. 2007). New field and laboratory observations as well as historical data are assembled and addressed for the catchments of the Ebro, Meuse, Elbe and Danube Rivers and the Brevilles Spring. In the same project, Barth et al. (2007) showed that for sediment transportation highest deposition rates were given for β-hexachlorocyclohexane (β-HCH) in river sediments at hot-spot areas of the Mulde River in the Bitterfeld region (Elbe Basin, Germany). However, no clear answer was provided with respect to contaminant entry of floodplain soils in the groundwater.

Meanwhile, conflicts of interests emerge at different sites when, e.g., drinking water protection areas and flood retention areas overlap. On the one hand, retention areas have to be provided to manage in particular extreme flood events (Frerichs et al. 2003); on the other hand, these areas frequently overlap with other land usages. Such a conflict of interests is given next to Karlsruhe, were a waterworks (Kastenwoert) shall be constructed close to a retention area (Bellenkopf-Rappenwoert), which has been designed to be used in the case of an extreme flood event (events with a return period of more than a 100 years and a highly destructive potential). This potential for conflict was subject of a joint BMBF research project: “Flood retention and drinking water supply − Preventing conflict of interest” (Maier et al. 2006).

1.5 Objectives of the study

This study aimed to investigate on the hazard potential of contaminants that are bound to suspended particulate matter (SPM), in particular with respect to impact of flood events. Therefore, in a first part flood SPM was sampled during floods with recurrence intervals of one and eight years at the rivers Rhine and Neckar, Germany, and assessed using a broad battery of *in vitro* biotests to detect both, acute and mechanism-specific adverse effects. Exposure of RTL-W1 cells of the rainbow trout (*Oncorhynchus mykiss*) were used to determine cytotoxicity in the Neutral Red retention assay. Dioxin-like and aryl hydrocarbon receptor (AhR)-mediated activity was assessed with the EROD assay. Samples causing elevated effects were chosen for effect-directed analysis (EDA) and fractionated in order to reduce the complexity of the environmental samples. Subsequent, fractions were investigated with biotests to identify biologically active fractions and possibly the effective contaminants.

Thus, hypotheses to be verified were:

- SPM sampled in flood events indicate elevated toxicities
- Elevated toxicities can be determined using *in vitro* biotests and different (eco-)toxicological endpoints
- Effect-directed analysis is a powerful tool that can be used to identify active fractions and effective compounds in complex environmental mixtures
- Chemical analysis allows to determine active compounds within the fractions
- The concept of toxicity equivalency concentrations (TEQs) can be used to determine the quota of the analyzed compounds to the total biological activity of the extracts and fractions

In a second part of this thesis, the angle of view was extended to the impacts of river contaminants on inundated sites, in particular areas that are planned to be used as retention basins in the case of extreme flood events. In a pilot study, a potential hazard of river contaminants to the groundwater and, thus, drinking water resources, was indicated at one of these projected retention basins that, therefore, was used as model site. Therefore, a long-term study was carried out and samples were taken throughout two years. Further, soil was sampled at inundated and non-inundated sites at the basin and groundwater at wells within the retention basin.

Samples were assessed using an extended set of *in vitro* biotests. Next to the tests named above, the Ames Fluctuation assay using the bacterial tester strains TA 98 and TA 100 (*Salmonella typhimurium*) was used to determined mutagenic potentials. Further, the Yeast Endocrine Screen (YES) assay with bakery yeast (*Saccharomyces cerevisiae*) was used to investigate endocrine activities. These investigations aimed to show hazard potentials of contaminants that are transported in the fluent wave in floods to inundated sites and are deposited there. Further, contaminant introduction in the groundwater and drinking water resources was in the focus of research. Thus, hypotheses to be verified were:

- Biological hazards and chemical loads increase with more extreme flood events
- Long-term assessment of SPM allows to determine biological hazard potentials in flood events that can be ranked with hazards in times without floods
- Deposition of flood SPM can be shown with soil sampled at inundated sites and compared to soil of non-inundated sites
- Long-term investigation of groundwater shows modifications in contaminant composition and biological activity in consequence of flood events
- The application of a recently developed fractionation method allows usage of EDA in order to determine active fractions and to compare contamination pattern among samples
- Further, this fractionation method provides new findings with respect to more polar and polar compounds

- Finally, these investigations assist to evaluate the risk of contaminant transfer from the fluent wave to retention basins and the hazard of groundwater contamination caused by flood events

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Section A

Impact of sediment dynamics and hazard potentials of suspended particulate matter

Chapter 2

Influence of hydrodynamics on sediment ecotoxicity

H. Hollert[1], M. Duerr[2], I. Haag[3], J. Wölz[1], K. Hilscherova[4], L. Blaha[4], S. Gerbersdorf[5]

[1] Department of Zoology, Institute of Zoology, University of Heidelberg, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany

[2] Hygiene Institute Halle, Johann-Andreas-Segner Street 12, 06097 Halle (Saale), Germany

[3] Consultant Engineer Dr.-Ing. Karl Ludwig Water Economy - Hydraulic Engineering, Herrenstr. 14, 76133 Karlsruhe, Germany

[4] Masaryk University, RECETOX, Kamenice 3, 62500 Brno, Czech Republik

[5] University of St. Andrews, School Biology, Gatty Marine Laboratory, East Stands, Fife, St. Andrews, Scotland, KY16 8LB, United Kingdom

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Hollert H, Duerr M, Haag I, Wölz J, Hilscherova K, Blaha L, Gerbersdorf S (2007): Influence of hydrodynamics on sediment ecotoxicity. In: Westrich B , Foerstner U (Editors), Sediment hydraulics and pollutant mobility in rivers. Springer-Verlag, Heidelberg, pp. 401-416; ISSN 1863-5520, ISBN 978-3-540-34782-8

2.1 Role of sediments in freshwater quality

There is general agreement that sediment-bound substances are of major importance for the fate and effects of trace contaminants as well as water quality in aquatic systems. Sediments can act as sinks for various pollutants but could also become a contamination source under certain circumstances such as dredging or flood events (Ahlf et al. 2002a,b, Foerstner and Müller 1974, Hollert et al. 2000a, 2003a). Contaminated sediments are known to cause various adverse effects on organisms even when contaminant levels in the overlying water are low (Chapman 1989). Thus, monitoring and assessment of sediment quality is of prime significance for national legislation in general and for the implementation of the European Water Framework Directive in particular (SedNet 2004). Especially through the activities of SETAC North America (Wenning et al. 2005, Wenning and Ingersoll 2002) and the European SedNet network (Salomons and Brils 2004, SedNet 2004) sediments related issues were given increasing attention in both science and the public. While water quality has notably improved over the past three decades, the sediments in many European river basins still retain the toxic heritage from the past era of uncontrolled industrial production, and which will continue to influence the quality of waters significantly for many years to come (Salomons and Brils 2004, SedNet 2004).

Since the 1970s several chemical analytical studies revealed elevated concentrations of dominant environmental contaminants such as heavy metals and organic pollutants in marine and riverine sediments using chemical analyses (Foerstner et al. 2004, Foerstner and Müller 1974, Giger et al. 1974, Haag et al. 2001, Stoffers et al. 1977). These hazardous contaminants are often accumulated in deeper layers covered by relatively unpolluted sediments, and, thus, are sequestered from the bioavailable oxic sediment surface zone (Haag et al. 2001, Ziegler 2002). However, these chemicals are mostly persistent in the natural environment, and can enter the oxic water column after an erosion events such as bioturbation (Chapman et al. 1992), flood events (Hollert et al. 2000a, 2003b) or dredging and relocation of sediments (Koethe 2003). Consequently, toxicants can become bioavailable (Calmano et al. 1993, Simpson et al. 1998, Ziegler 2002) and may result in detrimental effects on aquatic organisms at various trophic levels. Furthermore, downstream transport and deposition of contaminated particles in inundated areas may also result in negative effects on biota in these regions (Japenga and Salomons 1993).

With a delay of more than one decade to the first geochemical studies, the assessment of biological consequences of particle-bound pollutants has become a major topic in international water research (Burton 1991, Giesy and Hoke 1989, Power and Chapman 1992). To date, most studies focused on the development of suitable bioanalytical methods and the assessment of their potential to investigate sediment-bound contaminants. However, the role of sediment remobilization and possible ecotoxicological effects of contaminants bound to suspended material has been scarcely investigated.

2.2 Factors affecting mobilization of sediments and (bio-)availability of contaminants

In many river systems, hazardous contaminants are predominantly transported in association with suspended particulate matter. The suspended particles and the sediment-bound pollutants accumulate in regions of low turbulence, such as groyne fields, harbors, and river reservoirs forming thus sites with high levels of contamination.

An important issue related to the role of sediments in water quality is their potential to be subject to remobilization, transport and redistribution during certain environmental events such as floods. Although these processes increase accessibility and bioavailability of contaminants, the conditions under which these processes occur, their amplitude and possible role in contaminant accessibility and effects are still poorly understood. The complexity of cohesive sediments, which are biologically active and chemically reactive, precludes the definition of a general analytical theory for their resuspension behavior. Moreover, the sediment properties of cohesive sediments vary on a number of spatial, temporal and vertical scales (Gerbersdorf et al. 2005, 2007) and empirically based field and laboratory experiments are needed to elucidate the mechanisms which govern the erosion resistance of cohesive sediments. As well, interdisciplinary studies are needed, to obtain better and realistic conceptual understanding of natural sediments and their inherent physical and biological complexity (Black et al. 2002). However, either physico-chemical or biological sediment properties have been in the focus of research on their impact on sediment stability, and only recently, the first comprehensive investigative approach to derive master-variables affecting sediment stability was published (Gerbersdorf et al. 2005, 2007).

Over the past decades a numerous studies have been conducted that primarily addressed isolated aspects of sediment pollution issues. Recently the fate of particle-bound pollutants and hydrodynamic transport processes has been addressed increasingly in interdisciplinary joint projects. These studies documented that particle-bound priority pollutants (e.g., EPA-PAHs) are major contributors to both the overall contamination and transport of lipophilic pollutants in rivers. Work that significantly contributed to these findings were, among others, the DFG-Research Group 371 or the interdisciplinary BMBF-funded joint project, SEDYMO (Foerstner et al. 2004, Foerstner and Westrich 2005). However, the questions regarding physico-chemical surface properties of suspended particles, chemical mobilization and biological degradation of pollutants as well as regarding the related bioavailability of contaminants and their toxicity have not been satisfactorily addressed to date. Especially, the important link between the erosion potential and hazard potential of sediments/distinctive sediment horizons originating from contaminated riverine sites, need to be addressed in future studies if a realistic risk assessment is to be derived.

The fate of the contaminants associated with sediments is strongly influenced by the amount and type of the sedimentary organic matter, which reflects the environmental evolution in the drainage area and fluviative or lake depositional systems (Martínek et al. 2006, Stout et al. 2002). The geochemical parameters of organic matter are controlled by the interplay of biomass productivity, weathering during transport, and microbial reworking during and shortly after deposition (Peters et al. 2005).

Valuable monitoring data have been collected on the contaminants in sediments. However, only limited data exist on the associated organic matter and the role of different organic matrices for the fate of pollutants is insufficiently documented and not well understood (Stout et al. 2004). Fresh sedimentary particles behave in a different way when compared to the re-deposited older sediments, even if the content of pollutants is similar. It is, therefore, highly desirable to integrate the role of natural organic matter of different biological origin, mainly terrestrial plants, woody material, bacteria and algae (Gonzáles-Vila et al. 2003, Meyers 2003) into the ecotoxicological assessment of complex sedimentary systems of rivers and their relevance for potential contaminant bioavailability. The extracellular polymeric substances (EPS) excreted by microorganisms such as microalgae or bacteria, can be a significant part of the total organic pool. These polymeric substances have received more and more attention over the last years due to their role in biostabilization of sediments (e.g., De Brouwer et al. 2000, Paterson et al. 2000). Only recently, the importance of EPS for the erosion resistance could be shown for several contaminated freshwater sites (Gerbersdorf et al. 2005, 2007). Concerning the fate of the contaminants, these polymeric substances influence as well the nature of the eroded material, but this work is at an early stage (Perkins et al. 2004). Depending on floc characteristics such as floc size and floc strength, the adsorption/degradation processes of the associated contaminants will change as well as the lateral particle transport until deposition (Droppo 2004). Thus, the binding capacity of the polymeric substances, as well as their influence on the nature of the erodible material should be addressed by investigating the quantity and the quality of the EPS in order to contribute to the questions on the bioavailability of contaminants.

2.3 Ecotoxicological methods to assess sediment contamination

As discussed in the previous paragraphs, cohesive stability of sediments and their mobilization leads to increased bioavailability of hazardous contaminants. Sediment mobilization is affected by numerous physico-chemical, geochemical and biological parameters that are poorly understood and that have been scarcely investigated by complex interdisciplinary research projects. In spite of intensive research and development of numerous model testing systems, little is still known about possible ecotoxicological consequences of mobilized sediment contaminants. To evaluate adverse effects on ecosystems, neither biotests nor chemical-analytic techniques alone are sufficient. In contrast, a combination of biotests and chemical methods allows comprehensive insights into the hazard caused by sediment contamination.

To monitor the sediment quality, ecotoxicological bioassays are first applied to screen if contamination had significant effects on biological functions of the model organisms/ systems. A broad spectrum of test batteries of standardized bioassays has been used to assess the possible hazardous effect of particulate matter and elutriate. The bioassays included *in vivo* tests at different levels of the aquatic food chain and *in vitro* tests. Various microbiological toxicity tests have been developed and validated for use in sediment risk assessment during past 20 years (Ahlf et al. 1989, van Beelen 2003). It was shown that contamination correlates with the shift in microorganism communities toward toxicant-resistant species and that persistent toxic effects on the micro flora caused for example by zinc, cadmium and copper often occur at concentrations lower than European Community limits (van Beelen 2003). Other assays for ecotoxicological studies include the algae growth inhibition assay, the bacterial bioluminescence bioassay, and the Daphnia assay (den Besten et al. 2003, Koethe 2003). Since fish are representing vertebrates, and can be linked via bioaccumulation to humans, large efforts have been undertaken to develop fish-based test systems for the assessment of sediment bound substances (Chen and White 2004, Davoren et al. 2005, Hilscherova et al. 2000, Hollert et al. 2000a, 2005, Kammann et al. 2005a, Kosmehl et al. 2004, US-EPA 2002). In addition to *in vivo* sediment exposure tests with fish, a number of suborganismal assays are in use such as cell-based *in vitro* systems (Davoren et al. 2005, Hollert et al. 2000a, Kosmehl et al. 2004, Segner 1998), the fish egg assay with *Danio rerio* (Hallare et al. 2005, Hollert et al. 2003b). While acute toxicity was of major concern in the last decades, recently for many river basins a change in focus to more subtle specific chronic non-lethal effects occurred (Brack et al. 2005a). While these effects are difficult to assess using *in vivo* tests, they can be relatively easily determined by *in vitro* techniques that allow to predict toxic potentials of complex environmental mixtures (Janošek et al. 2006). The *in vitro* bioassay approach serves as efficient, fast and cost effective screening for evaluation of the receptor-mediated activities of the complex mixtures (Hilscherova et al. 2002). We have successfully used this approach to prioritize contaminated sediment sites (Hilscherova et al. 2003, Hollert et al. 2002a) and to study novel endocrine disruptive effects observed in situ (Blaha et al. 2006). A further advantage of a bioassay approach is, that the combination of different bioanalytical methods allows to investigate multiple endpoints such as genotoxic or mutagenic (Chen and White 2004, Kosmehl et al. 2006), dioxin-like (Hilscherova et al. 2002, Hilscherova et al. 2001, Hilscherova et al. 2000), or various endocrine effects (Ankley et al. 1998, Sumpter and Johnson 2005) in parallel in the same sample.

2.4 Combined approaches to investigate the influence of hydrodynamics on sediment ecotoxicity

Recently, in several studies toxicity has been evaluated at various sediment depths (Burton Jr. et al. 2001, Hollert et al. 2003a, Kosmehl et al. 2004), showing for at least some of the locations a dramatically increase of chemical contamination and toxicity with the sediment depth. For several European river basis, including Neckar, Rhine and Elbe, highly contaminated old sediments can be described as 'potential chemical time bombs' (Cappuyns et al. 2006, Japenga and Salomons 1993). An important process which may remobilize such sediments and which is of still increasing importance in relationship to the global climate change is more often occurrence of stronger floods in Europe as well as in other parts of the world. To understand and predict possible toxicological and ecotoxicological consequences of contaminants mobilized from sediments by flood events it is necessary to develop scientific approaches for the assessment of regularly flooded rivers. The combination of hydrodynamics and ecotoxicological investigations is devolving to an emerging field of research. Recently, it was shown that hydrodynamic aspects can be involved as additional Line-of-Evidence in Weight-of-Evidence studies assessing the impact of sediments (Chapman and Hollert 2006). In the last five years several studies were published addressing the ecotoxicological impact of flood events (Brack et al. 2002, Grote et al. 2005, Hollert et al. 2000a, Oetken et al. 2005, Matthaei et al. 2006, Sect. 10.2) or using combined approaches for evaluating flood events and the risk of erosion (Babut et al. 2006, Haag et al. 2001, Hollert et al. 2000b, 2003a).

In this context, studies on the Elbe flood in 2002 indicated elevated effects in bioassays (Heise et al. in prep). Moreover, cellular changes could be found in livers from flounder (*Platichthys flesus*) and digestive glands of blue mussels (*Mytilus edulis*), 5 month after the flood disaster in the Elbe Estuary and the Wadden Sea (Einsporn et al. 2005). In comparison to earlier data from long-term studies at the same stations, a significant impairment in the function of cell organelles (lysosomes), involved in the detoxification and elimination of pollutants in fish liver, was found. In addition, in a long time study, EROD activity was measured in livers of dab (*Limanda limanda*) from the German Bight (North Sea) from 1995 to 2003 (Kammann et al. 2005b). In autumn 2002, significantly elevated EROD activities were detected, possibly related to effects of the river Elbe flood event.

These findings support the hypothesis that extreme flood events can affect not only freshwater ecosystems but also marine systems and have deleterious effects on animal health. Furthermore, flood events can influence floodplains and wetlands negatively (Schwartz et al. 2006, Ulrich et al. 2002). Consequently, the risk of extreme flood events for drinking water supply will be an emerging topic in the future (Maier et al. 2006).

In conclusion, research should consider the potential of sediments to serve as sources of contamination for the aquatic ecosystem, for drinking water supply but also for the floodplain soils and other flooded areas. In the following case studies, two examples for such integrated approaches addressing the risk of erosion are presented briefly.

2.5 Case Study River Neckar (Germany)

During the seventies, the river Neckar in Southern Germany ranged among the most strongly contaminated rivers in Germany with high loads of both organic pollutants and heavy metals (Förstner and Müller 1974). For instance, cadmium loads were increased by a factor of up to 300, when compared to pre-industrial clay stone sediments. As a consequence of sewage treatment, the quality of water and sediments improved significantly, and today the Neckar can be classified among Germany’s moderately contaminated rivers, however, with heavily loaded old sediments at some sites (Hollert et al. 2000a). Hence, earlier studies within the Neckar catchment area or the river Neckar itself, revealed moderate to strong ecotoxicological effectiveness in several bioassays for mutagenic, genotoxic, endocrine, teratogenic, and dioxin-like responses as well as correlations between biological effects and concentrations of organic pollutants (Hollert et al. 2005, 2002a,b, 2003b).

The objective of the presented study was to develop a combined ecotoxicological and hydraulic approach by the cooperation between the Universities Stuttgart and Heidelberg to elucidate the ecotoxicological implications associated with the risk of erosion of contaminated sediments (Hollert et al. 2000b, 2003a). This integrated strategy was applied to the lock-regulated river Neckar in Southern Germany (Haag et al. 2002, 2001, Hollert et al. 2000b, 2003a, Knauert et al. 2004). For this purpose, sediment cores of the heavily contaminated Lauffen reservoir/river Neckar were investigated (A) as well as suspended particulate matter during a flood event in the river Neckar (B) in order to give the potential and effective pollution risk under different hydraulic scenarios (Fig. 1).

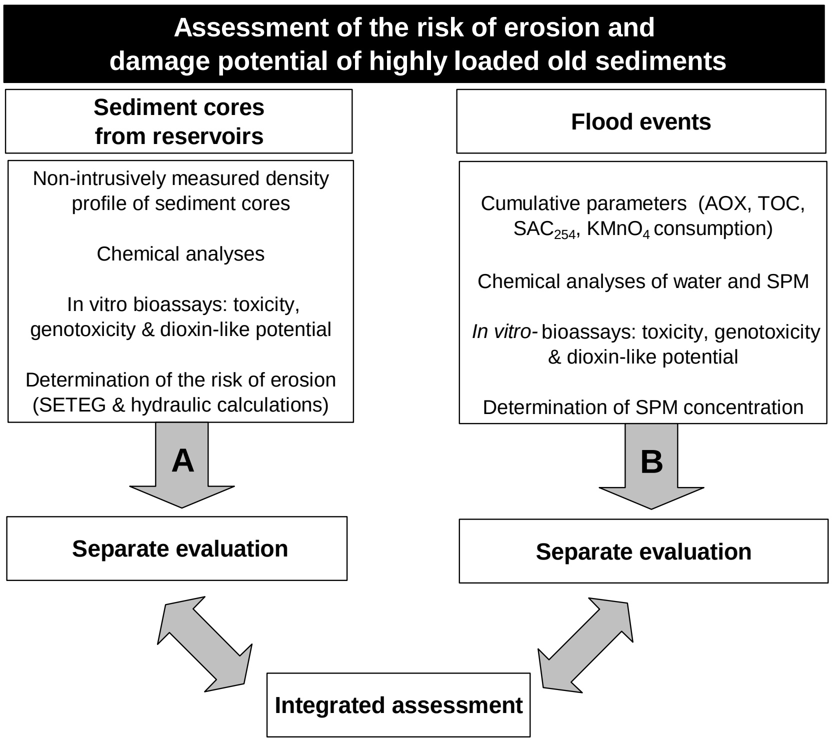

Fig. 1 Test strategies for examination and evaluation of the remobilization risk of old sediments in lock-regulated river systems (redrawn from Hollert et al. 2000).

2.5.1 Methods

a) Two undisturbed sediment cores (13.5 cm in diameter and 150 cm in length) were taken from one location in the backwater region of the Lauffen reservoir/river Neckar in south-west of Germany (in total 7 locations and 16 sediment cores). In both cores, vertical profiles of bulk densities were measured in 1 cm steps non-intrusively by using a γ-ray-densitometer. Thus, similar sediment layering within the parallel cores was ensured as well as subsequent sampling of the appropriate sediment layers (Haag et al. 2001). If, on the basis of the density profiles, parallel cores were considered to be similar, one of them served to experimentally determine the critical shear stress of mass erosion ($\tau c,e$) as a function of sediment depth. Erosion experiments were carried out in a rectangular water flume, the so called SETEG-system (Kern et al. 1999). The second one of the parallel cores was sectioned into layers of almost uniformly texture, thus, the core was cut at depths of significant bulk density changes. From this material, concentrations of heavy metals and PCBs were identified by chemical analyses while the cytotoxicity, dioxin-like activity and mutagenicity were investigated by bioanalytical methods (Kosmehl et al. 2004, Seiler et al. 2006). By comparison of the critical shear stress/sediment stability of the investigated sediment cores with the natural occurring bottom shear stresses, calculated by the 1-D flow and transport model COSMOS (Kern and Westrich 1997), the possible resuspension risk of contaminated sediment layers could be predicted.

b) In order to gain insight into the ecological effects of a possible remobilization of heavily contaminated old sediments, suspended particulate matter (SPM) was collected in SPM traps from two sites of the lock-regulated section of the river Neckar: downstream the Lauffen reservoir with its high cadmium contaminations and downstream the less polluted Heidelberg reservoir (reference site). Parameters investigated are presented in fig. 1.

2.6 Results and Discussion

The combined hydraulic and ecotoxicological approach revealed the high risk of erosion down to depth of 70 cm as well as an ecotoxicological hazard potential of the associated contaminants (Haag et al. 2002, Hollert et al. 2000, 2003).

Clear cut changes in bulk densities, the percentage of particles size $d < 20$ µm and ^{137}Cs content support the hypothesis of an erosional unconformity (Fig. 2).

An erosional unconformity is the result of a flood event, where fine grained sediments are resuspended and non-cohesive particles are re-deposited (Haag et al. 2000). In the vertical sediment profiles, layers with coarse particles, low TOC and consequently increased bulk densities could be detected mostly below 25 cm depth. Often these layers were also characterized by sudden decreases of $\tau c,e$ in the corresponding parallel core, indicating the predominance of non-cohesive particles (Fig. 2).

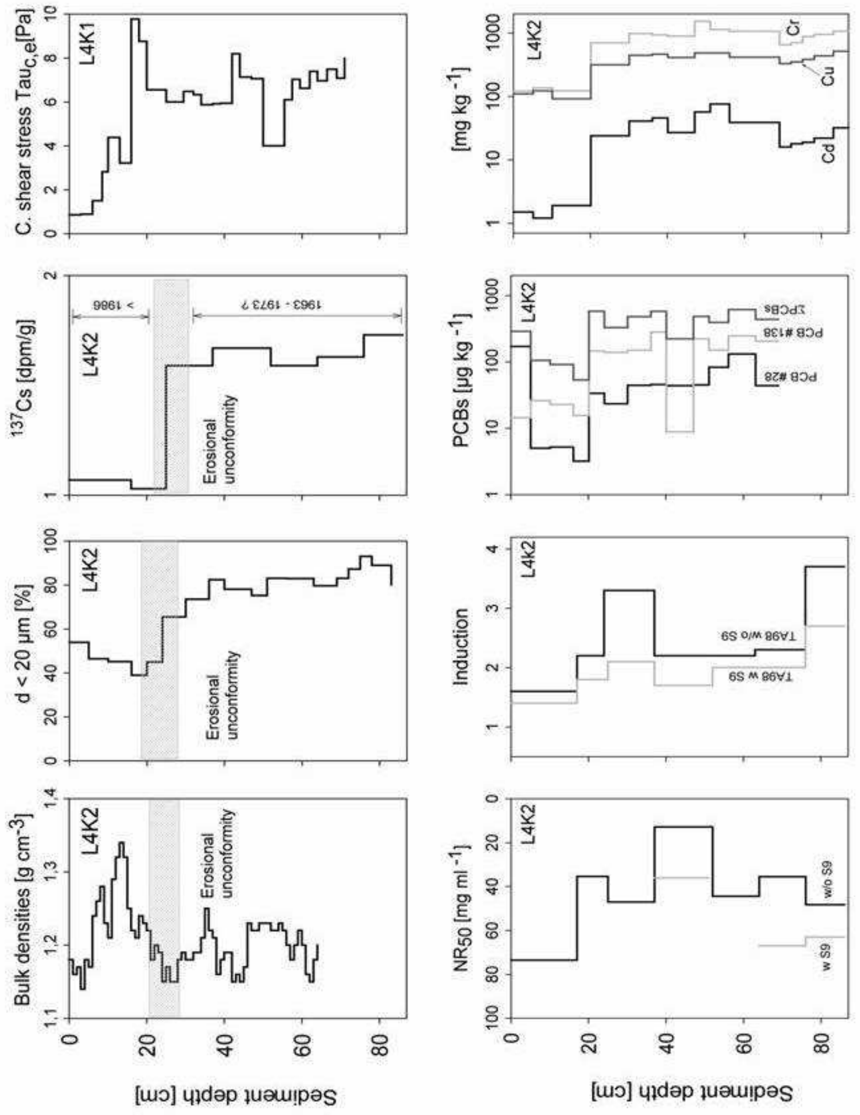

Fig. 2 Non-intrusively measured density profile, d < 20 µm, critical shear stress, cytotoxicity, mutagenicity, heavy metals and PCBs of core LN4K2 from the Lauffen Reservoir on the Neckar River depending on the depth (according to Hollert et al. 2003).

Bioanalytical and chemical investigations (Fig. 2) were showing clear-cut changes of the ecotoxicological hazard potential below the depth of the erosional unconformity. The younger sediments within the top 25 cm depth revealed neither strong cytotoxicity nor mutagenicity. In contrast, for the older sediments below that zone, a strong cytotoxicity, dioxin-like and mutagenic potential could be determined. PCBs and anthropogenic influenced heavy metals such as Cd and Pb showed up to 100 times higher concentrations in the sediment layers below the erosional unconformity. Concentrations above 10.8 mg/kg of cadmium and 193 mg/ kg copper, respectively, allowed the classification of these sediment layers to the older, highly contaminated sediments (HCS). In contrast, the upper layers represented low contaminated sediment layers (LCS, Haag et al. 2001, Hollert et al. 2003a). Since this unconformity happens in a transition zone between younger, less contaminated and older, heavily contaminated sediment layers, the last flood must have exposed not only deeper sediment layers but also their contamination load.

The suspended matter of the high discharge - return periods of 15 to 20 years (Hollert et al. 2003a) - exerted significantly higher cytotoxicity and mutagenic activity (Fig. 3) than a moderate flood with a 1-year return period (Hollert et al. 2000a). These findings supported the conclusion that the observed ecotoxicological effects during major floods may be due to the in-stream erosion of highly contaminated bottom sediments.

Recently, SPM of a flood event at the Neckar in 2004 with a recurrence interval of five year was sampled using a sediment trap. Highest EROD activities of the extracts could be found for the peak of the flood, with a ten time higher dioxin-like activity when compared to other SPM samples (Wölz et al. 2008). The two samples with the highest effects have been used for effect directed analyses. Using the shown strategy it is possible to investigate the risk of erosion. However, the identity of the pollutants causing effects in the bioassay is still unknown.

Effect-directed analyses is a strategy to gain insight into the character of the noxious substances (Brack 2003, Brack et al. 2005b). Organic extracts from SPM sampled during the 2004 flood events was fractionated for polarity and aromatic properties according to a previously developed methodology (Brack et al. 2005b). Only the fractions revealing high toxicity on bioassays are used for chemical analyses in order to identify the toxic substance class or substance. Using this approach, it was possible to elucidate PCBs and dioxins/furans to contribute only for less than 1 % of the biologically derived EROD activities. The EROD activities of the fractions with PAHs explained the major part of the Dioxin-like potential of the crude extracts. However, the measured US-EPA priority PAHs contributed less than 20 % to the total EROD activities (Wölz et al. 2008).

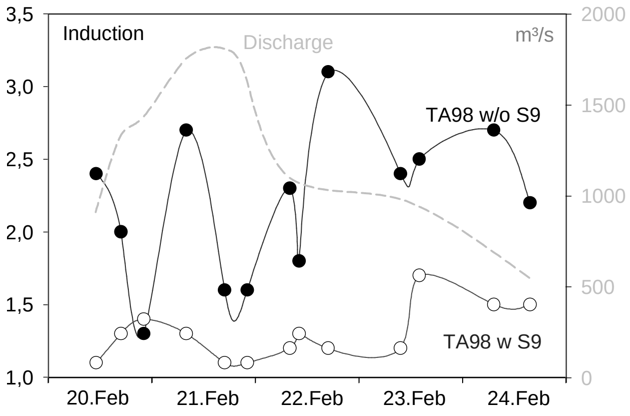

Fig. 3: Time-course of the mutagenicity during the flood event of Oct/Nov 1998. Since for a moderate flood event (HQ = 1) no mutagenicity could be found, several SPM extracts revealed genotoxic effects in the Ames-Test without S9 mix.

2.7 Case Study Morava Catchment Area (Czech Republic)

Major flooding events also occur regularly in the catchment area of the river Morava (Czech Republic). Water and sediment quality in this area has been impacted by historical industrial activities within the watershed. In July 1997 the region was affected by disastrous floods caused by two periods of exceptionally heavy rainfalls that resulted in great material and ecological damages. Extensive rainfall plagued the north part of the Morava River basin and the situation was even more complicated by the second flood wave within 10 days period. In historical context it was very rare event but due to human landscape interventions it is possible to expect similar events still more frequently. During the flooding period lasting for several days, older sediments were washed away and new silt materials were deposited up to several centimeters layer. Because of our earlier monitoring of this area, the situation brought unique opportunity to evaluate the changes in contaminant levels and the toxic effects in relation to flood events. Initial evaluations of the target contaminant profiles in sediment and water samples from several sites revealed that there was a gradient of concentrations along the Morava River from upstream to downstream, and suggested that the tributary of the little stream Drevnice serves as a source of pollution to the Morava River (Hilscherova et al. 2001, Holoubek et al. 1998). There are no limit values for sediment contaminants in the Czech Republic but the concentrations of polycyclic aromatic hydrocarbons (PAHs) as well as other organic compounds were above the maximal permissible limits that apply for instance in the Netherlands, as were the concentrations of Cd and Zn for soils. Most studies have been performed with the freshly contaminated top sediment layers, but still there is only little information on the deeper layers that might be mobilized during frequent floods.

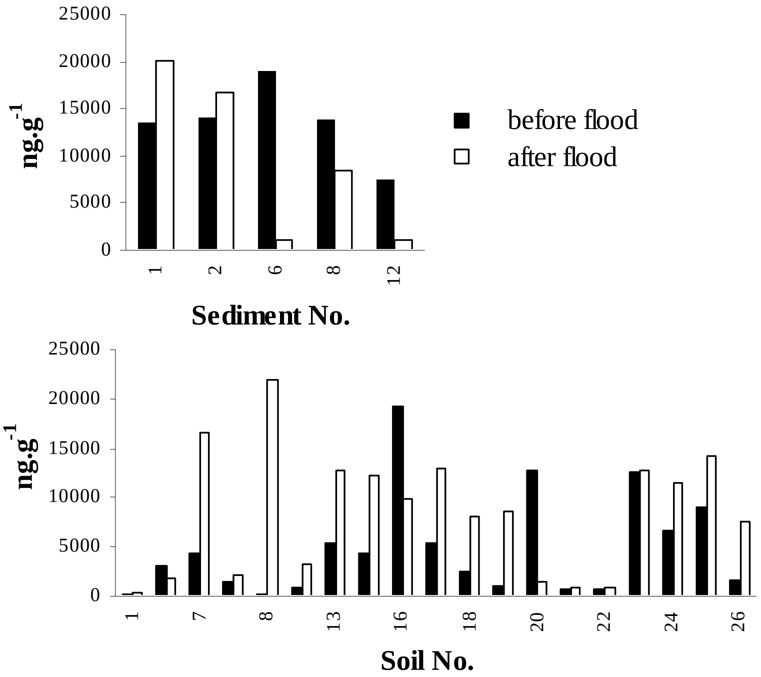

Fig. 4: Effect of floods in 1997 on concentrations of PAHs (sum of 16 US-EPA PAHs) in sediments and floodplain soils of the river Morava Catchment area.

Previous investigations have also shown the impact of floods on the periodically flooded soils with significantly elevated contaminated levels namely with persistent organic compounds (Hilscherova et al. 2001, Holoubek et al. 1998), and some heavy metals (recent unpublished data). The most obvious changes related to major floods in 1997 were observed for PAHs, the dominant contaminants in the area. The results clearly showed that in some regions there was significant decrease in PAHs concentrations in riverine sediments after the floods while the concentrations in the surrounding soils at most sites within the flood affected area significantly increased (Fig. 4). Application of *in vitro* biotests has shown significant toxic, genotoxic, dioxin-like, and estrogenic potentials in sediments collected from numerous sites (Hilscherova et al. 2002, 2001) and the bioassay results confirmed significant effects of floods. Both dioxin-like and estrogenic activities in sediments were generally either unaffected or significantly decreased after floods (Fig. 5 and 6) showing removal of upper contaminated layers and their transport downstream by the flood water.

The greatest added value of *in vitro* assays is that they provide an integrative measure of the potential of the complex mixture of compounds within the sample that may cause a negative effect through the specific mechanism of action. They serve as rapid, sensitive and relatively simple screening systems evaluating the presence of chemicals and their mutual interactions with specific mode of action. Fractionation of extracts enables separation of compounds present in the complex mixture and allows determination of the most active classes of compounds. In the study in part of the Morava catchment area, the simple fractionation procedure also revealed the important role of mediate polar PAHs and pesticides for both the estrogenic and dioxin-like effects (Hilscherova et al. 2001, 2002) which was confirmed by the mass balance calculations (Hilscherova et al. 2002, 2001). Further, mechanism-specific bioassays were confirmed to be an effective tool in initial screening of river sediments compared to the more time- and cost-demanding instrumental analyses.

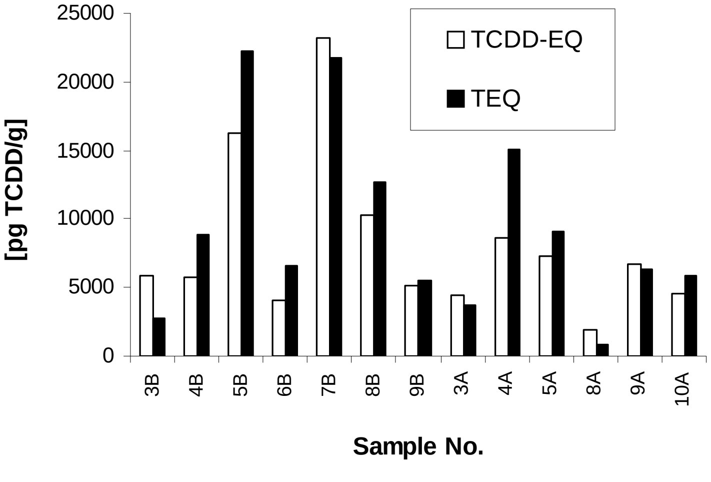

Fig. 5: Dioxin-like equivalents determined in bioassay with H4IIE.luc cells (TCDD-TEQs) and by chemical analysis (chem-TEQs) of organic extracts from sediments sampled in Morava Catchment area before ('B' samples) and after ('A' samples) the major floods in 1997 (Hilscherova et al. 2001).

In vitro biotests have shown significant toxic, genotoxic, dioxin-like, and estrogenic potentials in sediments collected from numerous waters (Hilscherova et al. 2002, 2001). However, most studies have been performed on the freshly contaminated top sediment layers, and there are no information regarding the deeper layers of sediments that might be mobilized during frequent floods.

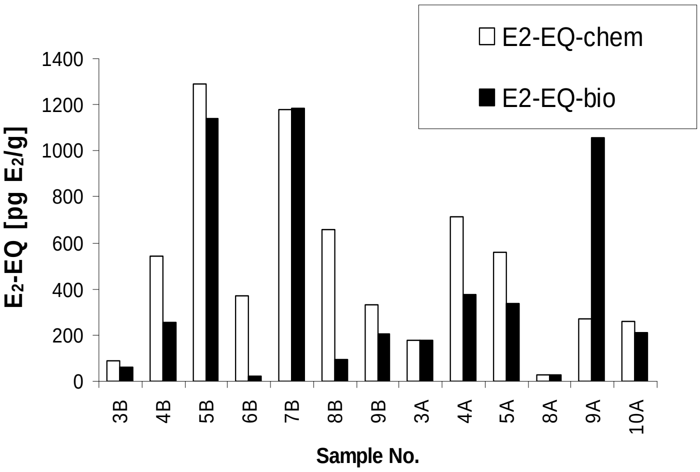

Fig. 5: Estrogenic equivalents determined in bioassay with MVLN cells (E_2-EQ-bio) and by chemical analysis (E_2-EQ) of organic extracts from sediments sampled in Morava Catchment area before (B) and after (A) the major floods in 1997 (according to Hilscherova et al. 2002)

2.8 Conclusions

The present article features the urgent need to cross disciplinary boundaries in order to derive a realistic assessment regarding the erosion risk of old deposited sediment layers as well as the bioavailablitiy and hazard potential of their associated contaminants at different aquatic sites. Especially the combination of hydrodynamic and ecotoxicological methods will give (i) comprehensive insights into the effects of flood events on biota and ecosystems and (ii) allow evaluation of sediment and, thus, water quality with regard to the global change and the expectations of more severe floods in the near future.

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Chapter 3

Changes in toxicity and Ah receptor agonist activity of suspended particulate matter during flood events at the rivers Neckar and Rhine

Jan Wölz[1,2] & Magnus Engwall[2] & Sibylle Maletz[1,2] & Helena Olsman Takner[2]
& Bert van Bavel[2] & Ulrike Kammann[3] & Martin Klempt[4] & Roland Weber[2,6]
& Thomas Braunbeck[2] & Henner Hollert[1]

[1] Department of Ecosystem Analysis, Institute for Environmental Research (Biology V), RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

[2] Man-Technology-Environment Research Centre (MTM), Department of Natural Science, University of Oerebro, 70182 Oerebro, Sweden

[3] Institute for Fishery Ecology, Federal Research Institute for Rural Areas, Forests, and Fisheries, Johann Heinrich von Thuenen-Institute (vTI), Palmaille 9, 22767 Hamburg, Germany

[4] Department of Safety and Quality of Milk and Fish Products, Federal Research Institute of Nutrition and Food, Max-Rubner-Institute (MRI), Hermann-Weigmann-Straße 1, 22767 Kiel, Germany

[5] Department of Zoology, Aquatic Toxicology and Ecology Section, University of Heidelberg, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany

[6] POPs Environmental Consulting, Ulmenstraße 3, 73035 Goeppingen, Germany

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3.1 Abstract

Background, aim, and scope

As a consequence of flood events, runoff and remobilized sediments may cause an increase of ecotoxicologically relevant effects from contaminant reservoirs. Aquatic and terrestrial organisms as well as cattle and areas of settlement are exposed to dislocated contaminants during and after flood events. In this study, the impact of two flood events triggered by intense rain at the rivers Neckar and Rhine (Southern Germany) were studied. Effects in correlation to flood flow were assessed at the river Neckar using samples collected at frequent intervals. River Rhine suspended particulate matter (SPM) was sampled over a longer period at normal flow and during a flood event. Three cell lines (H4L1.1c4, GPC.2D.Luc, RTL-W1) were used to compare Ah receptor agonist activity in different biotest systems. Multilayer fractionation was performed to identify causative compounds, focusing on persistent organic contaminants.

Materials and methods

Native water and SPM of flood events were collected at the river Neckar and at the monitoring station (Rheinguetestation, Worms, Germany) of the river Rhine. Water samples were XAD-extracted. SPM were freeze-dried and Soxhlet-extracted using acetone and finally dissolved in dimethyl sulfoxide. Resulting crude extracts were analyzed for cytotoxicity with the neutral red assay. Aryl hydrocarbon receptor (AhR)- agonist activity was measured in a set of biological test systems (DR-CALUX, GPC.2D, and ethoxyresorufin-*o*-deethylase (EROD) assay and different cell lines. In addition, crude extracts were fractionated using a combined method of multilayer (sequence of acidified silica layers) and carbon fractionation. Fractions from the multilayer fractionation contained persistent organic compounds (polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and some polycyclic aromatic hydrocarbon (PAHs), fractions from the carbon fractionation were separated into a PCDD/F and a PCB fraction. Dioxin-like activity of multilayer and carbon fractions was determined in the EROD assay and expressed as biological toxicity equivalency concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; bio-TEQs). The calculation of chemical equivalency concentrations (chem-TEQs) and comparison to bio-TEQ values allowed the determination of the contribution of the analyzed persistent compounds to the total biological effects measured.

Results

Soluble compounds in native and extracted water samples resulted in no or minor activity in the toxicity tests, respectively. Filter residues of native water caused increased AhR-mediated activity at the peak of the flood. Activities of SPM of the river Neckar correlated well with the flow rate indicating a flood-dependent increase of toxicity culminating at the peak of flow. River Rhine SPM showed a decrease of activity regarding an SPM sample of the flood event compared to a long-term sample. Excellent correlations with AhR-agonistic activity were determined for DR-CALUX and EROD assay, while the GPC.2D assay did not correlate with both other biotests. The activity of persistent dioxin-like acting compounds in multilayer and carbon fractionated PCDD/F and PCB fractions was low if compared to corresponding crude extracts. The congener pattern of PCDD/Fs revealed that the contaminations mainly originated from products and productions of the chlorine and organochlorine industries.

Discussion

Native and extracted water samples could be shown to contain little or no cytotoxic or AhR-agonistic compounds. In contrast, particle-bound compounds were shown to be the relevant effective fraction, as indicated by the activities of filter residues of native water and SPM. Compounds other than fractionated persistent PCBs and PCDD/Fs were more relevant to explain AhR-mediated activities of crude flood SPM at both rivers assessed. Biologically detected activities could at least in part be traced back to chemically analyzed and quantified compounds.

Conclusions

The calculation of the portion of persistent PCBs and PCDD/Fs in multilayer fractions causing the high inductions in the EROD assay in combination with chemical analysis provides a suitable tool to assess dioxin-like activity of persistent compounds in SPM sampled over the course of flood events. Depending on the catchment area and annual course of flood events, end points may either indicate an increase or a decrease of activity. In order to determine the ecological hazard potential of mobilized contaminants during flood events, the focus should be set on particle-bound pollutants. Furthermore, PCDD/Fs and PCBs, commonly expected to be the most relevant pollutants in river systems, could be shown to contribute only to a minor portion of the overall AhR-mediated activity. However, they might be most relevant for human exposure when considering persistence and bioaccumulation/biomagnification in the food chain.

Recommendations and perspectives

As a consequence of climate change, flood events will increase in frequency and intensity at least in some regions such as Central Europe. Thus, it is crucial to identify the potential hazard of (re-)mobilized contaminants from reservoirs dislocated via floods and threatening especially aquatic organisms and cattle grazing in flood plains. Since other less persistent compounds seem to be more relevant to explain AhR-mediated activities in flood SPM, nonconventional PAHs and more polar compounds also need to be considered for risk assessment. Effect-directed analysis using broad-range fractionation methods taking into account compounds from polar to non-polar should be applied for identification of pollutants causing biological effects, thus integrating biological and chemical parameters.

3.2 Introduction

Anthropogenic changes of the atmosphere are expected to cause profound climate changes and the first consequences are assumed to occur at present (Cracknell and Varotsos 2007, Wittig et al. 2007). In particular, an intensification of global water cycling associated with an increased risk of floods is anticipated (Hulme 2003, Wilby et al. 2006). Climate change will result in a further increase of both extent and frequency of floods in many regions across the globe (Ikeda et al. 2005, Kay et al. 2006a, b, Senior et al. 2002, Wilby et al. 2006). In Central Europe, increased flooding is expected at least for most major rivers such as the rivers Rhine and Elbe. Severe weather conditions with rainstorms in 2002 in the Alps, the Erz Mountains, and the Grand Mountains in Eastern Germany resulted in an extreme flood event along the Elbe River. The Elbe River, however, has been known to be severely contaminated by, e.g., magnesium chloride electrolysis, and organochlorine production in the Bitterfeld area since the 1940s via the tributary Mulde (Goetz et al. 1998, 2007, Wilken et al. 1994). Over the last 70 years, these contaminants have repeatedly been translocated in-stream and deposited on inundated land and flood plains (Goetz et al. 2007). During the 2002 flooding event, contaminated sediments were remobilized from the riverbed of the Elbe and were deposited on flood plains (Umlauf et al. 2005). As one of the many consequences, the milk produced by two farms had to be destroyed since the thresholds of admitted dioxin levels set by European regulation have been exceeded. Currently, a research project has been initiated to evaluate if some of the flood plains can again be used for grazing cows or for sheep farming (Wojahn 2007).

Furthermore, during the 2002 flood, highly contaminated Mulde River sediments were remobilized and deposited near Bitterfeld, Germany. Brack et al. (2002) were able to document an increased ecotoxicological impact for the deposited sediments, with metal and organic compound burdens exceeding threshold values for sewage sludge. As a consequence, the sediments had to be categorized as potentially hazardous.

One strategy of governmental authorities to cope with the increased risk of floods is to construct retention areas along major river systems to temporally retain and, thus, defuse the peak flow of extreme flood events (Disse and Engel 2001, Maier et al. 2006). In 2000, the European Union passed the Water Framework Directive (WFD, 2000/60/EG) as a basis for the reconstitution of an ecologically good status for European freshwater systems by the year 2015. Meanwhile, the WFD has been complemented by a daughter directive, listing 33 so-called priority pollutants which are important contaminants of river sediments. Thus, the contamination of sediments and suspended particulate matter (SPM) were enhanced in political perception (Foerstner 2007, Hollert et al. 2007b). At least in this context, compounds adsorbed to sediments and SPM have to receive special attention. Sediments of major rivers in Germany (e.g., Elbe, Rhine, Main, Neckar) are known to contain elevated loads of contaminants released mainly from the chlorine and organochlorine industries from the 1940s to 1970s or via their products (Einsporn et al. 2005, Haag et al. 2001, Heinisch et al. 2004, 2007, Kosmehl et al. 2007, Weber et al. 2008a). These historic reservoirs are relevant for contemporary polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) contamination (Weber et al. 2008b). Today, fewer contaminated sediment surface layers are known to cover older contaminated sediments, deposited at sites with low flow in the rivers, such as flood plains, river reservoirs, and groyne fields, e.g., at the river Rhine.

Nevertheless, the risk of resuspension of old contaminated sediment layers as well as the transport of particle-bound downstream translocation in the river system *via* in-stream erosion increases with water discharge and the frequency of flood events. Furthermore, remobilized sediments may intoxicate organisms and facilitate contaminant translocation in the water column *via* SPM (cf. Gerbersdorf et al. 2007, Gerhardt 2007, Haag et al. 2001, Heise et al. 2004, Heise and Foerstner 2006, Hofmann and Wendelborn 2007, Hollert et al. 2007a, Lick and McNeil 2001, Schwartz et al. 2006, Witt and Westrich 2003).

Contaminants in sediments and SPM of the rivers Neckar and Rhine are usually expected to include chlorinated hydrocarbons comprising a large group of ubiquitously persistent organic pollutants including polychlorinated biphenyls (PCBs), PCDDs, and PCDFs (Habe et al. 2001, Safe 1990, 1994). Due to their hydrophobic character and persistence, these chemicals tend to adsorb to mineral and organic surfaces, e.g., SPM, which aquatic organisms are then exposed to and which accumulate along the food chain (Hollert et al. 2000). Furthermore, Heise et al. (2004) reported that hexachlorobenzene (HCB) contributes as a major toxicant to the overall contamination of the river Rhine. HCB concentrations in SPM were shown to be increased already at normal discharges. Concentrations of these contaminants have been reported to significantly increase as a consequence of flood events and increasing sheer stress. These compounds then are often translocated downstream and deposited in the riverbed or on inundated floodplains (Hollert et al. 2007a, Gerbersdorf et al. 2007).

In this study, extreme meteorological conditions, causing intense rainfalls in the southwest of Germany, were assessed in January 2004. As a consequence of the intensive precipitation, river flow rates increased and flood events resulted along the rivers Neckar and Rhine. Subsequent precipitation along the Neckar river lead to an intensive flood event with a hazard quotient (HQ) of 1:10 (HQ = ratio of a flood with a certain discharge at a certain site) and, at the rive Rhine, with an HQ of 1:2. Two flood events at the rivers Neckar and Rhine, two major German river systems, were investigated to assess their potential influence on the toxicity of water samples and SPM. For this end, a set of selected ecotoxicological end points was recorded in a variety of cell-based monitoring systems. In detail, basic toxicity was determined using the Neutral Red retention assay (NR assay) as detailed by Babich and Borenfreud (1992). Acute cytotoxicity is quantified via uptake and retaining of the neutral red stain in lysosomes of cells. In damaged cells, the stain is no longer retained in cytoplasmic vacuolar membranes and the plasma membrane does not act as a barrier to retain the dye within the cell (Babich and Borenfreud 1992). Furthermore, aryl hydrocarbon receptor (AhR)-mediated toxicity was measured using different mechanism-specific cell-based test systems. The 7-ethoxyresorufin-*o*-deethylse (EROD) assay was used to determine the overall and, in particular, the dioxin-like enzyme activity, caused by native and extracted water of the river Neckar and Soxhlet-extracted SPM sampled at the rivers Neckar and Rhine. The test is based on the increased *de novo* synthesis and activity of cytochrome P450 enzymes (CYP) by cells being exposed to contaminants, which is determined using the fluorescamine method to quantify protein and the photometric detection of the enzyme reaction product resorufin (Lorenzen and Kennedy 1993, Kennedy and Jones 1994). In addition, the GPC.2D assay and the DR-CALUX assay were used to determine AhR-mediated activity of SPM samples, quantifying the CYP activity via luminescence measurement.

Thus, the purpose of the present study was:

(1) to determine the portion of toxic effects of dissolved and particle-bound contaminants with samples of two flood events at different catchments

(2) to compare the hazard potential of the more active samples using a set of cell-based biotest systems and

(3) to identify and compare the portion of persistent compounds among the rivers Neckar and Rhine

3.3 Materials and methods

3.3.1 Suspended particulate matter sampling

Surface water of the river Neckar were sampled in the period from January 14, 2004, to February 3, 2004, (Tab. 1) with 20 L bottles or a water sampler according to Hollert et al. (2000). SPM were collected with an SPM trap installed to the floating bridge of a power station at Heidelberg. SPM was transferred to glass bottles, transported at 4 °C, and protected from light. Samples were frozen at -20 °C, freeze-dried immediately (beta 1 - 8 K; Christ, Osterode, Germany), and stored at 4 °C in darkness until analyzed.

Table 1 Sampling times and encoding of native and extracted water as well as SPM from the Neckar River and of SPM from the Rhine River

Sample code	Sampling date	Sampling period		
		Native water	Extracted water	SPM
Neckar 0	14.01.2003	10:00	10:00	n.a.
Neckar 1	14.01.2004	14:00	n.a.	10:00-14:00
Neckar 2	14.01.2004	17:00	n.a.	14:00-17:00
Neckar 3	14.01.2004	18:00	n.a.	17:00-18:00
Neckar 4	15.01.2004	22:00	n.a.	18:00-22:00
Neckar 5	15.01.2004	02:00	02:00	22:00-02:00
Neckar 6	15.01.2004	08:00	08:00	02:00-08:00
Neckar 7	15.-16.01.2004	12:00	n.a.	08:00-12:00
Neckar 8	16.-20.01.2004	13:30	n.a.	12:00-13:30
Neckar 9	20.01.-03.02.2004	12:00	n.a.	13:30-12:00
Neckar 10	03.02.2004	14:00	n.a.	12:00-14:00
Rhine 1	November 2003 to February 2004			
Rhine 2	15.-19.01.2004			

n.a.—not assessed

The sampling site at the river Rhine is located within the Rhine monitoring station at Worms, a governmental institution founded for the continuous monitoring of water quality of the river Rhine. Along the pillars of the bridge, four pumps were installed to provide a continuous water supply from four lanes inside the river (for details, cf. Pawlowski et al. 2003). SPM was allowed to settle down in a tank. Two mixed samples were collected, one of which was sampled over an extended period from November 2003 to February 2004, including the flood event (subsequently termed 'Rhine 1'). The other sample contains SPM over the complete flood event (January 15-19, 2004; subsequently termed 'Rhine 2').

3.3.2 Sample extraction

Twenty grams of each freeze-dried SPM samples were extracted with 250 ml acetone (Merck, Darmstadt, Germany) for 14 h using standard reflux (Soxhlet) extractors at approximately eight to ten cycles per hour. The solvent was reduced in volume, and residues were evaporated under a gentle N_2-stream close to dryness. Finally, extracts were reconstituted in 1 ml dimethyl sulfoxide (DMSO, Sigma- Aldrich, Deisenhofen, Germany) and stored at -20°C until testing. Empty extraction thimbles were extracted and processed in parallel to serve as process controls. Subsequent provider and location of chemicals and medium used in this study will only be named when differing from Sigma-Aldrich.

3.3.3 Water samples

Sample volumes of 20 L each were used for the preparation of XAD water extracts and extracts of the filter residues according to the methods by Keiter et al. (2006) and Hollert et al. (2005). All samples were cooled in a refrigerator to 4 °C immediately after return to the laboratory and filtered using a 0.4 µm fiber glass filter (Sartorius, Goettingen, Germany) at a pressure of approximately 1 bar. The effluent particle phase was extracted as detailed above for SPM samples. In each case, the sample filtrate was adjusted to pH 2 using 1 M HCl. 1 L methanol (Fluka, Buchs, CH) was added to each sample before organic compounds were extracted by using a 1:1 (v:v) mixture of Amberlite™-XAD 4 and XAD 7 resins (Serva, Heidelberg, FRG). After extraction, the solvent volume was reduced close to dryness by evaporation with N_2, the extract was reconstituted in DMSO and stored at -20 °C until analysis. Due to the reduction of the volume (20 L to 2 ml), a concentration factor (CF) of 10,000 was achieved for each sample. Additionally, three 20 ml subsamples of each water sample were stored at -20 °C for the investigation of native water samples.

3.3.4 Multilayer fractionation

In order to provide information on the identities of unknown substances inducing AhR-mediated activities in whole extracts, a multilayer fractionation was performed according to the methods shown by Keiter et al. (2008) to remove acid-degradable non persistent compounds (e.g., polycyclic aromatic hydrocarbon (PAHs)). The complete fractionation method is shown in Fig. 1. Samples were cleaned on an open 'sandwich' silica column (mesh 15 mm, Merck, Darmstadt, Germany) packed with KOH silica (30 mm), neutral silica (5 mm), 40 % H_2SO_4 silica (30 mm), 20 % H_2SO_4 silica (15 mm), neutral silica (10 mm), and Na_2SO_4 (10 mm) and were eluted with *n*-hexane (Riedel-de Haen, Seelze, Germany).

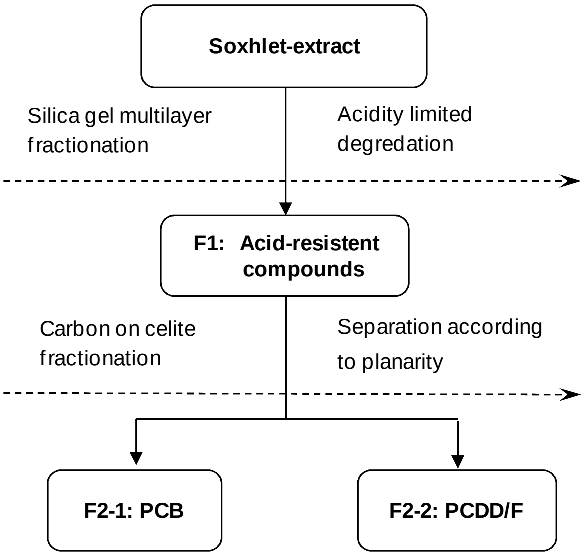

Fig. 1 Fractionation procedure to separate Ah receptor-activating and persistent compounds in complex flood SPM samples using multilayer and carbon on celite fractionation. F1 contained all compounds being resistant to oxidation by sulfuric acid. Finally, fractions solely contained PCBs (F2-1) and PCDD/Fs (F2-2). Dashed arrows describe the methodology, drawn through arrows describe those related to fractionation

The remaining fraction contained persistent dioxin-like active compounds and included, e.g., PCDD/Fs and PCBs. Some PAHs, however, are known not to be retained in the multilayer fraction (Windal et al. 2005). The solvents were divided into two equal portions, and the first portions were evaporated under a nitrogen stream, and the sample was transferred to DMSO (Fluka) for subsequent bioassays. To separate planar and non planar compounds, the second portions of the samples were fractionated using an open carbon column (Carbopack C, Fluka). Adsorbent (1.5 g), carbon on Celite™ (1:9, Celite 545, 20 - 45 µm, Fluka), and a layer of Na_2SO_4 were packed in glass columns. The sample was eluted into two subsequent fractions with 10 ml of hexane (non-planar compounds) and 80 ml of toluene (coplanar compounds). After the addition of recovery standards (^{13}C-labeled 1,2,3,4-TCDD, 1,2,3,7,8,9-HxCDD), the samples were evaporated and transferred to amber glass autosampler vials in 25 µl of tetradecane. The extracts and standards were stored at -18 °C until high-resolution gas chromatography (HRGC)-high-resolution mass spectrometry (HRMS) analysis.

3.3.5 PCBs and PCDD/Fs − HRGC-HRMS analysis

HRGC-HRMS analysis was performed with a Micromass Autospec Ultima instrument (Autospec Ultima, Waters Micromass, Manchester, UK) operating at greater than 10,000-12,000 resolution using electron impact ionization at 35 eV. All measurements were performed in the selective ion recording mode, monitoring the two most abundant ions in the chlorine cluster. Splitless injection of 1 µl of the final extract was used on a 60 m Rtx dioxin 2 column (0.25 mm inner diameter, 25 µm). In addition to a blank sample with each set of samples (five to ten), a procedure control that was treated like the samples was analyzed.

3.3.6 Neutral Red Retention assay

The acute cytotoxicity of the sediment extracts was determined with the neutral red retention assay as detailed by Babich and Borenfreund (1992), with modifications described by Klee et al. (2004) and Seiler et al. (2006). The permanent cell lines RTL-W1 (Lee et al. 1993) and RTG 2 (Wolf and Quimby 1962), both from the rainbow trout (Oncorhynchus mykiss), were used for biotesting. Cell culture was carried out as described by Klee et al. (2004). Sediment extracts were serially diluted in L15 medium along seven wells in six replicates of a 96-well microtitre plate (TPP, Trasadingen, Switzerland) to give a final concentration range of 0.78-50 mg/ml 3,5-dichlorophenol (Riedel-de-Haën) was used as a positive control at a maximum concentration of 80 mg/L medium. Confluent cultures of RTL-W1 cells were trypsinized and the resulting cell suspension was added to each well of the microtitre plate. After incubation at 20 °C for 48 h, cells were incubated with neutral red (2-methyl-3-amino-7-dimethylaminophenanzine) for 3 h, and neutral red retention was measured at 540 nm with a reference wavelength of 690 nm using a Spectra™ III multiwell plate reader (Tecan, Crailsheim, Germany). Second-order polynomial dose-response curves expressing the viability of the cells, compared to controls, were plotted using Prism 4.0 (GraphPad, San Diego, USA), and the cytotoxic potential of individual extracts was subsequently calculated as NR_{50} values (= effective concentration for 50 % cell death in the neutral red test compared to the negative controls with non-exposed cells). Subsequent NR_{50} values, with concentration units of milligram SPM equivalents (SPM-EQ) per milliliter test medium in the well, will be given as milligram per milliliter.

3.3.7 DR-CALUX assay

The DR-CALUX assay utilizes a rat hepatoma cell line (H4L1.1c4), with a luciferase reporter gene controlled by the AhR (Biodetection Systems, Amsterdam, the Netherlands, Murk et al. 1996), and was applied with the protocol shown by Gustavsson et al. (2004, 2007). Cell culture was carried out as described by Aarts et al. (1995). DR-CALUX cells were seeded in 96-well plates (TPP), 24 h prior to exposure under standard conditions of 37 °C and 5 % CO_2, and allowed to attain 100 % confluence. Thereafter, sample dilutions were prepared in culture medium supplemented with 10 % fetal calf serum (FCS) and added to the cells in triplicate wells. The extracts were tested in ten concentrations in 3-fold dilution series, with a maximum concentration of 2 mg/ml. In each assay, a calibration curve of TCDD (0 - 300 pM) was tested. The final concentration of DMSO (Fluka) was 0.8 %. After 24 h of exposure, the medium was removed and the cells were washed twice with phosphate buffered saline (PBS) and lysed in PBS at -20 °C overnight. After the substrate luciferin had been added, followed by incubation for approximately 1 h in darkness at 20 °C, the activity of luciferase was measured using the Luclite™ assay kit (PerkinElmer, Upplands Vaesby, Sweden). Cell lysates were transferred to a white 96-well plate (TPP), and luminescence was determined in a multiwell plate reader (Wallace 1420, Victor2, PerkinElmer, USA). Dose-response curves were analyzed with GraphPad Prism 4, using the Hill equation (Olsman et al. 2007). The bottom value was set to the response of the solvent controls. The luciferase-inducing potency of the samples was converted to biological toxic equivalents (bio-TEQs) as described below.

3.3.8 GPC.2D assay

The guinea pig GPC.2D.Luc liver cells were provided by Dr. T.A. Gasiewicz (University of Rochester, NY, USA) and cultivated in Dulbecco's modified Eagle's medium containing 10 % FCS and 2 mM glutamine in 75 cm^2 culture flasks (Sarstedt AG and Co, Nuembrecht, Germany) at 37 °C and 5 % CO_2 (Gasiewicz et al. 1996). Cells were trypsinized with trypsin-ethylenediaminetetraacetic acid (EDTA) and transferred to 96-well plates (25,000 cells per well, TPP). The cells were allowed to grow overnight. Subsequently, cells were exposed to a series (0.0014 - 80 nM, 0.0042 - 25 pg/ml) of TCDD (Ehrenstorfer, Augsburg, Germany) or sediment extracts (DMSO concentration 0.6 %) for 24 h. The maximum sediment concentration in the assay was 0.12 mg SEQ per milliliter test medium. All samples were tested in triplicates of five different concentrations according to the protocol by Olsman et al. (2007). Every experiment was repeated independently at least three times. After incubation, cells were washed with PBS and lysed in 50 µl lysis buffer (25 mmol glycoglycerine, 1 % Triton X-100, 10 % glycerine). Luminescence measurements were performed using a FLUOstar Optima plate reader (BMG Labtech, Offenburg, Germany) before and after injection of 30 µl of substrate (Tris-(hydroxymethyl) aminomethane (20 mM, pH 7.8), $MgCl_2$ (5 mM), EDTA (0.1 mM), dithiothreitol (33 nM), coenzyme A (270 µM), luciferin (470 µM) and ATP (530 µM)). Data were calculated by subtracting the arithmetic mean for 5 s before injection of the substrate from the mean for 13 s after substrate injection. The data were analyzed using the GraphPad Prism 4 program. EC_{25} values were calculated from log-transformed data using a sigmoid curve fit with variable slopes. The results were expressed relative to TCDD as bio-TEQ values as described below.

3.3.9 7-ethoxyresorufin-*o*-deethylase assay

Induction of EROD was measured in the CYP1A-expressing cell line RTL-W1, with slight modifications to the method described by Behrens et al. (1998). RTL-W1 cells were kindly provided by Drs. Niels C. Bols and Lucy Lee (University of Waterloo, Canada) and cultured at 20 °C in 75 cm^2 plastic culture flasks (TPP) in L15 medium supplemented with 8 % FCS, 1 % penicillin-streptomycin, and 1 % neomycin sulfate (Keiter et al. 2006). Before exposure to the standards, cells were seeded in 96-well plates (TPP) and allowed to grow to 100 % confluence for 72 h. Subsequently, the medium was removed and the cells were exposed for 72 h to the SPM extracts diluted in medium using eight dilutions with six replicates each. The DMSO content in the wells was less than 0.1 %. Cytotoxicity of DMSO with RTL-W1 cells can be determined with concentrations above 2-3 % in the well (analyzed in this study). TCDD (Promochem, Wesel, Germany) was serially diluted to give a final concentration range of 3.13 - 100 pM on two separate rows of each plate as a series of positive controls. Induction was terminated by removing the growth medium and freezing at -70 °C to lyse the cells. The deethylation of exogenous 7-ethoxyresorufin was initiated by adding the substance to each well and incubating in the dark at room temperature for 10 min before addition of NADPH. The plates were incubated for a further 10 min, and the reaction was stopped by adding fluorescamine dissolved in acetonitrile. EROD activity was measured fluorometrically after another 15 min using a GENios plate reader (Tecan, Crailsheim, Germany, excitation 544 nm, emission 590 nm). Protein was determined fluorometrically using the fluorescamine method (excitation 355 nm, emission 590 nm, Lorenzen and Kennedy 1993, Kennedy and Jones 1994) with the protocol detailed in Hollert et al. (2002). The concentration-response curves for EROD induction in the RTL-W1 bioassay were computed by nonlinear regression (GraphPad Prism 4) using the classic sigmoid curve or Boltzmann curve as model equations. The luminescence-inducing potency of the samples was converted to bio-TEQs as described below.

3.3.10 Bio-TEQ values

Bioassay-derived TCDD equivalents (bio-TEQs) were calculated by relating biological activities (luminescence in the DR-CALUX assay and GPC.2D assay, fluorescence in the EROD assay) caused by samples to the positive control 2,3,7,8-TCDD (cf. Keiter et al. 2008). Bio-TEQs for concentration-response curves were calculated following the fixed effect level quantification method, using the EC_{25} of the maximum response in the TCDD standard curves as the fixed level (Brack et al. 2000, Engwall et al. 1996). The bio-TEQs given in this study are means of $n = 3$ (EROD assay) and $n \geq 3$ (GPC.2D assay) individual assays. Bio-TEQs of the DR-CALUX assay were calculated using EC values of $n = 1$, as remaining extract volumes were insufficient for more individual assays. Data from the bioassays were evaluated with the GraphPad Prism 4.0 program.

Mean TCDD-EC_{25} values were determined as well as standard deviations (SD) between individual EROD assays and used to calculate bio-TEQs (Eq. 1). In contrast, TCDD-EC_{25} values of each individual assay were used to calculate equivalent concentrations with the DR-CALUX assay and the GPC.2D assay. Subsequent, bio-TEQs with concentrations in picogram TCDD per gram of SPM-EQ will be given as picogram per gram.

Eq. 1 $$\text{Bio-TEQ [pg TCDD /g SPM-EQ]} = \text{TCDD}_{25}\text{[pg/ml]} / \text{EC}_{25}\text{[g SEQ/ml]}$$

3.3.11 Chem-TEQ values

Safe (1990) described the AhR-pathway as structure dependent, as the most potent congeners were 2,3,7,8-substituted tetra- and penta-chlorinated PCDD/Fs as well as meta- and para-substituted coplanar PCBs. Based on *in vivo* and *in vitro* data to each compound included, relative toxic potencies (REP values) were assigned (Eadon et al. 1986, NATO/CCMS 1998). REPs are given as values which are related to the toxic potency of 2,3,7,8-TCDD (REP = 1).

Aiming to explain the determined bio-TEQ levels, chemically derived TEQs (chem-TEQs) were calculated by using the relative potency (REP) factors shown in tab. 1. Chem-TEQs are calculated by multiplying compound concentrations and REP values that were applied as determined by Clemons et al. (1997), specific for RTL-W1 cells and each compound assessed (Eq. 2). When cell-specific REPs were not available, World Health Organization (WHO)-REPs were used as given by van den Berg et al. (1998). Subsequently, chem-TEQs with concentrations in picogram TCDD per gram SPM-EQ will be given as picogram per gram.

Eq. 2 Chem-TEQ [pg TCDD / g SPM-EQ] = Conc. [pg/g SPM-EQ] x REP

3.4 Results

3.4.1 Neutral Red retention assay

Concentration-response curves of native water samples, XAD-extracted water samples, and XAD filter residue extracts of the water samples Neckar 0 and Neckar 5 are illustrated in fig. 2. Viability of the cells is given in relation to the viability of cells that were not exposed to samples. Significantly increased cytotoxic effects could not be observed for native Neckar water samples, both in RTL-W1 and RTG 2 cells. In contrast, XAD-extracted water caused a 50 % diminished cell viability with RTL-W1 cells (Neckar 5) when exposed to water concentrated 84.6 times (concentration factor: CF_{50} = 84.6) and a CF_{50} = 120.6 with RTG 2 cells (details not shown). Further on, extracted water filter residues caused CF_{50} = 50.3 (Neckar 0) and CF_{50} = 48.3 (Neckar 5) using RTL-W1 cells. Exposure of Neckar 5 extracts to RTG 2 cells caused CF_{50} = 65.8. No effect was observed with extracts of water sample Neckar 6. Neckar 0 and 5 indicated higher toxicities by extracts of particle-bound contaminants and filter residue extracts. Dissolved compounds in native and extracted water were shown to be significantly less toxic.

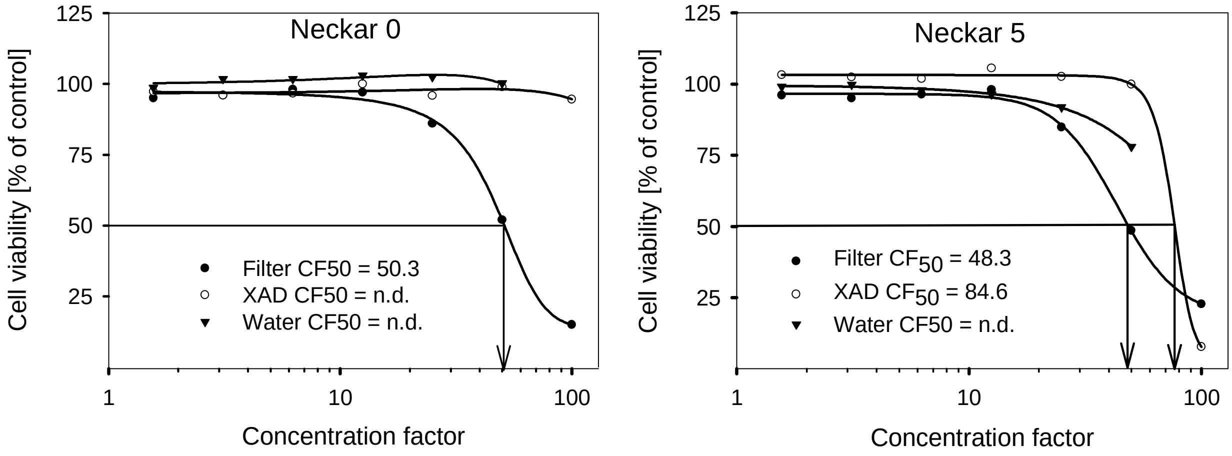

Fig. 2 Cytotoxicity of native water samples (filled inverted triangles), XAD-extracted water (empty circles) and the XAD filter residue extract (filled circles) of the samples Neckar 0 and Neckar 5, illustrated as concentration-response curves with common logarithmic scale. A 50 % decrease of cell viability is given as CF_{50} (CF = concentration factor) for native water, XAD-extracted water, and filter residues. Native water samples were diluted 1:2 in the test, and, therefore, highest concentrations

correspond to 50 % of native waters. The highest concentration of XAD extracts is corresponding to 100-fold concentrated water and water filter residue extract. n.d. − not detectable

Cytotoxic effects of river Neckar SPM collected during the flood event as well as corresponding discharge is shown in fig. 3, using RTL-W1 and RTG-2 cells. SPM extracts damaged RTL-W1 (average $NR_{50} = 22$ mg/ml) cells significantly (≥ twofold) more as compared with RTG-2 cells (mean $NR_{50} = 50$ mg/ml). After the flood event, cytotoxicity is decreased by a factor of 3 in both RTL-W1 ($NR_{50} = 97$ mg/ml) and RTG-2 cells ($NR_{50} = 144$ mg/ml).

Comparing both cell lines used, RTL-W1 cells were shown to be more sensitive than RTG-2 cells by a factor 1.5 to 2. Cytotoxicity of the Rhine 1 extract was not significantly different in RTL-W1 cells (mean $NR_{50} = 50.6$ mg/ml) and RTG-2 cells ($NR_{50} = 74.1$ mg/ml). In contrast, cell toxicity significantly differed after exposure of RTL-W1 and RTG-2 cells to the extract of Rhine 2 SPM to ($NR_{50} = 40.6$ and 97.8 mg/ml, respectively).

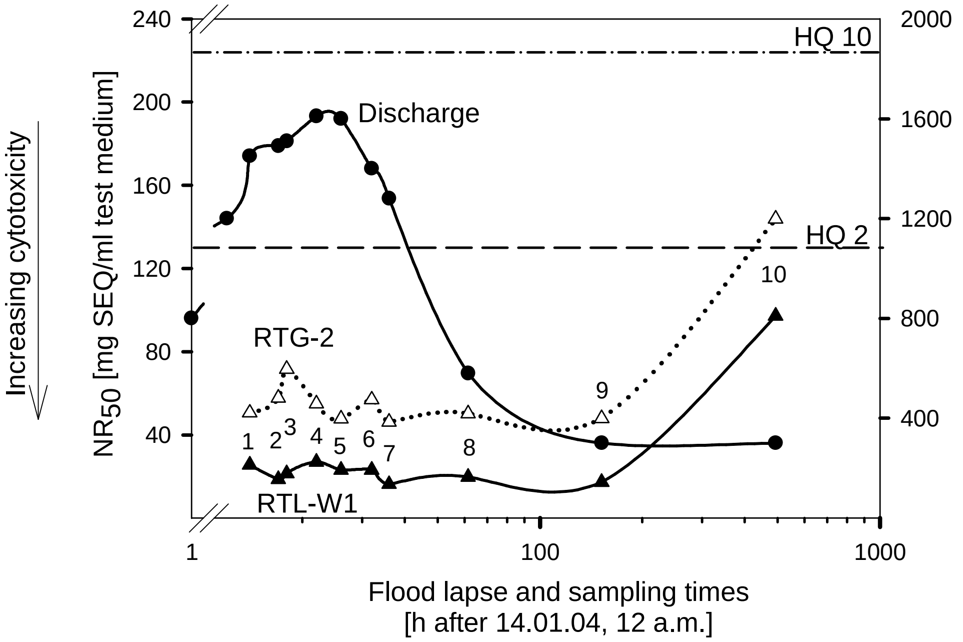

Fig. 3 AhR-agonistic activity of SPM samples of the river Neckar derived from the EROD assay and RTL-W1 cells in the context of the flood discharge.

3.4.2 AhR-mediated activity

An increase of EROD activity could not be observed with native water samples and XAD-extracted water samples from the Neckar. Filter residue extracts of the water sample Neckar 0 ($CF_{25} = 10 \pm 1.9$) and Neckar 5 ($CF_{25} = 3.7 \pm 1.3$) were shown to contain high quantities of compounds interacting with the AhR-activity. No activity was observed with water sample Neckar 6 (details not shown). AhR-mediated activity in RTL-W1 cells upon exposure to SPM total extracts from the river Neckar is illustrated in fig. 4. Enzyme activities corresponding to 25 % of the maximum induction of the positive control (effective concentration, EC_{25}) are shown as normalized bio-TEQs and are given in picogram TCDD per gram sediment. SPM extracts caused a concentration-dependent increase of AhR-mediated activity following the flow at the Neckar. Bio-TEQs indicated two peaks of AhR-mediated activity with 5,457 ± 2,213 pg/g (Neckar 2) and the maximum flow 8,341 ± 2,817 pg/g (Neckar 6), respectively. At the end of the flood event, reaching mean flow levels, bio-TEQs decreased significantly but still reached 3,313 ± 135 pg/g (mean value of Neckar SPM 9 and 10). Finally, Neckar 11, sampled another 14 days later, displayed an EROD activity of 930 ± 119 pg/g. Highest and lowest measured enzyme inductions differed by a factor of 6.7, indicating a flood-dependent increase of AhR-mediated activity. The SPM bio-TEQ of the Rhine SPM sampled over a longer period (Rhine 1 3,693 ± 520 pg/g) was elevated compared to the flood sample (Rhine 2 2,331 ± 328 pg/g), indicating a decrease of activity through the investigated flood event.

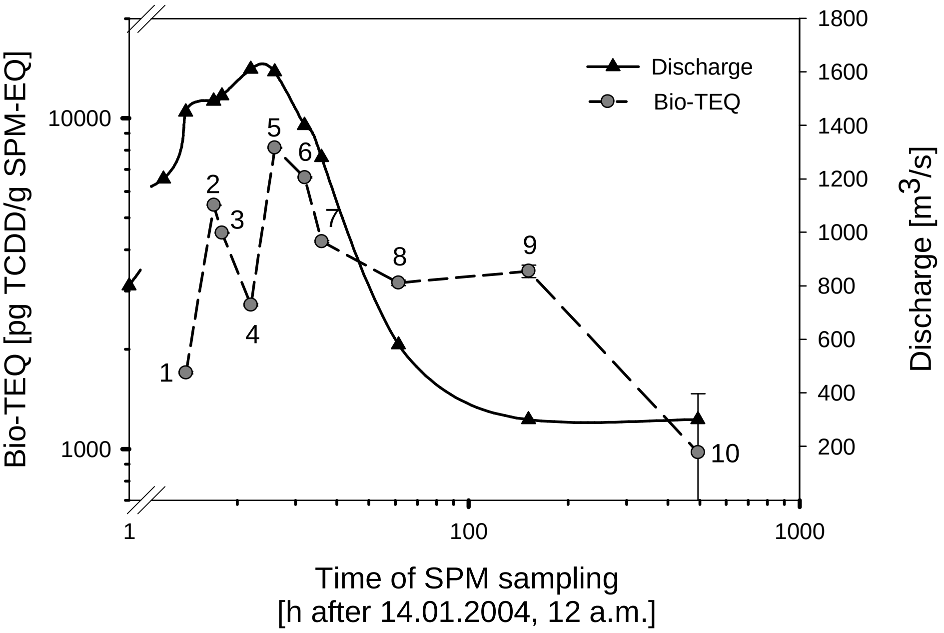

Fig. 4 Acute cytotoxicity of flood SPM from the river Neckar in the neutral red retention assays determined with the acute Neutral Red retention test after 48-h exposure, using the RTL-W1 and RTG-2 cell lines. Time of sampling is shown in hours on a common logarithmic scale, beginning 14 January 2004, 12 a.m. (= 1 on x-scale). Furthermore, discharge is given and highlighted by dots at the times of SPM sampling. HQ values, illustrated by dashed lines, are provided to permit a classification of the recent flood event discharge. Cell viability is given as percent of controls. Numbers indicate Neckar SPM sample encodings (cf. Table 1).

3.4.3 DR-CALUX and GPC.2D assay with SPM

Bio-TEQ values of the SPM extracts determined in the DR-CALUX and the GPC.2D assays in comparison with results of the EROD assay are given in fig. 5. All SPM extracts caused a concentration-dependent increase of AhR-mediated activity. The three cell lines displayed differential sensitivity to the SPM extracts. Highest activities were detected using the EROD assay. The AhR-mediated activity of SPM extracts in the H4L1.1c4 cells correlated well with RTL-W1 cells, with the Neckar 6 sample (bio-TEQ = 3,833 pg/g) displaying the highest activity. In contrast, for H4L1.1c4 cells, no significant increase could be observed for the

Neckar 2 sample. The lowest induction rates for all samples were obtained in the GPC.2D assay. Furthermore, the DR-CALUX and the GPC.2D assays were used to assess Rhine SPM. Using H4L1.1c4 cells, the bio-TEQ of Rhine 1 equaled a concentration of 2,007 pg/g, while Rhine 2 was significantly less toxic (908 pg/g). Rhine 1 and GPC.2D cells demonstrated a bio-TEQ of 145 ± 73 pg/g. Rhine 2 showed a significantly decreased bio-TEQ of 29 ± 30 pg/g. Thus, all applied assays indicated higher AhR-agonist activities for Rhine 1.

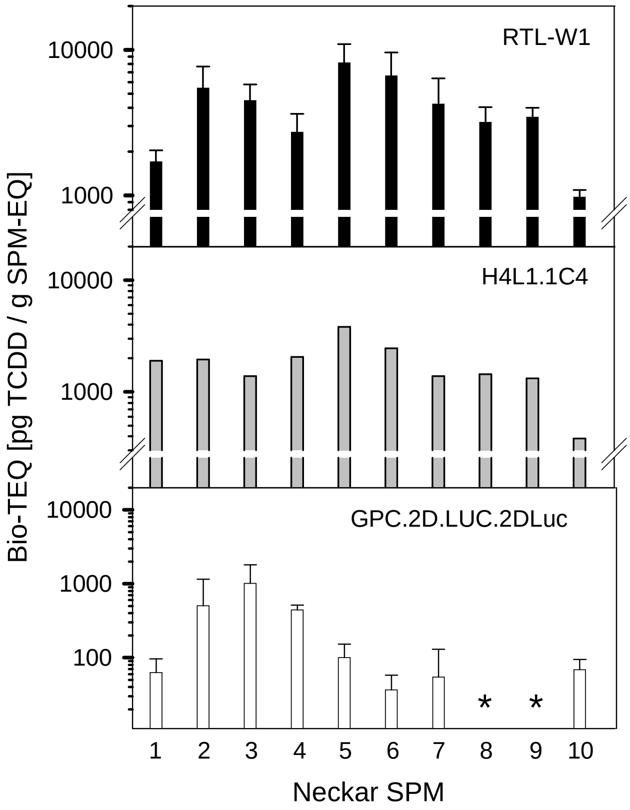

Fig. 5 AhR-mediated activity of SPM extracts of the Neckar River as determined in the DR-CALUX® (H4L1.1C4 cells, n = 1), GPC.2D (GPC.2D.Luc cells, n = 3-8) and 7-ethoxyresorufin-*o*-deethylase (EROD, RTL-W1 cells, n=3) assays. Bio-TEQ values were calculated as the concentration resulting in 25 % of the maximum induction of 2,3,7,8-TCDD. Data are presented as means ± SD in the case of the DR-CALUX® and EROD assays. * − activity not detectable

3.4.4 Multilayer and carbon on celite fractionation

Fractions from silica multilayer fractionation contain persistent organic and dioxin-like active compounds being resistant to oxidization by sulfuric acid. Neckar 1 and Neckar 6 as well as Rhine 1 and Rhine 2 were selected for multilayer open column chromatography. Primary multilayer fractions (F1), containing persistent sulfuric acid oxidation-resistant compounds (PCBs and PCDD/Fs), and secondary fractions (F2-1 containing PCBs, F2-2 containing PCDD/Fs) were analyzed by means of biological and chemical methods. Data about chemically analyzed and quantified persistent chemical compounds are provided in tab. 2. Concentrations of PCDDs increased with the degree of chlorination. Octachlorodibenzo-*p*-dioxin (OCDD) was concentrated the highest, reaching a maximum concentration of 5,013 pg/g (Rhine 1). Regarding PCDFs, concentrations were highest for octachlorodibenzo-

p-furans (OCDFs) in sample Rhine 1 (999 pg/g). Among all PCBs detected, #77 had the highest concentrations of all samples, 31,998 pg/g (Rhine 2).

Table 2 Concentrations of priority PCDD/Fs and PCBs given for selected samples from the rivers Neckar and Rhine

Compounds analyzed	Specific REP[1]	WHO REP[2]	Neckar 1 [pg/g SPM-EQ]	Neckar 6 [pg/g SPM-EQ]	Rhine 1 [pg/g SPM-EQ]	Rhine 2 [pg/g SPM-EQ]
PCDD						
2,3,7,8-TCDD	1.0	-	9	8	18	10
1,2,3,7,8-PeCDD	2.6	-	5	4	3	2
1,2,3,4,7,8-HxCDD	1.1	-	9	7	5	1
1,2,3,6,7,8-HxCDD	0.2	-	23	29	15	5
1,2,3,7,8,9-HxCDD	-	0.1	16	20	9	4
1,2,3,4,6,7,8-HpCDD	0.2	-	343	497	230	67
OCDD	-	0.0001	2719	4597	5013	3278
Sum PCDD			3123	5162	5292	3367
PCDF						
2,3,7,8-TCDF	0.2	-	48	58	109	23
1,2,3,7,8-PeCDF	0.2	-	28	24	94	16
2,3,4,7,8-PeCDF	1.9	-	34	33	59	14
1,2,3,4,7,8-HxCDF	1.1	-	55	57	61	25
1,2,3,6,7,8-HxCDF	-	0.1	21	18	27	8
2,3,4,6,7,8-HxCDF	-	0.1	15	18	12	4
1,2,3,7,8,9-HxCDF	-	0.1	5	5	7	3
1,2,3,4,6,7,8-HpCDF	-	0.01	164	181	104	34
1,2,3,4,7,8,9-HpCDF	-	0.01	17	14	9	3
OCDF	-	0.0001	432	511	999	746
Sum PCDF			819	918	1479	874
PCB		-				
PCB 77	0.0034	-	890	14327	610	31998
PCB 126	0.023	-	39	121	14	0
PCB 169	0.00016	-	7	5	2	0
Sum PCB			936	14454	627	31998
Total sum			4878	20535	7398	36239

In order to elucidate whether the analyzed compounds were responsible for the dioxin-like activity of each fraction tested in the bioassay applied, measured concentrations were expressed as chem-TEQs. Bio- and chem-TEQs of fractionated SPM extracts of the rivers Neckar and Rhine are illustrated in fig. 6. Regarding all multilayer fractions, both TEQ values showed good accordance.

Furthermore, the multilayer fractions of both Neckar samples (including PCDD/Fs and PCBs) showed similar inductions of dioxin-like activity. However, alterations in compound concentrations within these fractions were obvious using chemical analysis. Bio- and chem-TEQ values of PCBs increased by a factor of 13 during the flood event in both Neckar samples, whereas bio-TEQ values of PCDD/F remained constant (approximately 200 pg/g). Similarly, Rhine samples showed an increase of PCB bio- and chem-TEQs in Rhine 1 with a bio-TEQ increasing by not detectable amounts to 268 pg/g in Rhine 2. Total PCDD/F bio-TEQs were similar for Rhine 1 (289 pg/g) and Rhine 2 (278 pg/g).

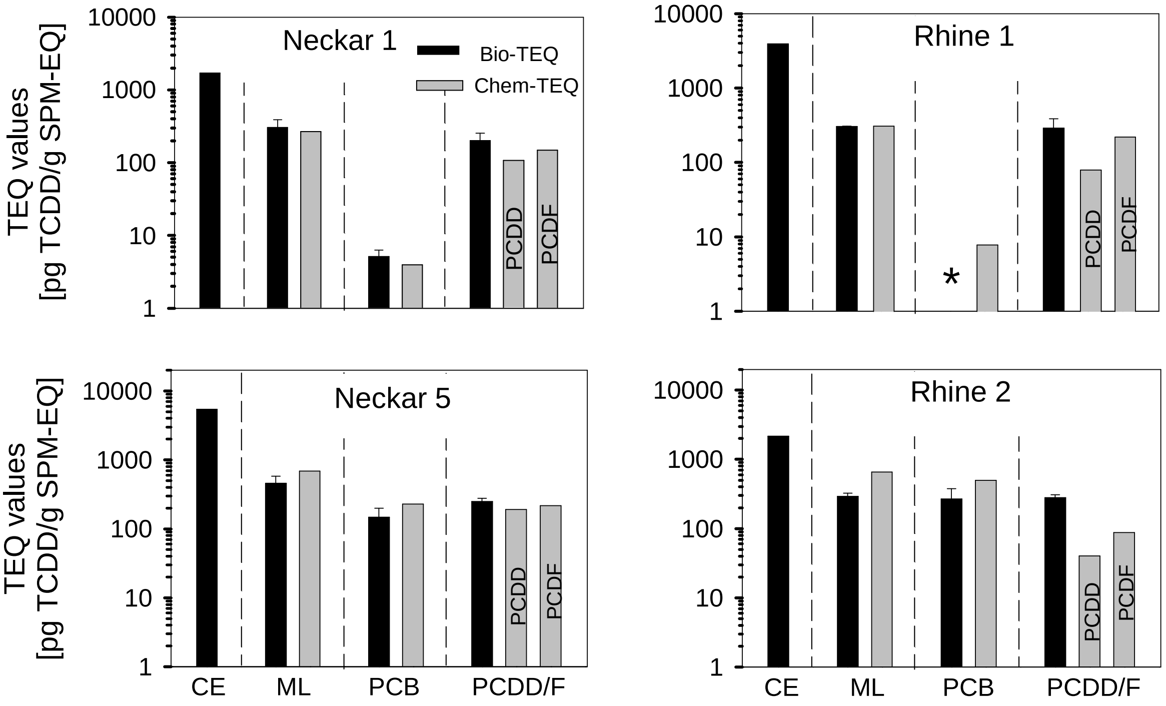

Fig. 6 Bio-TEQ values determined using EC_{25} values from the EROD assay (black bars) and chem-TEQs calculated by multiplying compound concentrations and REPs (gray bars) for SPM samples from the Neckar and Rhine rivers are shown. Neckar samples were selected as indicating highest dioxin-like activities at around the discharge peak of the flood. Comparison of TEQs for each fraction allowed for an explanation of the determined bio-TEQ levels related to the contribution of the chemically analyzed dioxin-like activity for the overall biological activities. Bio-TEQs of total extracts allow grading of each fraction activity. Bio-TEQs are given as a mean of three independent experiments ± SD on a common logarithmic scale. CE − crude extract, ML − multilayer fraction, * − activity not detectable

3.5 Discussion

3.5.1 Cytotoxic effects of complex samples

Cytotoxic effects with RTL-W1 cells were not detected for native water samples from the river Neckar, indicating that concentrations of water-borne toxic compounds were low during the flood event. Cytotoxic effects were only observed at the peak of flow with XAD-extracted water samples. The impact of extracted water-soluble compounds seemed negligible for cytotoxicity. In contrast, filter residue extracts of the samples Neckar 0 and Neckar 5 produced toxic effects, indicating particle-bound contaminants to be more relevant than dissolved compounds. Since the particle-bound contaminants were more toxic than the water samples, further analyses had been focused on SPM. RTL-W1 and RTG-2 cells revealed constantly dose-dependent decreased cell viabilities with SPM sampled during flood discharge. SPM extracts were also toxic when sampled at mean annual discharge directly after the flood event but became less toxic thereafter. Hollert et al. (2003b, 2007a) showed that SPM from the 1998 flood event at the Neckar (HQ = 1:20) caused NR_{50} values for RTG-2 cells between 20 and 60 mg/ml test medium. These results were comparable to the toxicity caused by the 2004 flood SPM assessed in this study. Compared to a moderate Neckar flood event (HQ = 1:1) with NR_{50} values for RTG-2 cells between 40 and 150 mg/ml, cytotoxicity was increased. As cytotoxic effects could not be correlated with the discharge in all three studies, toxic effects seem to increase in the beginning of flood events, but to remain on a certain level with increasing discharge.

Cytotoxic effects in this study indicated a good correlation between the RTL-W1 and RTG-2 cell line ($r_{Pearson} = 0.93$, c. f. fig. 7). The NR_{50} values of all but three samples were within the 95% confidence interval. Furthermore, RTL-W1 cells were shown to be more sensitive to SPM extracts, possibly indicating effects of metabolic processing. Cytotoxicity by Rhine 1 was similar in both cell lines, whereas Rhine 2 proved to be 2.6-times more toxic to RTL-W1 cells.

3.5.2 Ah receptor agonist activity of water samples

As for the neutral red assay, an increase of activity could not be observed with native and XAD-extracted water samples of the Neckar flood event, indicating a minor relevance of water-dissolved compounds for dioxin-like activity. This finding is in accordance with earlier studies investigating basic toxicity (Hollert et al. 2000, 2003a). In contrast to the solved Ah-agonists, EROD activities of fig. 6 Bio-TEQ values determined using EC_{25} values from the EROD assay (black bars) and chem-TEQs calculated by multiplying compound concentrations and REPs (gray bars) for SPM samples from the Neckar and Rhine rivers are shown. Neckar samples were selected as indicating highest dioxin-like activities at around the discharge peak of the flood.

Comparison of TEQs for each fraction allowed for an explanation of the XAD filter residue extracts and particle-bound compounds were significantly increased. An increased AhR-mediated activity was observed for the extracted and SPM sample Neckar 0, whereas the SPM sample Neckar 5 was even threefold more toxic than minimally concentrated water. Thus, EROD activity was increased, indicating that flood events resulted in an elevated exposure of aquatic organisms to dioxin-like compounds. Uptake of toxic compounds into organisms can be intensified by exposure *via* external surfaces and by ingestion in the digestive tract. Uptake conditions of contaminants *via* gills have been addressed in other studies (Erickson et al. 2006a, b), also regarding influences of natural organic matter (Klinck et al. 2005). So far, little research has been performed to bridge the gap between *in vitro* tests in the laboratory and effects on organisms and populations in the field involving the context of flood events. However, there is an urgent need to investigate the relevance of in vitro results for the situation in the field and to answer the question if a short flood event period with an elevated hazard by particle-bound pollutants may be able to cause adverse effects in fish in the field.

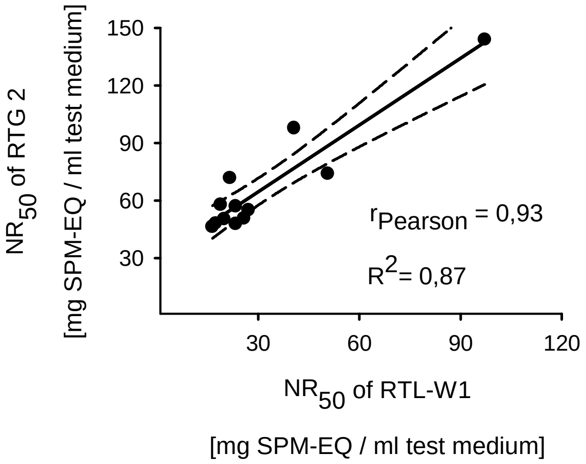

Fig. 7 Regression and correlation analysis for cytotoxicity data obtained with the rainbow trout cell lines RTG 2 and RTL-W1 in the Neutral Red cytotoxicity assay, using ten acetonic Neckar SPM extracts, are shown with the 95 % confidence interval (dashed lines). Correlation coefficients were calculated and are given as an r value (Pearson) with 95 % confidence interval and R^2 values.

3.5.3 AhR-mediated activity of SPM

Compounds causing AhR-mediated activity and more specific dioxin-like activity were the focus of this study since they are ubiquitous and highly concentrated in aquatic ecosystems (Weber et al. 2008b). This end point was mainly measured by means of the EROD assay, which is also accepted to verify AhR-mediated activity by the WHO In contrast to the neutral red assay, AhR-mediated activity of SPM was strongly increased in correlation with the flood flow and indicated a maximum activity during the peak of the flood event (bio-TEQ = 6,300 pg/g). In the present study, decreasing activities were observed upon average flow after the flood (Neckar 8 and 9), remaining on a significantly elevated level of 930 pg/g (Neckar 10) another 14 days later. However, it should be mentioned that elevated activities and high loads of SPM could be documented for several days following the maximum flood flow, indicating the high relevance of flood events for the mass transport of particle-bound contaminants causing cytotoxic and dioxin-like activity within the investigated Neckar flood event. Floods with intensified flow were shown to increase the toxicity associated with SPM assessing different biological end points (Hilscherova et al. 2007, Hollert et al. 2007a, Keiter et al. 2006, Rao et al. 1990). Whereas Neckar SPM induced an increase of activity, Rhine samples indicated a twofold decrease of AhR-mediated activity by flood flow comparing the time integrated SPM sample (including SPM of the flood event) and the SPM sample of the flood event alone. Most likely, the EROD activity of the Rhine flood sample was decreased as a consequence of dilution, alternatively, it might be suppressed by the presence of antagonistic compounds as was assumed in other studies (Chen and Bunce 2004, Peters et al. 2006). Apart from this, Rhine 2 SPM mainly consisted of significantly larger grain sizes, mostly sand, providing less adsorptive surfaces for contaminants. Thus, decreasing AhR-mediated activity might also have been caused by SPM composition. Sand load could be traced back to sediment erosion. Erosive processes seemed to be realistic regarding findings of Witt and Westrich (2003), who developed a method to determine erosion rates directly from experiments conducted with undisturbed sediment cores of the Rhine in a laboratory flume.

3.5.4 Modification of pollutant composition

PCB-TEQs of Neckar SPM increased 12-fold at the peak of flood flow, and Rhine PCB-TEQs increased even 40-fold, indicating a significant influence of flow on the amount and composition of the compound mixture. PCDD/F-TEQs of Neckar SPM were constant with flood flow, whereas TEQs of Rhine PCDD/F decreased 2.5-fold. As discussed above, compound mixtures might mainly be influenced by sediment erosion and remobilization of contaminants. Keiter et al. (2008) assessed near-surface sediment samples at the river Danube, showing that concentrations of persistent PCBs #77, #126, and #169 varied with a minimum of 23 pg/g and a maximum of 243 pg/g of dry sediment. All Neckar SPM indicated that concentrations of PCB #77, #126, and #169 increased by 4 to 52 pg/g at the peak of the discharge and were between 2 and 109 pg/g in samples collected from the Rhine. Thus, concentrations in Neckar and Rhine flood SPM were in a similar range to those in Danube near-surface sediments. Furthermore, Keiter et al. (2008) measured mean PCDD/F concentrations of 1,620 pg/g and maximum concentrations of 5,419 pg/g.

Both in the Neckar 6 and the Rhine 1 sample, PCDD/F concentrations were four to fivefold higher (6,080 and 6,771 pg/g, respectively) than mean concentrations at the peak of the flood than in the Danube sediments. While Danube sediment contamination levels highlight the high possible burden of sediments with organic chemicals, Neckar SPM indicate increased loads in consequence of the flood event (surface runoff, sediment erosion). In contrast, Rhine SPM showed decreased AhR-mediated activities, which might be traced back to dilution effects caused by the larger catchment area.

3.5.5 Comparison of AhR-mediated activity

Luminescence-based measurement of activities with the two mammalian cell lines GPC.2D and H4L1.1c4 confirmed the high toxicities of flood-borne SPM samples as determined by the EROD assay with RTL-W1 cells. Similarly to RTL-W1 cells, the rat hepatoma cell line H4L1.1c4 revealed a significant peak of activity at maximum flood flow (Neckar 6). The GPC.2D cell line indicated one peak of activity with SPM sampled before the peak of flood water flow (Neckar 3), thus differing from both other biotest systems used. Regression analysis with all bio-TEQ values of Neckar SPM indicated a good correlation between RTL-W1 and H4L1.1C4 cell line (Fig. 8, Pearson rank correlation coefficient r = 0.81). In contrast, there was no correlation between RTL-W1 and GPC.2D (r = 0.096) and H4L1.1c4 and GPC.2D (r = 0.004). Keiter et al. (2008) assessed Danube river sediment extracts and showed comparable regression coefficients using the same cell lines: RTL-W1 and H4L1.1C4 (r = 0.84), RTL-W1 and GPC.2D (r = 0.04), and H4L1.1C4 and GPC.2D (r = 0.21). Both studies indicated good correlations between RTL-W1 and H4L1.1C4 cell lines when testing

SPM and sediment extracts with respect to AhR-mediated activity, whereas no correlation was found for results of GPC.2D cell lines and the two other cell lines. These differences might be explained by species-specific induction mechanisms and

different exposure times as well as substrate inhibition in RTL-W1 cells at higher concentrations (Keiter et al. 2008). Furthermore, the GPC.2D assay indicated a 100-fold less induction than the other two bioassays. Reasons for this low induction might be differences in the transfected responsive elements as well as cell-specific and, therefore, chemical-dependent trans-acting factors and receptors (Garrison et al. 1996, Zhou et al. 2003).

In the DR-CALUX assay, the AhR-mediated activity of SPM samples ranged between 100 and ≥ 1,000 pg/g. Each year, authorities of The Netherlands have to decide whether 25 × 10^6 m^3 sediments of the port of Rotterdam get permission for disposal at sea. It has been suggested that 100 pg/kg dry weight, as measured in the DR-CALUX assay subsequent removal of acid-degradable non-persistent compounds, could serve as a threshold for prohibiting the disposal (Stronkhorst et al. 2002). In the present study, the bio-TEQs for SPM contributed by persistent compounds in the ML fractions are significantly increased compared to these values and, thus, the contamination levels of the river sediments would be too high for dumping at sea.

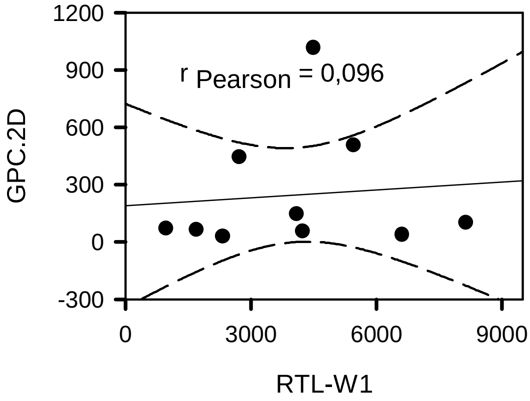

Fig. 8 Regression and correlation analysis for AhR-mediated activity, as determined using the EROD assay (RTL-W1) cells and the DR-CALUX assay H4L1.1c4. Correlation coefficients were calculated and are given as r values (Pearson) with a 95 % confidence interval and R^2 values

3.5.6 Sources of the remobilized PCDD/PCDF

The high contribution of the two fully chlorinated OCDD and OCDF (90 %, 85 %, 84 %, and 45 %, respectively, of the total 2,3,7,8-substituted PCDD/Fs) indicate that processes and products from the chlorine and organochlorine industry were responsible for a large extent of the PCDD/F contamination. Since only the 2,3,7,8-substituted congeners were analyzed in this study, a more detailed identification of sources, e.g., by principal component analysis, was not possible. However, the dominating 1,2,3,4,7,8-HexaCDF in all of the samples measured (Fig. 9a) also shows, for the lower chlorinated homologues, that elemental chlorine containing processes were also the source for most of the toxic HexaCDFs. Specifically, processes containing elemental chlorine favor the formation of the 1,2,3,4,7,8-HexaCDF *via* chlorination of dibenzofurans, whereas the formation of the 1,2,3,4,7,8-HexaCDF is not favored in incineration processes or processes from the metal industry (Fig. 9b).

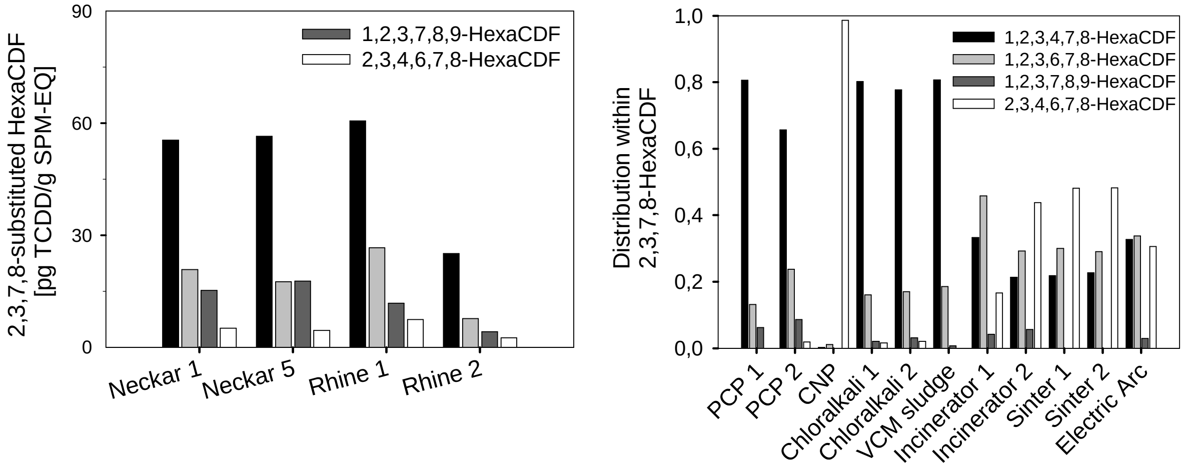

Fig. 9 a Concentrations and pattern of 2,3,7,8-HexaCDFs in SPM samples from the rivers Neckar and Rhine during the flood events of the present study. 9 b Pattern of 2,3,7,8-HexaCDFs congeners in some organochlorines (pentachlorophenols (PCP), chloronitrophene (CNP)) and processes (chloro-alkali process, vinyl chloride monomer (VCM) production) from the chlorine-organochlorine industry in comparison to patterns from incineration and metal industry

This indicates, for most of the PCDD/Fs in the monitored flooding events of the measured sectors of the Rhine and Neckar river, that the historical releases of the chlorine and organochlorine industry are still likely to be responsible for the major portion of the PCDD/F load (which, of course, also holds true for the PCB contamination). During the 1970s, the Neckar River in southern Germany ranked among the most strongly contaminated rivers in Germany, with high loads of both organic pollutants and heavy metals. These particle-bound contaminants accumulate in river-bottom sediments and, thereby, decrease the bioavailability of toxicants for a broad range of aquatic organisms. Sediments which act as contaminant sinks are more or less immobile and cohesive under normal hydrological conditions. The release of adsorbed compounds to the free water column is usually of minor relevance. Admittedly, increasing discharges as related to flood events may remobilize sediments *via* in-stream erosion and remobilize highly contaminated sediments.

Hollert et al. (2003a) worked on bottom-sediment cores elucidating ecotoxicological implications associated with the risk of erosion of contaminated sediments. It could be shown that samples below an erosional unconformity revealed a clear increase of chemical and biological indices. Furthermore, flood events with an HQ 1:5 and higher were in principle said to erode even older, well-consolidated, and highly contaminated sediments. Thus, deposited contaminants can pose a serious threat in the future, with increasing incidences of extreme flood events caused by climate change (Hollert et al. 2007a). Hence, a comprehensive erosion risk assessment of contaminated sites is of crucial importance (Gerbersdorf et al. 2007).

3.5.7 Relevance of persistent compounds analyzed

The major aim of the chemical analysis was to obtain an overview of concentrations of persistent organic pollutants in SPM from the Neckar and Rhine River, which are commonly expected to be most relevant in sediments and SPM. To this end, PCDD/Fs and PCBs were investigated which are known to induce dioxin-like activity. Bio- and chem-TEQs of multilayer PCB and PCDD/F fractions were compared with one another. In fig. 6, chem-TEQs of the multilayer PCB and PCDD/F fractions in the EROD assay were shown to equal bio-TEQ values very well, thus confirming the concept of possible additive effects in complex compound mixtures. The persistent compounds analyzed could, thus, be made responsible for the AhR-mediated activity caused by compounds in the multilayer fractions and PCB, as well as by PCDD/F fractions and analysis comprised relevant persistent pollutants in the river SPM assessed. The bio-TEQs of the multilayer fractions were low compared to crude extracts of bio-TEQs of each SPM: 5.6 % (Neckar 1), 11.8 % (Neckar 2), 12.8 % (Rhine 1), and 7.4 % (Rhine 2). Hence, less persistent AhR agonists have to be mostly effective. Other studies showed comparable or even lower contributions of these compounds quantified analytically. Keiter et al. (2008) determined bio-TEQs of PCBs and PCDD/Fs fractions clearly below 8 % assessing sediment crude extracts. Nevertheless, high proportions of the effects by crude extracts cannot be explained by persistent organic pollutants in the latter and the present study.

Furthermore, Brack et al. (2005) worked on sediments of the river Neckar and used the approach of effect-directed analysis (EDA) to prove that less persistent non-priority compounds caused the majority of AhR-mediated toxicity, underlining the recent results. Hence, in a subsequent study of Woelz et al. (2008a) that is currently underway, a combined EDA approach was used to identify the portion of less persistent compounds to the overall effect of crude extracts.

3.6 Conclusions

The combination of multilayer fractionation and biological and chemical analysis is a suitable tool to assess AhR-mediated activity by persistent PCDD/Fs and PCBs in flood event SPM samples. Flood events translocate considerable amounts of SPM and, thus, particle-bound contaminants. Depending on differences in the catchment area and the intensity of floods, the load of erodible old sediments, as well as biological effects, can be significantly increased, as shown in assessing a river Neckar flood event with high resolution sampling. In contrast, a total sample of a river Rhine flood indicated a decrease. Thus, the assessment of flood events needs a highly elaborated sampling design and intensive sampling, regarding contaminant translocation and remobilizing behavior in the context of surface runoff and discharge. The use of total samples to evaluate complete flood events is inadequate and may result in undervaluation of pollutant release, translocation, and impact on aquatic organisms. Furthermore, PCDD/Fs and PCBs were shown to contribute only to a minor portion to the AhR-mediated activity of the raw extract without an oxidative cleanup step. As a consequence, there is a need for the identification of other compounds (classes) with AhR agonistic activities and, in our study, to investigate the contribution of these unknown compounds to the overall AhR-mediated toxicity of the SPM extracts investigated in this study, and, more generally, to complex environmental samples in aquatic systems (Brack et al. 2005, 2007).

3.7 Recommendations and perspectives

As it cannot be excluded, an increase of flood events in the context of climate change has to be regarded in future flood risk management, at least in some regions such as Central Europe. Thus, it is crucial to determine the potential hazard of (re-)mobilized contaminants dislocated via floods and posing a threat to organisms and man. Furthermore, the majority annual load of particle-bound contaminants has been determined to be translocated within a few days of flood events in many catchment areas. Thus, floods remobilizing older highly contaminated sediments, may pose a risk at these rivers and lead to the fact that the good surface water status, as demanded by the EU-Water Framework Directive until the year 2015, will not be achieved (Hollert et al 2007b, Heise and Foerstner 2006, Netzband et al. 2007). Consequently, sediment mobility and particle-bound contaminants adsorbed to sediments ought to be more in the focus of recent monitoring (Babut et al. 2007, Chapman and Hollert 2006, Hilscherova et al. 2007, Hollert et al. 2007a, Westrich and Foerstner 2005). Since other less persistent compounds seem to be more relevant to explain AhR-mediated activities in flood SPM, the focus should be on PAHs and more polar compounds. Thus, effect-directed analysis using broader fractionation methods, integrating biological and chemical analysis methods, and covering compounds from polar to non-polar will be applied for the identification of causative pollutants in a subsequent study.

In order to determine the possible ecological relevance of (re-)mobilized contaminants during flood events, more focus should be put on particle-bound contaminants, at least with respect to the fate and effects of more lipophilic compounds such as those inducing AhR-mediated activity. The use of organic extracts and *in vitro* assays allows one to evaluate the potential worst-case scenario with contaminants being detached from adsorptive surfaces easily available for cellular systems. Nevertheless, the link of *in vitro* to *in vivo* approaches has been poorly subjected in the context of flood events so far. There is a need for studies closing this gap and assessing the possible effect to aquatic organisms being exposed to contaminants originating from surface runoff and sediment erosion. Recently, a feasibility study was started that connects the assessment of sediment erosion and effects on fish using a circular flume (Pathfinder Project FLOODSEARCH, Woelz et al. 2009).

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Chapter 4

Effect-directed analysis of Ah receptor-mediated activities caused by PAHs in suspended particulate matter sampled in flood events

J. Wölz[1,4], W. Brack[2], C. Moehlenkamp[3], E. Claus[3], Th. Braunbeck[4], H. Hollert[1,4]

[1] RWTH Aachen University, Institute for Environmental Research (Biology V), Department of Ecosystem Analysis, Worringerweg 1, 52074 Aachen, Germany

[2] UFZ Helmholtz Centre for Environmental Research, Permoserstraße 15, D-04318 Leipzig, Germany

[3] German Federal Institute for Hydrology, Am Mainzer Tor 1, D-56068 Koblenz, Germany,

[4] Department of Zoology, Aquatic Toxicology and Ecology Section, University of Heidelberg, Im Neuenheimer Feld 230, D-69120 Heidelberg, Germany

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4.1 Abstract

Suspended particulate matter (SPM) sampled during a flood event in the year 2004 at the rivers Neckar and Rhine (Southwest Germany) was assessed for aryl hydrocarbon receptor (AhR)-mediated activities using EROD-induction in the rainbow trout liver cell line RTL-W1. All EROD-inductions were normalized to the positive control TCDD and given as bio-TEQ values. Since all samples indicated elevated AhR-mediated toxicities, an effect-directed analysis (EDA) was applied to identify compounds causing the effects. In three primary fractions (F1 to F3) non-polar aliphatics, non-polar aromatic compounds and more polar compounds were separated. Fraction F2, co-eluting with non-polar polyaromatic compounds (PACs) including polycyclic aromatic hydrocarbons (PAHs) gave highest AhR-agonistic effects and, thus, were sub-fractionated into seven secondary fractions (F2-1 to F2-7). Fractions F2-1, co-eluting with PCBs and PCDD/Fs, did not cause AhR-agonist activities. F2-2 to F2-4 containing PACs of less than 16 aromatic C-atoms produced minor activities. Highest inductions were detected with fractions F2-5 to F2-7, containing compounds of more than 16 aromatic C-atoms (bio-TEQs up to approximately 4,500 pg/g).

Concentrations and relative potencies (REPs) of priority EPA-PAHs allowed the calculation of chemical toxicity equivalent concentrations (chem-TEQ values). Based on the chem-TEQs, EPA-PAHs explained between 16 and 58 % of crude extract bio-TEQs from both rivers. Whereas fractions F2-1 to F2-4 indicated no biological activities, EPA-PAHs in fraction F2-5 to F2-7 accounted for 2 to > 100 % of AhR-related activities.

4.2 Introduction

There is a general agreement that sediment- and particle-bound substances play an important role for the water quality in aquatic systems. Sediments can act as sinks for pollutants in the river system which are, thus, abstracted from the water column and thereby become less or non-available for aquatic organisms. In fact, worldwide, rivers are loaded with a multitude of particle-bound biologically active and toxic contaminants as a legacy of the industrial past. Further, sediments can turn into sources of contaminants, when deposited materials are eroded and remobilized as a consequence of, e.g., increased discharge and sheer-stress during flood events (Gerbersdorf et al. 2007b). Therefore, remobilization of sediments was also in the focus of some studies in the context of flood events (Hilscherova et al. 2007, Zonta et al. 2005). However, with climate change, the impact by floods received further attention, as an increase of extreme weather conditions, e.g. intense rain, and subsequent extreme floods are predicted in certain regions (Ikeda et al. 2005, Kleinen & Petschel-Held 2007). Further, eroded sediments can be transported and dislocated downstream and on inundated areas in floods as suspended particulate matter (SPM). Hazards of remobilized and contaminated sediments can be evaluated sampling suspended matter in floods (Stachel et al. 2004).

In this study, suspended particulate matter (SPM) sampled in parallel flood events – caused by storm precipitation – at the rivers Neckar and Rhine (Germany) were assessed using the concept of effect-directed analysis (EDA). EDA has been shown to be a powerful tool for toxicant identification in complex environmental samples (Brack et al. 2005, Sundberg et al. 2005). In a previous study applying the concept of EDA, strongly persistent polychlorinated biphenyls (PCBs) as well as polychlorinated dioxins and furans (PCDD/Fs) were shown to be only responsible for a minor portion (< 10 %) of the total AhR-mediated activities (Wölz et al. 2008). Thus, the present study aimed at identifying less persistent substances causing activating Ah receptor-related activity.

Polycyclic aromatic hydrocarbons PAHs can act as mutagens or even carcinogens and are, hence, of general interest (Chen & White 2004). The present study put major emphasis on the 16 EPA-PAHs (US-EPA, Laboratory Test Protocol Number 610). However, hundreds of other PAHs are present in the environment, which may affect aquatic organisms in various ways, although they have not been registered as priority contaminants (Biselli et al. 2005, Neff et al. 2005).

Thus, the major aims of this study were (1) to identify fractions causing AhR-related effects in suspended materials collected during a flood event and (2) to determine the contribution of EPA-PAHs to the overall AhR-mediated activity.

4.3 Material and Methods

4.3.1 Chemicals used

Unless stated otherwise, all chemicals used were provided by Sigma-Aldrich (Deisenhofen, Germany).

4.3.2 Sampling and preparation

Suspended particulate matters (SPM) were collected with a SPM-trap installed on a floating bridge of a power station at Heidelberg, Germany and transferred in light-safe glass bottles at 4 °C to the laboratory (methodology cf. Hollert et al. 2000). Rhine samples were taken at the water quality monitoring station at Worms, Germany. Along the pillars of the bridge, four pumps were installed to provide a continuous water supply from four lanes across the river. In the laboratory, SPM were frozen at -20 °C, samples were freeze-dried as early as possible (beta 1 - 8 K; Christ, Osterode, Germany) and stored at 4 °C in darkness until analysis.

Aliquots of 20 g of freeze-dried SPM samples were extracted with 200 ml dichloromethane using Soxhlet extraction thimbles (Schleicher & Schuell, Dassel, Germany), and for 14 h at 8-10 cycles per hour. The solvent was reduced in volume, and residues were evaporated close to dryness under a gentle N_2-stream. Residues were re-dissolved in 1 ml *n*-hexane and stored at -20°C until fractionation. As process controls, empty extraction thimbles were subjected to extraction and processed in two parallel experiments.

4.3.3 Fractionation

SPM used for fractionation were selected on the basis of maximum AhR-related activities found in a previous study (Wölz et al. 2008): Neckar A and B were sampled on January 14, 2004 (2 pm) and January 15, 2004 (8 am): Rhine A is a sample collected over an extended period from November 2003 to February 2004, including the January flood event, whereas Rhine B was sampled exclusively over the flood period from January 15 to 19, 2004.

Fractionation was performed using a recently developed, two-step procedure with some modifications (Brack et al. 2005). In order to separate and characterize AhR-agonists in crude extracts a two step procedure was applied starting with open column chromatography on alumina followed by normal-phase high performance liquid chromatography (HPLC) on a nitro-phenyl phase (Fig. 1).

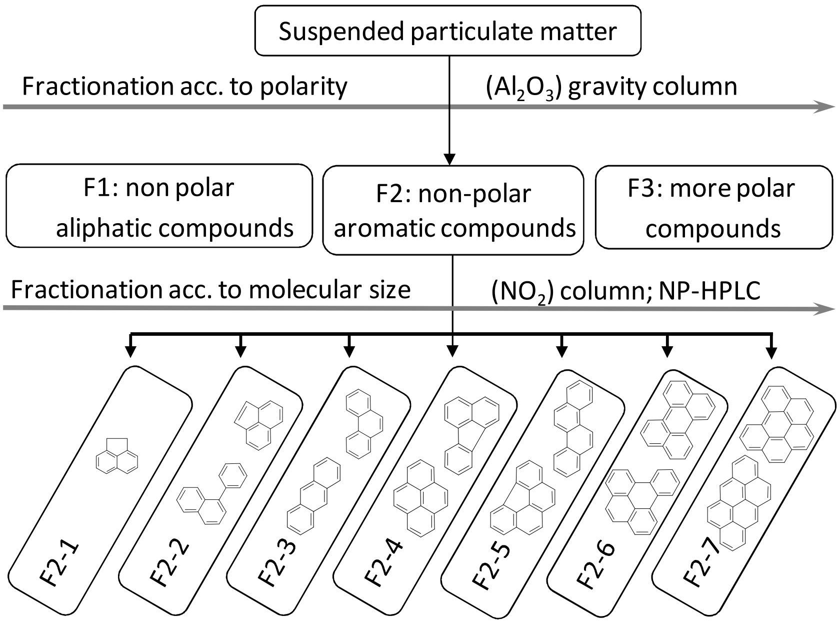

Fig. 1 Fractionation procedure for AhR-agonists in suspended particulate matter including a first separation step according to polarity providing three primary fractions and a second step for non-polar aromatic compounds, according to increasing numbers of aromatic rings (i.e., molecular size; seven sub-fractions: F2-1 to F2-7). In fraction F2-1, PCBs and PCDD/Fs may have been co-eluted. SPM − Suspended particulate matter, NP-HPLC − normal-phase high-performance-liquid-chromatography, F − fraction

4.3.4 Primary Fractionation

Crude extracts were fractionated in open glass columns with a diameter of 3 cm using 90 g of alumina (activity 1, ICN, Biomedicals GmbH, Eschwege, Germany) deactivated with 4.5 % of water per column as a stationary phase (1999). Compounds were eluted with solvents of increasing polarity: fractions F1 containing non-polar aliphatic compounds were eluted using 75 ml of *n*-hexane; fractions F2 characterized by non-polar polycyclic aromatic compounds were eluted with 250 ml *n*-hexane/dichloromethane (90/10, v/v); and fractions F3 containing more polar compounds were eluted with 250 ml of dichloromethane. All primary fractions were evaporated to dryness and re-dissolved in 1 ml dimethylsulfoxid (DMSO), corresponding to a final concentration of 20 g sediment equivalent/ml DMSO.

4.3.5 Secondary fractionation

All fractions F2 were separated into seven sub-fractions (F2-1 to F2-7) according to increasing numbers of aromatic C-rings using normal-phase high performance liquid chromatography (NP-HPLC) on a stainless steel column (21 x 250 mm) packed with nitro phenyl propyl silica (5 µm Nucleosil 100-5 NO_2, Macherey and Nagel, Düren, Germany) with a pore diameter of 0.1 nm. An isocratic solvent-mixture of *n*-hexane and dichloromethane (95/5, v/v) was used as mobile phase at a temperature of 10 °C and a flow rate of 10 ml/min.
According to Brack et al. (Brack et al. 2003a), secondary fractions are typically characterized by the following model compounds. F2.1: diaromatic compounds like polychlorinated biphenyls, dibenzo-*p*-dioxins, dibenzofurans and naphthalenes as well as the non-chlorinated parent compounds; F2.2: PAHs with MW 152-166, acenaphthylene and fluorene; F2.3: PAHs with MW 178, e.g. anthracene and phenanthrene; F2.4: PAHs with MW 202, e.g. pyrene and fluoranthene; F2.5: PAHs with MW 226-228, e.g. benzo[*ghi*]fluoranthene and chrysene; F2.6: PAHs with MW 252, e.g. benzo[*a*]pyrene and perylene and F2.7: PAHs with MW $\geq$ 276, e.g. anthanthrene and benzo[*ghi*]perylene. The elution was monitored with a diode array detector at 250 nm. Fractions were stored at 4°C in darkness until required.

4.3.6 PAH analysis

Gas chromatographic separation of the compounds of all fractions was performed on an Agilent 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany), equipped with a 30 m x 0.25 µm film HP-5MS fused silica capillary column (Agilent Technologies). Helium was used as carrier gas at a constant flow of 1 ml/min. 1 µl of each fraction was injected in split/splitless mode at 50 °C for 2 min and ramped to 130 °C at 20 °C /min and then to 320 °C at 4 °C/min. The injector and transfer line temperature were 220 °C and 310 °C, respectively. For quantification of PAHs, fractions were diluted at a factor of 15 and injected subsequently. Identification and quantification of compounds were carried out on a mass spectrometer (model 5973N, Agilent Technologies). All fractions were measured in scan and sim mode. Mass spectal data were collected in the mass range of 50 to 600 amu at full scan mode and a scan rate of approx. 2 scan/s. Electron impact (EI) at 70 eV were performed. The 16 EPA-PAHs were identified and quantified by using 6 standard solutions of different concentrations in the range grom 5 to 1,000 pg/µl (Dr. Ehrenstorfer GmbH, Augsburg, Germany) by single ion monitoring. The detection limit was 5 pg/µl. Some other standards of PAHs (Dr. Ehrenstorfer) were measured only for identification of these compounds. For further identification of unknown compounds by low resolution the spectra libraries NIST/EPA/NIH Mass Spectral Library (NIST 98) and Wiley/NBS Registry of Mass Spectral Data, 6th Ed. were used.

4.3.7 EROD induction assay

Method according to chapter 3.3.10.

4.3.8 Bio-TEQ values

Computation of bio-TEQs according to chapter 3.3.11.

4.3.9 Chem-TEQ values

Computation of chem-TEQs according to chapter 3.3.12.

4.4 Results

4.4.1 AhR-agonist activities in primary fractions

In fig. 2, AhR-agonist activities caused by primary fractions are compared to crude SPM extract inductions. Primary fractions 1 (F1) of each sample, containing non-polar aliphatic compounds, were shown to contain no AhR-inducing substances. Highest activities were associated with fractions F2, containing polycyclic aromatic compounds followed by F3, containing more polar compounds. Bio-TEQs of crude extracts and primary fractions both indicated a significant increase of activity in sample Neckar B, which was sampled at the peak of discharge during the flood in January 2004. In the Rhine samples, bio-TEQs reflected a lower AhR-agonist potency in Rhine B, if compared to the long-term sample Rhine A. Highest AhR-agonist activities were determined for Neckar B crude extract (bio-TEQ = 6,620 pg/g).

Total bio-TEQs for the crude extracts and the sum of combined primary fractions of each SPM were significantly different. At the Neckar River, primary fractions outranged total activities in the Soxhlet extract about two-times of Neckar A and 19 % of Neckar B. In contrast, bio-TEQs of crude extracts and added primary fractions were almost congruently in Rhine A, but 44 % decreased for primary fractions in Rhine B.

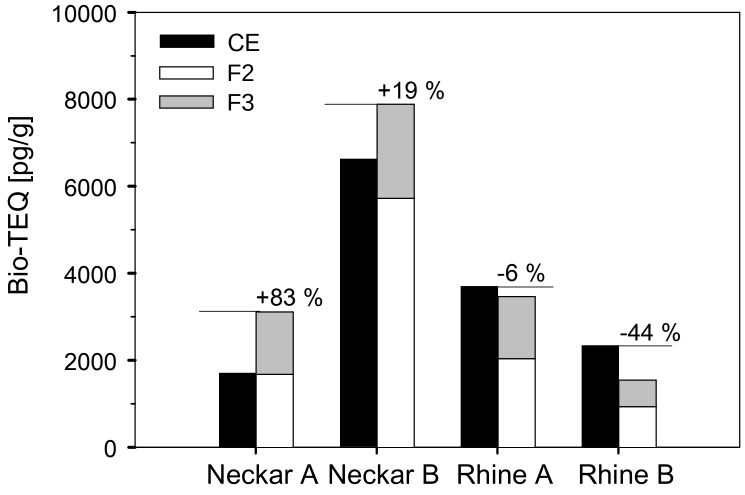

Fig. 2 AhR-mediated activities of crude extracts and each primary fractions (F1 to F3) based on EROD inductions in RTL-W1 cells are given as bio-TEQs. F1, containing non polar aliphatic substances did not cause any AhR-agonist activity. Numbers in percent give the share of added F1 to F3 to the crude extracts induction. Bio TEQs are given as means of n = 3 independent experiments. CE − crude extracts

4.4.2 Distribution of activities among secondary PAH fractions

NP-HPLC was used to fractionate F2 fractions according to their number of C-atoms in aromatic rings and, further, to identify effective secondary fractions (Fig. 3). Fractions F2-1 and F2-2 of Neckar A caused no detectable Ah receptor-agonist activity. Bio-TEQs were low in fractions F2-2 to F2-4 (bio-TEQ < 143 pg/g). Highest AhR-mediated activities were determined in F2-5 to F2-7 and a maximum bio-TEQ of 4,600 pg/g in F2-5 of Neckar B. However, except for F2-5 of Neckar B, fractions F2-6 gave the highest inducing potential among secondary fractions.

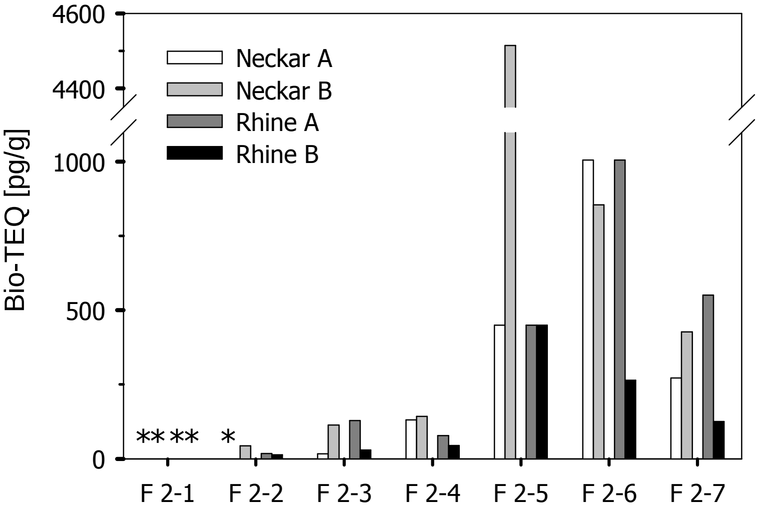

Fig. 3 Bio-TEQs of secondary fractions F2-1 to F2-7, containing non polar PAHs, determined with SPM samples from the rivers Neckar and Rhine. * − no AhR-mediated activities detected

4.4.3 Quantification of EPA-PAHs

Chemical analysis was applied to determine concentrations of the 16 EPA-PAHs that are given with relative toxic potencies (REPs) in tab. 1. Chemical analysis indicated a predominance of PAHs with molecular weights of 202 and more (≥ F 2-4). These compounds also showed high REP values and, accordingly, caused maximum AhR-agonistic activities in the EROD assay. Total PAH concentrations were highest in Neckar B (4,920 µg/kg) and lowest in Rhine B (1,020 µg/kg). Concentrations were increased following the maximum discharge in Neckar B, with the exception of indeno[1,2,3-*cd*]pyrene, dibenzo[*a*,*h*]anthracene and benzo[*ghi*]perylene. In contrast, Rhine samples indicated a decrease of concentrations for each compound after the peak discharge.

Tab.1 EPA-PAHs are shown in the order of elution in the NP-HPLC fractionation and according fractions (Brack 1999), as well as substance concentrations. REP values are given as specifically determined for used RTL-W1 cells by Bols et al. (1999).

EPA-PAH compounds	REP values	Fraction	Compound concentration [µg/kg]			
			Neckar A	Neckar B	Rhine A	Rhine B
naphthalene	n.i.	F2-1	8.6	15.2	7.5	1.6
acenapthylene	n.i.	F2-1	0.2	0.3	0.0	0.0
acenaphthene	n.i.	F2-1	11.9	12.8	6.8	2.5
fluorene	n.i.	F2-2	0,4	25,1	10,4	5,4
phenanthrene	n.i.	F2-3	244.5	290.5	141.0	60.6
anthracene	n.i.	F2-3	37.0	41.2	19.2	15.1
fluoranthene	n.i.	F2-4	680.0	844.0	404.7	226.5
pyrene	n.i.	F2-4	504.9	547.0	293.4	163.7
benzo[*a*]anthracene	0.043 x 10^{-3}	F2-5	308.1	518.5	162.0	84.4
chrysene + triphenylene	0.047 x 10^{-3}	F2-5	390.4	636.1	211.4	101.0
benzo[*b*]fluoranthene	0.193 x 10^{-3}	F2-6	404.0	507.6	146.3	87.9
benzo[*k*]+[*j*]fluoranthene	1.039 x 10^{-3}	F2-6	356.6	436.7	161.3	60,4
benzo[*a*]pyrene	0.302 x 10^{-3}	F2-6	449.1	610.6	163.0	91.9
indeno[1,2,3-*cd*]pyrene	0.278 x 10^{-3}	F2-7	291.2	240.0	96.1	55.8
dibenzo[*a,h*]anthracene	0.35 x 10^{-3}	F2-7	51.4	27.7	17.5	13.3
benzo[*ghi*]perylene	1.039 x 10^{-3}	F2-7	262.0	170.7	83.9	51.9
Sum of EPA-PAHs			4000	4920	1920	1020

F2-1 to F2-7 − secondary fractions no. 1 to no. 7, n. i. − not inducing

4.4.4 Contributions of EPA-PAHs to determined AhR-agonist activity

Finally, contributions of chemically analyzed EPA-PAHs to the Ah receptor-agonist activities in crude extracts and secondary fractions were determined comparing biologically and chemically derived TEQs (Fig. 4). Whereas EPA-PAHs analyzed in Neckar A, sampled before the peak of discharge, caused 58 % of the crude extract activity, EPA-PAHs in Neckar B, sampled shortly after the peak of discharge, were less dominant and contributed only 16 % to the crude extracts bio-TEQ. River Rhine SPM extracts gave EPA-PAH concentrations that were equal to Neckar B. In the flood sample Rhine B, priority EPA-PAHs were twofold increased compared to Rhine A and equaled half the contribution of Neckar A.

Since EPA-PAHs eluting in fraction F2-1 to F2-4 are considered as non-inducing AhR-related activities, chem-TEQs and contributions of priority PAHs could not be calculated. EPA-PAHs did induce AhR-associated effects- in each of the fractions F2-5 to F2-7 and contributed at rates between 2 and 96 % to the respective fraction bio-TEQs. F2-7 of Neckar A gave more than 100 % activity of EPA-PAHs to the biological activity.

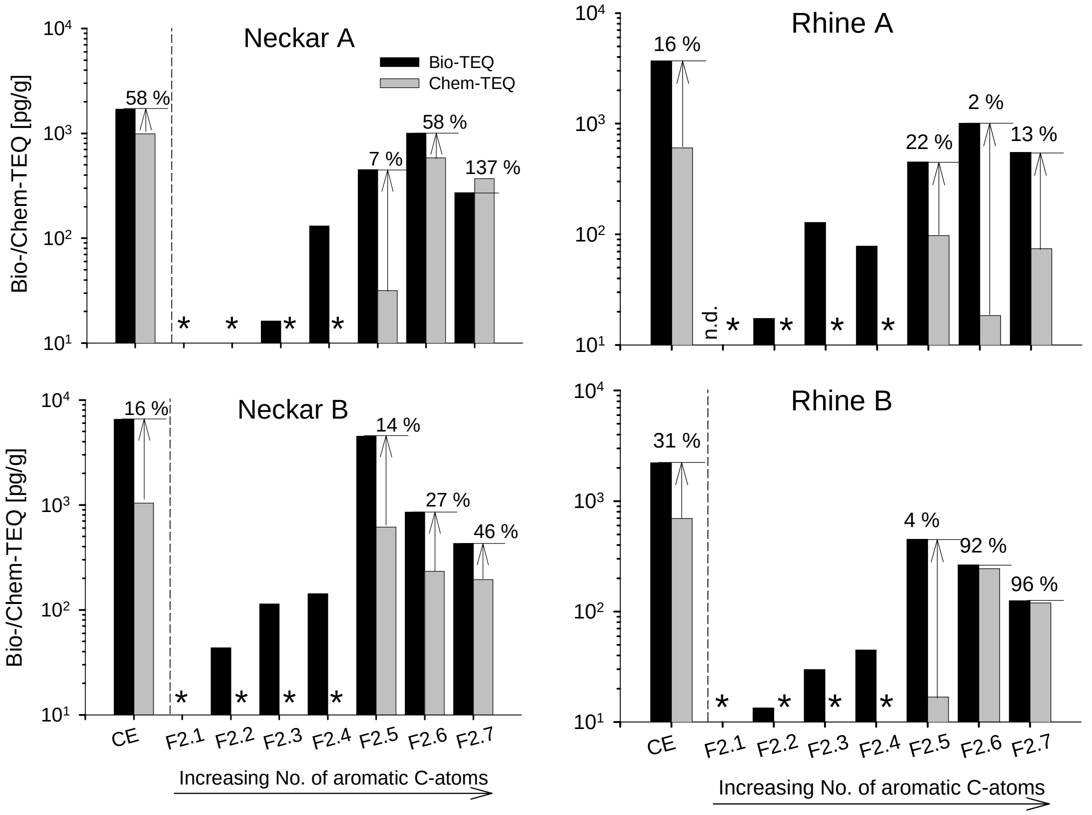

Fig. 4 AhR-mediated activities of crude extracts (CE) and secondary fractions (F2-1 to F2-7) induced by Neckar and Rhine rivers SPM are given as bio-TEQs determined in the EROD assay (black bars). EPA-PAH concentrations are given as chem-TEQs (grey bars). Contributions of EPA-PAHs of each fraction are given in percent. n.d. − no toxic effect/bio-TEQ determined, * − EPA-PAHs in this fraction are not EROD inducing with RTL-W1 cells

4.5 Discussion

4.5.1 Active fractions

Effect-directed analysis and a two-step fractionation allowed to determine and to compare shares of eluted compounds in fractions of each SPM sample. Non-polar aliphatic compounds in primary fractions F1 did not cause AhR-mediated activities in any of the samples, which corroborates conclusions drawn from other studies on Ah receptor-mediated activities (Brack et al. 2002b, Engwall et al. 1997). Fractions F2 showed the highest inducing potentials, followed by significantly increased activities in fractions F3. Thus, non-polar and often assumed PAHs (F2) and more polar compounds (F3) could be identified as major inducers of AhR-mediated activities in river SPM. These findings are in agreement with other studies on sediments, which next to non-polar polychlorinated compounds also indicated an induction potency of more polar compounds in the EROD assay (Keiter et al. 2008, Kleman et al. 1992).

However, site-specific differences were detected, and primary fractions of SPM from each river showed characteristic ratios of total and summed up bio-TEQs. In detail, activities caused by crude extracts of both Neckar samples were significantly lower than summed up primary fractions F1 to F3, in particular in Neckar A. In contrast, only Rhine B indicated a clearly decreased induction comparing bio-TEQs of the crude extract and added F1 to F3. EROD inductions with more than 100 % in fractions compared to crude extracts may be caused by retention of humic substances in the fractionation procedure. Thus, previously antagonistically acting humic substances may be separated and agonists can display their complete activity, respectively.

Since fractions F2 indicated the highest activities among all SPM assessed, secondary fractionation was focused on corresponding non-polar aromatic fractions and highest activities were determined in fractions with more than 16 aromatic C-atoms (F2-5 to F2-7). In SPM of both rivers, there was no EROD inducing potency in F2-1fractions. Thereby, a higher importance of less persistent compounds was obvious. Minor AhR-activities were determined in fractions with PAHs of lower molecular weights. These are more likely degraded, due to preferred physico/chemical- and bio-degradation (Cerniglia 1992). In contrast, higher molecular compounds are more persistent to biochemical processes. These compounds provide dense pi-electron clouds inhibiting nucleophilic substitutions and, accordingly, tend to accumulate in environmental compartments (Johnsen et al. 2005). Fractions containing higher molecular PAH compounds were also shown to induce the highest AhR-mediated activities in earlier studies on AhR-agonistic activity (Boxall & Maltby 1995, Maltby et al. 1995a). Furthermore, they cause various toxic effects in aquatic organisms (Johnsen et al. 2005, Villeneuve et al. 1997) and, therefore, are of elevated interest in ecotoxicology.

4.5.2 Evaluation of prioritized compounds

Chemical analysis was applied to identify compounds causing AhR-agonistic activity to aquatic organisms. Usually, analysis is focused on few and *a priori* selected compounds which are considered to be of high priority, e. g. the 16 EPA-PAHs. Hence, other compounds and substance classes are regarded as less relevant and are not among the commonly evaluated contaminants. In this study, most secondary fractions indicated elevated AhR-agonist activities. Nevertheless, bio-TEQs determined could often not be explained by chem-TEQs calculated from the concentrations of the analyzed 16 EPA-PAH. In fractions with PAHs of low molecular weight, only non-priority PAHs caused the total biological activity. Low molecular EPA-PAHs can even be excluded from an evaluation of Ah receptor-agonistic compounds, since they are not active with respect to AhR-related processes (Bols et al. 1999) and, thus, chem-TEQs cannot be determined. Compounds in fractions with higher molecular weights were consistently more potent inducers and showed significantly increased bio-TEQs. However, for several fractions, chem-TEQs of priority PAHs often explained only minor portions of the effects, whereas non-priority compounds caused were higher inducers. This finding emphasizes the potential contribution of non-priority pollutants to environmental hazards. In fact, in numerous earlier studies, it turned out that so far unknown and usually not analyzed non-priority PAHs were of higher relevance (Barron et al. 2004, Brack et al. 2005, Maltby et al. 1995a). Thus, an exclusive focus on prioritized pollutants may result in inadequate assessment of environmental samples, and analysis of a broader range of compounds needs to be considered.

Though EPA-PAHs are not necessarily major effective compounds, many studies provide data and allow a ranking with respect to the PAH-related burden of the SPM analyzed in the present study. However, it has to be mentioned that very little data are available for suspended particulate matter, so far. In fact, there is hardly any literature on EPA-PAH concentrations in SPM during the course of a flood. Some studies worked on PAH contamination in sediments with a focus on EPA-PAHs and determined concentrations between 0.008 x 10^{-3} and 8.7 x 10^{-3} µg/kg (El Nemr et al. 2007, Shen et al. 2008), while studies with more PAHs (including EPA-PAHs) varied between 0.01 x 10^{-3} to 25 x 10^{-3} µg/kg (Gaspare et al. 2009, Grundl et al. 2003). There are only few internationally published studies on EPA PAHs in suspended particles available. One of the few studies worked on river near Beijing and reported of 1.33 x 10^{-3} to 28 x 10^{-3} µg/kg (Shen et al. 2008).

For an attempt of SPM classification the ATV scheme as recommended by Ahlf et al. (2002b). The German ATV classification system was established for evaluation of dredged sediments. EPA-PAH concentrations of this study (1.02 x 10^{-3} to 4.9 x 10^{-3} µg/kg) are ranked in the ATV quality classes II - III (1 x 10^{-3} to 10 x 10^{-3} µg/kg) among 6 possible classes (with class VI being worst). Following this classification system, the quality goal would be reached with class II reflected by EPA-PAH concentrations of 1 x 10^{-3} to 4 x 10^{-3} µg/kg and, thus, the detected concentrations in this study indicate slightly increased compound concentrations. Ranking the findings of the present study, EPA-PAH concentrations of Neckar and Rhine SPM can be rated elevated, although maximum concentrations found in the other studies were not reached.

PAHs were listed as priority hazardous compounds in water by the Water Framework Directive (WFD) of the European Union (Annex X), focusing on concentrations in water. Meanwhile the WFD regulation implemented evaluation of particle-bound pollutants in sediment, whereas EQS are non-existing, so far. Further, the International Commission for the Protection of the Rhine (ICPR) listed namely benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene and benzo[*a*]pyrene as substances of concern, emphasizing the elevated significance of this compound class to reach environmental quality aims. Hazard assessment of PAHs in the context of the WFD and other approaches with environmental quality standards shall account for the processes and impact of flood events and the fate of particle-bound substances in SPM.

4.6 Conclusions

Effect-directed analysis using two steps of fractionation has been proved to be a meaningful tool to identify compound categories and active PAH fractions causing AhR-related effects in suspended particular matter (SPM) collected during a flood event. In the assessment of AhR-related processes in SPM samples, non-polar aliphatic compounds may be excluded, since they do not possess any AhR-inducing potential. PAHs were determined as the class with highest inducing potential and should, thus, be in the focus of AhR-related analyses on contaminations of rivers. In most SPM fractions, however, priority EPA-PAHs contributed only to a minor extent to the determined Ah receptor-mediated activities. Thus, so far non-prioritized PAH gave the higher inducing potential. Further, focus should be on high molecular weight PAHs with more than 16 aromatic C-atoms, since they could be identified as the compounds with the highest inducing potential. Since they are also more resistant to degradation, such high molecular weight PAHs are of primary ecotoxicological concern.

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Section B

Impact of flood SPM on floodplains and linked conflicts of interests

Chapter 5

Flood Retention and Drinking Water Supply − Preventing Conflict of Interests

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The RIMAX Joint Research Project HoT

M. Maier[1], D. Kühlers[1]*, H.-J. Brauch[2], M. Fleig[2], D. Maier[2], G. H. Jirka[3], U. Mohrlok[3], E. Bethge[3], H. H. Bernhart[4], B. Lehmann[4], G. Hillebrand[4], J. Woelz[5], H. Hollert[5]

[1] Stadtwerke Karlsruhe GmbH, Daxlander Str. 72, 76127 Karlsruhe, Germany

[2] DVGW-Technologiezentrum Wasser (TZW), Karlsruher Straße 84, 76139 Karlsruhe, Germany

[3] Institute for Hydromechanics, Universität Karlsruhe (TH), Kaiserstraße 12, 76128 Karlsruhe, Germany

[4] Institute for Water and River Basin Management, Universität Karlsruhe (TH), Kaiserstraße 12, 76128 Karlsruhe, Germany

[5] Department of Zoology, University of Heidelberg, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany

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5.1 Background

Diverse studies were able to identify a toxicological risk potential of suspended particles at high water (Brack et al. 2002, Hollert et al. 2000, 2003, Oetken et al. 2005). However, there are significant scientific deficiencies with regard to the influence of extreme floods, in particular on the extraction of drinking water within areas which are inundated at high water.

Numerous studies indeed show high contaminant loads of surface water samples, in part, and deposited and suspended sediments at differing water levels (Breitung 1999, Brauch et. al. 2001, Foerstner & Westrich 2005, Hollert et al. 2000, 2005, Kosmehl 2004, Maier et. al. 1997, LfU 1996a). However, there is only one pilot study so far with regard to the toxicological risk potential of suspended particulate matter during flood events on the production of drinking water. This study indicates that suspended particulate matter during flood events cause an increase of the (eco-)toxicological hazard potential (in several biotests and chemical analyses) of near surface soil samples in a riparian area which is frequently inundated as compared to rarely inundated regions (Ulrich et al. 2002). Present knowledge does not provide an answer to the question of whether either the contaminants which are partially sorbed to the soil are eluted and negatively influence the groundwater and drinking water, respectively, or are reduced or adsorbed along their passage through the unsaturated zone.

Conflicts of interests are to be expected for virtually all major rivers in Germany. On the one hand, retention areas have to be provided to minimize the risks associated with extreme flood events. On the other hand, the groundwater and bank filtrate of many riparian areas are used for the production of drinking water. Along the river Rhine, between the cities of Basel and Duisburg alone, there are 15 sites where projected retention basins and water protection areas overlap (IKSR, IAWR 1998). Water suppliers – who provide drinking water directly or indirectly taken from the Rhine for more than 20 million people – are concerned about the potentially increased risk of pollution of the groundwater resource by the establishment of a retention area in the vicinity of their water extraction facilities: Organic pollutants could be a danger to the extraction of drinking water by means of the retained water and the transported, suspended particulate matter at flood events (LfU 1996b). Besides a general degradation of the quality of the groundwater by nearby flood plains, the actual operation of water collection facilities may be endangered over longer periods, especially during extreme flood events.

5.2 Aim of the joint research project

Within the project, which is supported by the German Federal Ministry of Education and Research (BMBF), the dominant processes and mechanisms along the transport path from flood wave via retention area and groundwater to the waterworks are investigated. On the one hand, it is aspired to estimate whether contaminants and micro-organisms are able to migrate from the river into the aquifer and a nearby waterworks under the specific conditions of extreme floods. On the other hand, it is investigated whether substances and micro-organisms may reach the nearby waterworks during a regular operation of the retention basin, which is necessary for providing the retention area during extreme flood events.

In this project, the transport paths from the flood wave to the nearby waterworks are regarded as a multi-barrier system. The first barrier is the transport of contaminants and micro-organisms into the retention area. The second barrier is the unsaturated zone with its transport and retardation mechanisms. The third barrier is the flow and transport behavior within the saturated zone.

On the basis of the achieved knowledge, strategies are to be established in order to minimize mutual impairments of flood retention and drinking water supply. These strategies will be summarized to a guideline, which highlights and helps to prevent or minimize the predominant majority of present and future conflicts between flood management and drinking water supply by providing a corresponding package of measures.

5.3 Framework of investigation

The following studies are carried out within the joint research project:

- Analysis of chemical and toxicological characteristics of the water quality data and studies on suspended and deposited sediments depending on the spatiotemporal development and the discharge situation. The results of this work package will be published as a literature review in JSS.
- Chemical and toxicological testing of sediment and water samples taken from the river Rhine at diverse water levels and depending on the method used for collecting the suspended particulate matter using chemical and bioanalytical methods (e.g., Gustavson et al. 2004, Klee et al. 2004, Reifferscheid et al. 2005, Seiler et al. 2006)
- Identification of unknown contaminants with biological impact by means of effect-directed analyses (cooperation with the Helmholtz Centre for Environmental Research Leipzig, Dr. Werner Brack; cf. Brack et al. 2005).
- Estimation of particle retention in retention areas by hydrodynamic modeling and approaches of the pelit research.
- Chemical and toxicological field investigations for different soil horizons at the project study area Bellenkopf/Rappenwoert (projected retention area at the upper Rhine, river kilometer 354.5 to 359.5) on the differing loads at frequently inundated and not inundated regions. Laboratory studies to achieve parameters of several characteristic compounds on their behavior during elution and microbial decomposition.
- Determination of hydraulic soil characteristics in retention areas and modeling of the transport processes in the unsaturated zone.
- Chemical and toxicological investigations at several groundwater observation wells for documenting the spatio-temporal change of the contaminant load.
- Numerical groundwater modeling for the determination of conditions on which contaminants transported into the retention area may reach a nearby waterworks.

The projected retention area Bellenkopf/Rappenwoert (retention volume of 14 Mio. m^3) near Karlsruhe is used for the field studies. A major part of this area is situated within a drinking water protection area, which has been created for a projected waterworks. At the model site, there are regions which are already irregularly inundated at present and are therefore suited for the investigations in the unsaturated zone.

As there are partly sparse investigations (known to us) carried out or published concerning the topic of the project, the project collaborators are grateful for references to publications and also gray literature on chemical and toxicological loads of suspended and deposited sediments in the context of the literature study (fleig@tzw.de, Henner.Hollert@urz.uni-heidelberg.de).

5.4 Structure of the joint research project

The joint research project is a cooperation of five partners from four organizations:

- The Stadtwerke Karlsruhe GmbH is the project coordinator and operating company of the projected waterworks within the investigation area. It is responsible for the numerical modeling of the groundwater flow.
- The DVGW-Technologiezentrum Wasser (TZW) Karlsruhe carries out the chemical analyses of the soil and suspended sediment samples gathered in the field, and, furthermore, investigates the behavior of the contaminants in the retention area by comprehensive laboratory studies.
- The Institute for Water and River Basin Management, Universität Karlsruhe (TH) is responsible for simulations as well as field and laboratory studies on the suspended load transported into the retention basin.
- The Institute for Hydromechanics, Universität Karlsruhe (TH) carries out laboratory studies and field investigations to determine the hydraulic characteristics of the unsaturated zone in the study area and numerical modeling of transport and transformation of characteristic compounds.
- At the Heidelberg Institute of Zoology (HIZ), toxicological studies and effect-directed analyses are used to determine the biological hazard potential of suspended particles from the flood wave up to within the unsaturated zone and to identify unknown groups of contaminants.

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Chapter 6

Impact of suspended particulate matter sampled at the river Rhine with respect to operation of retention basins and drinking water safety

J. Wölz[1], M. Fleig[2], T. Schulze[3], S. Maletz[1], U. Lübcke-von Varel[3], G. Reifferscheid[4],
D. Kühlers[5], T. Braunbeck[6], W. Brack[3], H. Hollert[1]

[1] Department of Ecosystem Analysis, Institute for Environmental Research, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

[2] DVGW-Water Technology Center (TZW), Chemical Analysis Department, Karlsruher Strasse 84, 76139, Karlsruhe, Germany

[3] UFZ Helmholtz Centre for Environmental Research, Department of Effect-Directed Analyses, Permoserstrasse 15, 04318 Leipzig, Germany

[4] German Federal Institute for Hydrology, Am Mainzer Tor 1, D-56068 Koblenz, Germany

[5] Stadtwerke Karlsruhe GmbH (SWK), Karlsruhe, Germany

[6] Department of Zoology, Aquatic Toxicology and Ecology Section, University of Heidelberg, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany

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6.1 Abstract

This study investigated suspended particulate matter (SPM) sampled at the river Rhine barrage of Iffezheim, Germany. SPM were collected within the RIMAX-HoT joint research project (2005 - 2009) that worked on the question whether flood management may conflict with drinking water supply, since for example projected retention basins often overlap with water protection areas. To answer this question, SPM were sampled periodically throughout the year 2006 and more frequently in the course of a flood event with a recurrence interval of 10 years in August 2007.

GC-MS analysis was used to determine concentrations of polychlorinated biphenyls (PCBs) as well as those of the historical river Rhine contaminant hexachlorobenzene (HCB). PCB concentrations remained more or less constant in 2006 (30 µg/kg) as well as during the flood (maximum 51 µg/kg). In contrast, the sediment contaminant HCB was constantly detectable in 2006, but concentrations were clearly increasing in the August flood (maximum: 110 µg/kg).

SPM crude extracts were further assessed in order to determine dioxin-like and aryl hydrocarbon receptor (AhR)-mediated activities using the EROD assay and RTL-W1 cells form rainbow trout (*Oncorrynchus mykiss*). EROD induction, given in biological toxicity equivalent concentrations (bio-TEQs), showed elevated contamination levels in 2006 with bio-TEQs between 1,159 pg/g and 6,639 pg/g. Further, flood SPM showed a maximum bio-TEQ of 6,141 pg/g in the course of the flood. Further, mutagenic potentials were determined using the Ames Fluctuation assay with the bacterial strains TA98 (frameshift mutation) and TA100 (base pair substitution). Crude extracts indicated no significantly increased mutagenic activity with the Ames Fluctuation assay, but caused high maximum induction factors in the Comet assay ($IF_{max} = 13$).

Since hazard potentials were increased with SPM, effect-directed analysis (EDA) was used to determine effective compound classes in flood SPM; target analysis was applied to identify shares of EPA-PAHs to total biological effects. Fractionation showed that PAH fractions were highly EROD inducing. However, EPA-PAHs contributed to less than 1% to the overall biological activity. Fractions containing more polar to polar compounds gave highest inductions, at least with SPM sampled after the flood peak. Mutagenic activities of fractions was increased with SPM sampled after the flood peak, reflected by $IF_{max} = 14.7$ with the bacterial strain TA 98 without metabolic activation by S9 supplement (rat liver homogenate).

With respect to the anticipated conflict of interests between flood retention and drinking waters supply, it was shown that contaminant concentrations and biological activity were clearly increased with a flood recurrence interval of 10 years. The retention basins in question will be operated with recurrence intervals of 100 years. Since these floods can be assumed to cause definitely higher contaminant (re-)mobilization a considerable compound deposition on soils of flooded basins has to be assumed.

6.2 Introduction

Assessment of particle-bound pollutants in suspended particulate matter (SPM) is of high relevance for a sound understanding of processes and hazard potentials caused by flood events. Flood water causes considerable physical damages to inundated sites and goods as well as further to human and environmental health, since it is loaded with contaminants of concern (Euripidou & Murray 2004). These compounds mainly originate from sediment erosion, remobilization and subsequent translocation and redistribution (Koethe 2003). Since sediments serve as sinks, but also as important secondary contaminant sources, increasing contaminant loads are expected with more extreme floods in the near future (Heise & Foerstner 2006). In this context, SPM was recognized as the carrier of contaminants and hazard potentials (Schulze et al. 2007).

At present, extreme flood events such as the Elbe flood in 2002 are still hydrological outliers causing considerable economic and ecological damage with minor recurrence intervals (Ikeda et al. 2005, Klok & Kraak 2008). Nevertheless, as a consequence of climate change, these events are expected to increase in frequency and intensity in many regions worldwide (Change 2007). Furthermore, it is assumed that changes in precipitation may even be amplified in river runoff (Chiew & McMahon 2002), and there is evidence that the magnitude of peak flows increases (Middelkoop et al. 2001). Thus, recurrence intervals of floods comparable to that of the Elbe in 2002 will become shorter (Bronstert 2003).

Under specific conditions, flood impact can cause conflict of interests when flood management and further interests, e.g. drinking water supply, are concerned. In this context, strategies to manage flood impact implement the operation of retention areas with higher retention volumes than available at present (Disse & Engel 2001, Hooijer et al. 2004). However, required retention basins can, e.g., overlap with water protection areas that are needed for the operation of waterworks (Maier et al. 2006). The resulting conflict of interests appears by now alone at 15 sites along the river Rhine between Basel (Switzerland) and Duisburg, Germany). 20 millions of the 50 millions of people who live in the Rhine watershed today drink treated Rhine water which in most cases is produced from riverbank filtration (ICPR − International Commission for the Protection of the Rhine 2009). Water suppliers are concerned about the potentially increased risk of pollution of groundwater resources by the establishment of retention areas in the vicinity of their water extraction facilities.

To date, many studies have shown high contaminant loads of sediments and suspended particulate matter in the context of floods (Hollert et al. 2003, Oetken et al. 2005, Hilscherova et al. 2007, Wölz et al. 2008). Further, many studies showed elevated contamination of floodplain soils (Hilscherova et al. 2007, Pies et al. 2007, Yang et al. 2008). However, so far there is only one pilot study with regard to the (eco-)toxicological hazard potentials of sediments, suspended particulate matter and floodplain soils to the production of drinking water, indicating an increase of the (eco-)toxicological hazard potential of near-surface soil samples at inundated sites (Ulrich et al. 2002). Since elevated impact was determined in this preliminary study, an interdisciplinary follow up project was initiated to investigate this hazard potential in more detail. The results, detailed in this study, are part of the outcomes of that joint research project 'Flood retention and drinking water supply – Preventing conflict of interests' (RIMAX-HoT, Maier et al. 2006, Kühlers et al. 2009). The project worked on the possible conflict of interests at the planned retention area Bellenkopf-Rappenwoert and a nearby projected waterworks, Kastenwoert, both located next to Karlsruhe, Germany.

In this first part of the study, outcomes of ecotoxicological exposure assessment are presented with respect to contamination of SPM. Therefore, results are detailed for SPM that was sampled once at monthly intervals in 2006, and more frequently during a flood event in August 2007 with a recurrence interval of 10 years. Samples were investigated with *in vitro* biotests. Dioxin like and aryl hydrocarbon receptor (AhR)-mediated activities were determined with the EROD assay and the rainbow trout liver cell line RTL-W1 (Lee et al. 1993). Further, mutagenic potentials were assessed with the Ames Fluctuation assay and the two bacterial tester strains TA 98 and TA 100. Whereas, many compounds were chemically analyzed, concentrations of PCBs and HCB were highest by far, and, thus, only these compounds are regarded in this study. A recently developed method of effect-directed analysis (Lübcke-von Varel et al. 2008) was applied to receive further insight into contaminant loads in SPM sampled in the context of the flood event assessed. The 18 factions obtained were assessed using the biotests listed above to identify effective compound classes and chemical analysis was focused on EPA-PAHs with higher molecular weight, since low-molecular compounds are not EROD inducers (Bols et al. 1999).

Thus, the present study aimed

(a) to identify *in vitro* hazard potentials and variations of SPM sampled throughout a year and with SPM sampled in a flood event,

(b) to apply effect-directed analysis to identify effective compound classes,

(c) to chemically analyze concentrations and pattern of selected compounds in total SPM and fractions and

(d) to project the results obtained on the assumed conflict of interest between water retention and drinking water production.

6.3 Material and methods

6.3.1 Chemicals used

Subsequent providers of chemicals will only be named if other than Sigma-Aldrich (Deisenhofen, Germany). Chemicals were at least reagent grade.

6.3.2 SPM sampling

In this study SPM was collected in 2006 using a continuous-flow centrifuge that was installed just above the hydro power plant at the river Rhine barrage of Iffezheim, Germany (for location, see fig. 1) at a depth of 0.8 m according to the method described by Babarowski (2005). The Padberg Z61 (Padberg, Lahr, Germany) centrifuge type gives a flow rate of 900 L/h, 17,000 U/min and was run for 4 to 6 h.

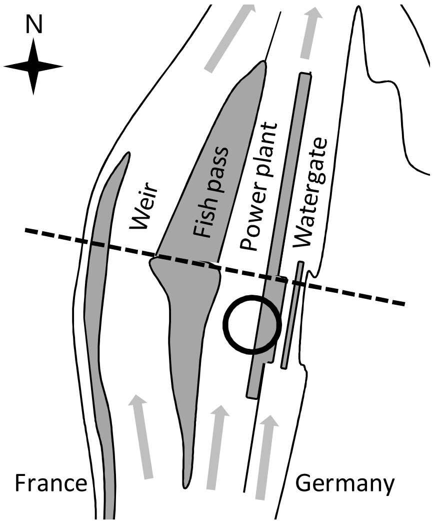

Fig. 1 Location of the continuous-flow centrifuge and the passive sedimentation boxes at the Rhine barrage of Iffezheim, Germany (circle). River flow direction is shown by light grey arrows; dashed line gives the road across the river.

Further, SPM was sampled at the same site with higher frequency in the course of a flood event with a recurrence interval of 10 years in August 2007 (Tab. 1 and fig. 2) using two passive sedimentation boxes (Schulze et al. 2007). SPM was transferred to glass bottles, protected from light and transported at 4 °C. SPM were then treated according to DIN 38414, part 22. Samples were freeze-dried in two steps using a BETA 2 - 16 (Christ, Osterode, Germany). Initially, SPM were dried for two days at 0.6 to 1 mbar and a temperature of 20 °C to 25 °C. Subsequently, SPM were post-dried for two days and at least 0.001 mbar to lower the residual moisture to < 0.5 %. SPM were than sieved at a mesh size of 600 µm for 15 min using a Bandelin Sonorex RK 255 H ultrasound bath (Schalltech GmbH, Moerfelden-Walldorf, Germany). SPM were stored at 4 °C in darkness until extraction.

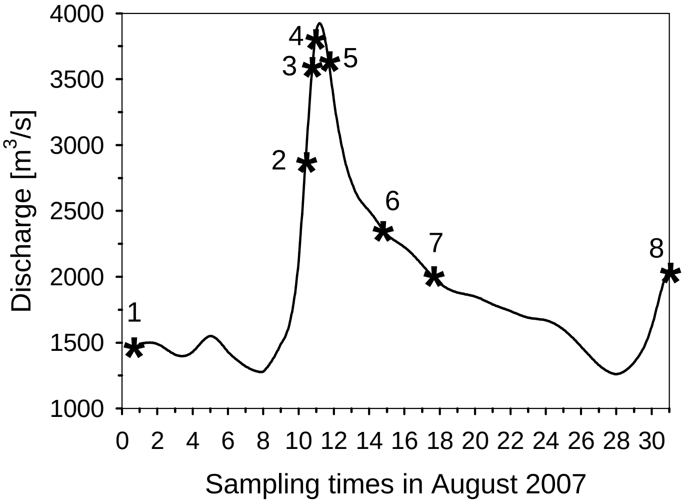

Fig. 2 Discharge at the gauge of Maxau, Germany, close to the sampling site and sampling periods in the flood course. * − sampling times of SPM in August 2007

Tab. 1 Sampling times of SPM collected at the river Rhine barrage of Iffezheim, Germany; fractionated samples are marked (X).

No.	Sampling from [date]	[time]	Sampling to [date]	[time]	Fractionated samples
1	14.07.07	12:00 a.m.	31.07.07	12:00 a.m.	
2	31.07.07	12:00 a.m.	09.08.07	21:00 p.m.	●
3	09.08.07	21:00 p.m.	10.08.07	10:40 a.m.	
4	10.08.07	10:40 a.m.	10.08.07	12:00 a.m.	●
5	10.08.07	12:00 a.m.	11.08.07	14:50 p.m.	●
6	11.08.07	14:50 p.m.	14.08.07	09:00 a.m.	●
7	14.08.07	09:00 a.m.	17.08.07	14:00 p.m.	
8	17.08.07	14:00 p.m.	31.08.07	12:00 a.m.	

6.3.3 Preparation of crude extracts

10 g of each freeze-dried SPM was weighed in 200 ml extraction thimbles (Schleicher & Schuell, Dassel, Germany), stoppered with glass wool, placed in 400 ml Soxhlet extractors and extracted with 250 ml acetone at 8 to 10 cycles/h for 14 h. The solvent was reduced in volume and residues were evaporated under a gentle N_2-stream close to dryness. Residues were re-dissolved in dimethylsulfoxide (DMSO) and stored at 20 °C until biotesting. Empty extraction thimbles were subjected to extraction and processed in two parallel experiments to serve as process controls.

6.3.4 Clean-up of extracts and automated fractionation

10 g of each freeze-dried SPM was Soxhlet extracted as detailed above using a dichloromethane (DCM):acetone (3:1; v/v) solvent mixture, reduced in volume, evaporated under a gentle N_2-stream and re-dissolved in *n*-hexane:acetone (7:3; v/v). Accelerated membrane-assisted clean-up (AMAC) was used for purification of SPM extracts (Streck et al. 2008). Briefly, 1 ml extract with a concentration of 10 g SPM equivalent/ml was transferred to dialysis membranes (low density polyethylene, 80 µm thickness; Polymer-Synthese-Werk, Rheinberg, Germany) and dialyzed using an ASE 200 device (Dionex, Sunnyvale, CA) with a mixture of DCM:acetone (3:1, v/v). The solvents used, temperature, pressure, number and duration of cycles was chosen as described previously (Lübcke-von Varel 2008). Extracts were collected in ASE glass vials closed by PTFE-coated screw caps, reduced in volume, evaporated under a gentle N_2-stream and re-dissolved in *n*-hexane:DCM (9:1; v/v) to a final concentration of 20 g/ml for subsequent fractionation.

AMAC-purified extracts were fractionated using an automated fractionation method (Lübcke-von Varel 2008). Initially, compounds of the AMAP extracts are loaded on three types of columns: More polar to polar compounds are trapped on a cyanopropyl (CN) silica column with *n*-hexane as mobile phase. Non-polar substances are flushed using a nitrophenylpropylsilica (NO) column and porous graphitized carbon (PGC) as stationary phase. Flushing of NO and PGC phase with *n*-hexane continues eluting the remaining chlorinated diaromatic substances from the NO to the PGC column.

Subsequent, sequential fractionation starts to elute compounds from each column. First of all, chlorinated diaromatic compounds are separated of PGC using *n*-hexane and toluene as mobile phase. Compounds trapped on the NO phase are successively eluted with *n*-hexane:DCM (95:5; v/v). Finally, *n*-hexane, DCM and acetonitrile are used to elute substances on the CN column. Fractions were collected in glass vessels, reduced in volume, evaporated under a gentle N_2-stream and re-dissolved in *n*-hexane (for GC-MS) and DMSO (for biotesting) to a final concentration of 10 g/ml. Model compounds for each fraction are detailed by Lübcke-von Varel (2008).

6.3.5 Chemical analysis of HCB and PCBs

The analyzed PCBs included: #28, #52, #101, #118, #138, #153, #170, #180 and #194. Analysis was performed with a Perkin Elmer Autosystem XL (Waltham, Massachusetts, USA) equipped with 63Ni electron-capture detector (ECD). The two columns used for analysis were: Column A (CLP, 30 m x 0.5 mm x 0.32 µm; Restek Corp., Bellefonte, PA, USA) and Column B (DB5, 30 m x 0.25 mm x 0.32 µm; J&W Scientific, Folsom, CA, USA). The analysis conditions were: initial column temperature 60 °C (1 min), increased at 20 °C/min to 180 °C, then increased at 3 °C/min to 207 °C and at 1.5 °C to 260 °C that were finally hold for 5 min. The carrier gas was helium. The injector temperature was 50 °C, 300 °C/min to 270 °C and the volume injected in splitless mode was 4 µl. The detector temperature was 310 °C. As internal standard 25 µl of TCX/P209, 1 ng/µl, were added to the sample prior to analysis. In addition to a blank sample with each set of samples (five to ten), a process control treated like the samples was analyzed.

6.3.6 GC-MS analysis for PAHs

GC-MS analysis was carried out on a HP 6890 GC coupled to a HP MSD 5973 (Agilent, Palo Alto, USA), equipped with a 30 m x 0.25 mm I.D. x 0.25 µm film HP-5 MS fused capillary silica column, a 5 m pre-column (Agilent J&W, Folsom, USA) and a splitless injector with deactivated glass wool. Chromatographic conditions were as follows: 280 °C injector temperature, 1 µl pulsed splitless injection at oven temperature of 60 °C (1 min isotherm), then programmed at 30 K /min to 150 °C, at 6 K/min to 186 °C and finally at 4 K/min to 280 °C (16.5 min isotherm). Carrier gas velocity (Helium 5.0, Air Liquide, Boehlen, Germany) was 1.3 ml/min at constant flow. The MS was operated in electron impact ionization mode (EI+, 70 eV) with a source temperature of 230 °C scanning from 30 to 500 amu (full scan mode) or single ion monitoring (SIM) for quantification. Target analytes were quantified using an external calibration in single ion monitoring (SIM). The results were corrected with an internal standard containing deutered PAH (Mix 35, Promochem, Wesel, Germany).

6.3.7 EROD-induction assay

Induction of 7-ethoxyresorufin-*o*-deethylase (EROD) was measured in the CYP1A-expressing cell line RTL-W1 (Lee et al. 1993) according to the method of Gustavsson et al. (2004) with the modifications given by Keiter et al. (2008). Cells were seeded in 96-well plates (TPP, Trasadingen, Switzerland) and allowed to grow to 100 % confluence for 72 h. Subsequently, the medium was removed and the cells were exposed for 72 h to the SPM extracts diluted in medium using eight dilutions with six replicates each as well as to the standards. Maximum DMSO concentration was 0.1 % since DMSO causes cytotoxicity at concentrations higher than 2 to 3 % in the well (Wölz et al. 2008). The positive control 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; Promochem, Wesel, Germany) was serially diluted to give a final concentration range of 3.13 to 100 pM on two separate rows of each plate. Exposure was terminated by removing the growth medium and freezing at 70 °C to lyse the cells.

7-ethoxyresorufin was added to each well as exogenous substrate and incubated in the dark at room temperature for 10 min. Subsequently, NADPH was supplemented to start the de-ethylation of the exogenous substrate and plates were incubated for another 10 min. The reaction was stopped by adding fluorescamine dissolved in acetonitrile. EROD activity was measured fluorometrically after another 15 min using a GENios plate reader (Tecan, Crailsheim, Germany; excitation 544 nm, emission 590 nm). Protein was determined fluorometrically using the fluorescamine method (excitation 355 nm, emission 590 nm; Lorenzen & Kennedy 1993, Kennedy & Jones 1994). The concentration-response curves for EROD induction in the RTL-W1 bioassay were computed by non-linear regression using GraphPad Prism 4 (GraphPad, San Diego, USA) and classic sigmoid or Boltzmann curves as model equations (Seiler et al. 2006, Olsman et al. 2007). The enzyme-inducing potential of the samples was converted to biological toxic equivalents (bio-TEQs) as described below.

6.3.8 Bio-TEQ values

Ah receptor agonist activities were determined as EC_{25} values of each sample and were normalized to the positive control 2,3,7,8-TCDD as biological toxicity equivalent concentrations (bio-TEQs; cf. Wölz et al. 2008). Bio-TEQs were calculated as given in eq. 1 given as mean values of n = 3 independent biotests. TCDD-EC_{25} were determined with each test plate and mean values were used for the calculating of bio-TEQ values. Subsequently, bio-TEQs with concentrations in pg TCDD/g of SEQ will be given as pg/g.

Eq. 1: Bio-TEQ [pg TCDD / g SEQ] = TCDD-EC_{25} [pg TCDD/ml] / Sample-EC_{25} [g SEQ/ml]

6.3.9 Ames Fluctuation assay

The Ames Fluctuation assay is a modification of the plate incorporation Ames test (Maron & Ames 1983) according to the method described by Reifferscheid et al. (2005). In contrast to the classic test, exposure was in liquid medium on 384-well microtitre plates. Mutagenic activity of SPM was determined with the two tester strains TA 98 (frameshift mutation) and

TA 100 (base pair substitution) as detailed by Maron & Ames (1983). Bacteria were cultured overnight in Oxoid Nutrient Broth No. 2 and ampicillin (50 µg/ml) at 37 °C ± 1 °C in a shaking water bath for not more than 10 h. Densities of the overnight inoculum were computed as formazine attenuation units (FAU) by relating measured optical densities ($\lambda = 595$ nm) to a standard (10 g/L hexamethylenetetramine, 1 g/L hydrazinesulfate; equals 1,800 FAU) according to the method described by (Hawe & Friess 2008). For testing overnight cultures were adjusted to 1,800 FAU (TA 98) and 450 FAU (TA 100).

Subsequent adjustment, bacteria were pre-incubated with exposure medium, containing low concentrations of histidine (6.45 µM per well), in 24-well microtiter plates (TPP) for 90 min at 37 °C to allow some cell divisions. Pre-incubated bacteria were 6-fold diluted in histidine deficient reversion indicator medium, containing bromocresol purple. Bacteria were distributed into 384-well plates (TPP) with 48 wells per replicate (controls and sample dilutions) for 48 h at 37 °C. Only reversed bacteria recover growth in minimal medium. Acidification by metabolic activity causes a definite switch of bromocresol from purple to yellow in the well. Wells that indicated reversions were counted. For the evaluation of metabolic activation, rat liver homogenate S9-fraction (RCC Rossdorf, Germany) from phenobarbital/ß-naphthoflavon-treated mice (protein concentration: 30.5 mg/ml S9) was added in a buffer mixture to each well.

For each test ± S9 negative and positive controls were used as validity control. Tests were valid when mean values of spontaneous revertants in negative controls counted for 0 to ≤ 5 per 48 wells (TA 98) and > 0 and ≤ 10 per 48 wells (TA 100) at all testing conditions with both strains ± S9. Positive controls were valid when no. of revertants were ≥ 25 per 48 wells as mean values for both bacterial strains ± S9 at all testing conditions. DMSO was added as solvent/negative control (maximum of 0.1 % per well). Positive controls were 4-nitro-*o*-phenylenediamine (20 nM per well) for TA 98 strain without S9, nitrofurantoin (1.67 nM per well) for TA 100 without S9 and 2-aminoanthracene for TA 98 and TA 100 with S9 treatment (0.87 nM per well).

Ames et al. (1975) used the rule that twofold induction versus the negative control indicate statistical significant mutagenic activity of a sample for the plate incorporation assay (Cariello & Piegorsch 1996). However, the Ames Fluctuation assay showed a low number of spontaneous reversions in the negative control and relatively high standard deviations. Futher, test replicates were low in number. Thus, the twofold rule may not be used. In contrast, Fisher`s Exact Binomial test that for low numbers of tests was chosen. Mutagenic activity was considered statistically significant when $p < 0.05$. This statistical method is also planned to be used in the recently developed ISO norm (International Organization for Standardization) of the Ames Fluctuation assay. Fisher`s Exact test allows to calculate NOEC values (no observed effect level/concentration). While NOEC values provide information on effects with respect to concentrations, intensities of effects are not addressed. Thus, in addition maximum induction factors (IF_{max}) were computed, that give the induction of the highest inducing sample concentration, referred to the negative control induction.

6.4 Results

6.4.1 SPM sampled in 2006

Whereas various compounds were analyzed (e.g. HCHs, DDT and metabolites), elevated concentrations were only determined for HCB and selected PCBs and showed concentrations of 7.4 to 29 µg/kg (HCB) and 4.7 to 28 µg/kg (PCBs) as given in fig. 3a. These compounds showed minor variations in concentration throughout the year 2006. Seasonal or discharge-dependent influences could not be observed. Further on, SPM were assessed with respect to Ah receptor-mediated activities and showed a bio-TEQ range of 1,160 to 6,640 pg/g (Fig. 3b). In contrast to PCB and HCB concentrations bio-TEQs of SPM showed a seasonal variation with highest inductions in June and the following month. These elevated activities were not correlated with discharge. SPM sampled in early and winter month of 2006 indicated comparably lower inductions.

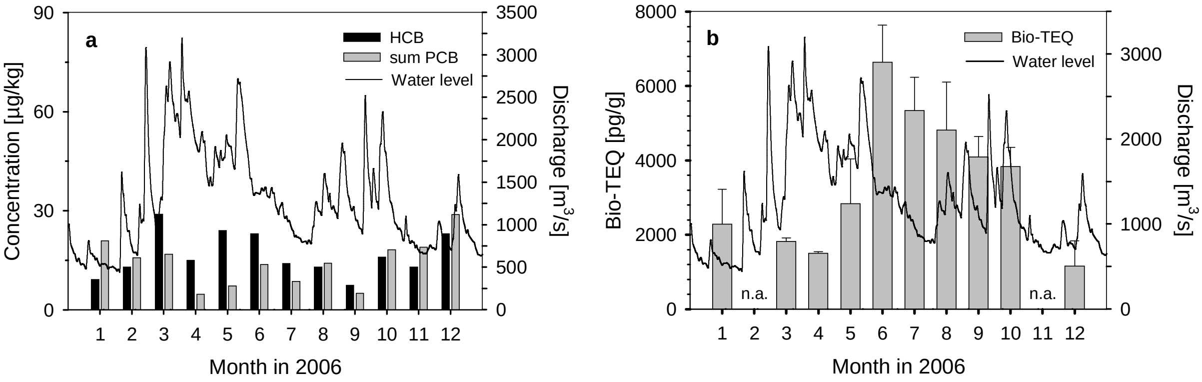

Fig. 3 (a) HCB and PCB concentrations determined with SPM sampled in 2006 using a centrifuge at the Rhine barrage of Iffezheim, Germany, in the context of the water level at Maxau, Germany, close to Iffezheim and (b) AhR-mediated activity with the same SPM samples determined with n = 3. n.a. − not assessed

6.4.2 SPM sampled in the context of the flood event in August 2007

In accordance to the data presented for SPM sample in 2006, HCB and PCBs remained the highest concentrated compounds with SPM sample at end of July and in August 2007. Concentrations of HCB and PCBs as determined in the timeframe of the flood event are given in fig. 4a. HCB concentrations were more than twofold increased in the first flood sample (August 9, 2007, 21.00 p.m.) compared to the concentrations determined before the flood at the end of July (July 31, 2007, 12.00 a.m.). Maximum concentrations of 110 µg/kg were measured at the peak discharge of the flood. HCB concentrations decreased clearly after the flood peak. However, SPM sampled subsequent indicated elevated concentrations that were higher than were about twofold increased compared to the SPM of end of July. In contrast, PCBs indicated only an increase at the beginning of the flood (67 µg/kg). Subsequent sampled SPM indicated lower concentrations (5 to 25 µg/kg) and showed no relation to the flood discharge.

Measured Ah receptor mediated activities, given as bio-TEQs, indicated a clear-cut increase of activity in accordance to the increasing discharge (Fig. 4b). TEQs indicated decreasing

AhR-agonist activities about one day after the flood peak (August 11, 2007, 14:50 p.m.) in accordance to HCB analysis. However, the maximum bio-TEQ was measured with the following sample (6,140 pg/g). SPM sampled subsequent indicated decreased, but still high bio-TEQs, that were comparable to end of July SPM.

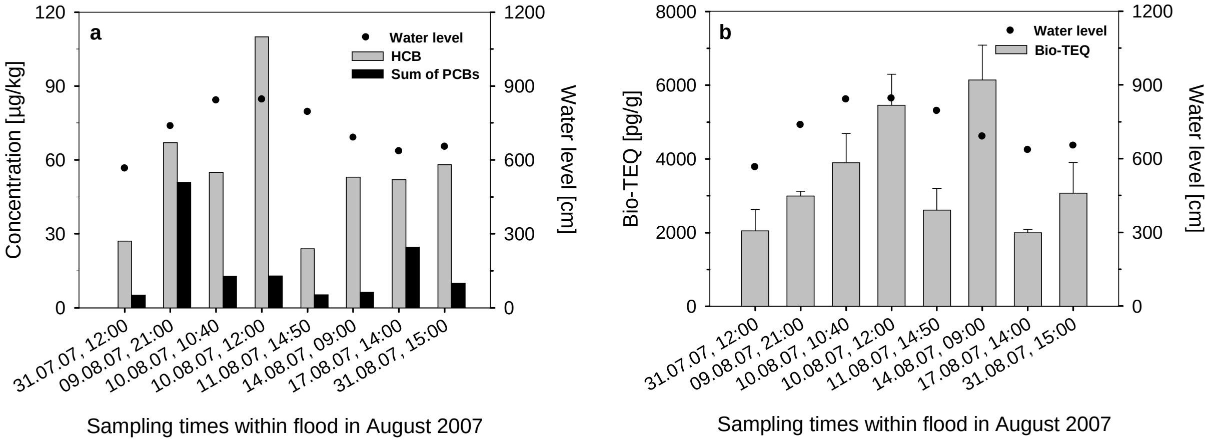

Fig. 4 (a) HCB and PCB concentrations for SPM of August 2007, sampled at the river Rhine barrage of Iffezheim (Germany) using a sediment trap. (b) Ah receptor-mediated activities for the corresponding SPM sample, given as bio-TEQ values in pg/g (n = 3).

6.4.3 Identification of effective fractions

For a more profound analysis and identification of effective compound classes, EDA was applied, providing 18 distinct fractions (Fig. 5). Fractions F1 to F4, containing for example PCBs and PCDD/Fs and fractions F5 to F7 with PAHs of ≤ 4 aromatic rings indicated minor dioxin-like and AhR-agonist potentials. Significantly increased TEQs were determined with each fraction F8 to F11. Fraction F12 mostly containing mononitro-PAHs was less inducing, while fraction F13, e.g. containing chinone, hydroxy-PAHs, was highest inducing. Fraction 14, containing e.g. (hydroxy-)quinones, keto-, dinitro-, hydroxy-PAHs, and N-heterocycles with rising polarity, gave minor bio-TEQs until the flood peak but increased activities thereafter. Fractions F15 to F18, containing e.g. 2 hydroxyanthraquinone, showed decreasing but nevertheless elevated bio-TEQs.

In order to determine contributions of compound categories to the total effect each 18 fractions were primarily added giving 11,800 pg/g (August 11, 2007) to 17,300 pg/g (August 14, 2007). Further, fractions containing PAHs (F5 to F12) and fractions containing more polar to polar compounds (F13 to F18) were added. First of all, added bio-TEQs of all 18 fractions showed a 3- to 7-fold increase of enzyme inductions compared to crude SPM extracts with all SPM assessed. Added PAH fractions were the highest inducing compound category with SPM sampled with increasing discharge at August 9, 2007. With each other sample, sums of bio-TEQs were higher with fractions containing more polar to polar compounds and a maximum bio-TEQ of 10,012 pg/g.

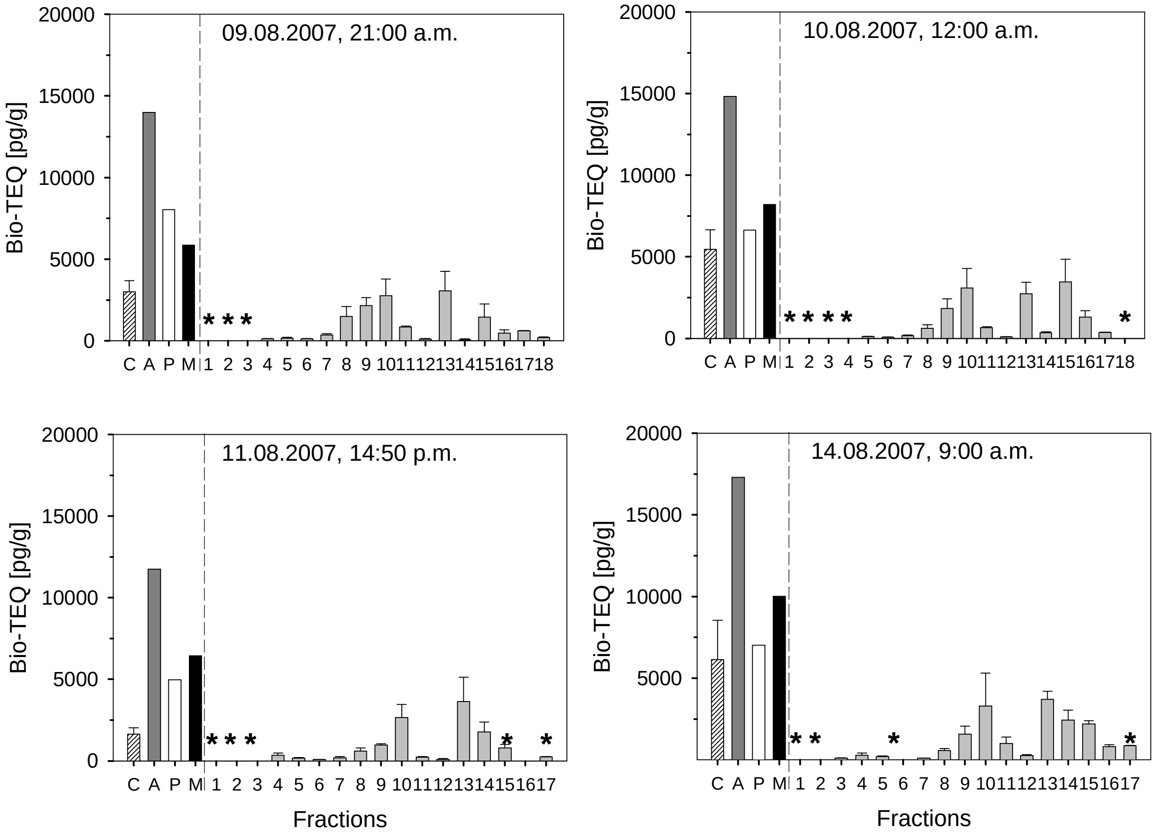

Fig. 5 Ah receptor mediated activity, given as bio-TEQ values for SPM crude extracts (C), added fractions F1 to F18 (A), added PAH fractions F5 to F12 (P), added fractions with more polar to polar compounds F13 to F18 (M) as well as for each single fraction (n = 3). * − No EROD induction detected

6.4.4 Mutagenic potentials of fractions

Mutagenic activity was measured with each SPM sample and with fractions. SPM sampled in 2006 indicated no significant mutagenic potentials. However, fractions of SPM sampled in August 14, 2007 at 12 a.m. caused significantly elevated effects. Fisher`s Exact Binominal test showed significant NOEC values ≤ maximum concentration with fractions containing more polar to polar compounds (Tab. 2).

Fraction F15 revealed the highest mutagenic potential with TA 98 without S9 metabolism and a NOEC < 2.08 mg/ml, the lowest concentration assessed. Thus, elevated potentials were caused by compounds that induce frameshift mutations. Further, mutagenic activity was highly increased with TA 100 without S9 metabolism in F13, showing that compounds causing base pair mutations were highest concentrated in this fraction.

Further, maximum induction factors were computed for fractions that were determined to show significant elevated mutagenic potentials. With respect to IF_{max} highest mutagenic activity was determined accordingly in F15 and TA 98 without S9 metabolism (IF_{max} = 14.7).

Tab. 2 Mutagenic potential of SPM fractions of August 14, 2007, 12 a.m. in the Ames Fluctuation assay using bacterial strains TA 98 and TA 100, determined with n = 1 and 48 replica per test. Mutagenic potentials are given as NOEC value and maximum induction factor (IF_{max}).

Fraction no.	NOEC [mg/ml]				Fraction no.	Induction factor (IF_{max})			
	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9		TA 98 -S9	TA 98 + S9	TA100 -S9	TA100 +S9
1	*	*	*	*	1	*	*	*	*
…	…	…	…	…	…	…	…	…	…
13	*	16.67	4.17	*	13	*	3	3.7	*
14	*	*	*	*	14	*	*	*	*
15	< 2.08	*	*	*	15	14.7	*	*	*
16	8.33	8.33	*	16.67	16	4.3	1.7	*	2.0
17	*	*	*	*	17	*	*	*	*
18	*	16.67	*	33.33	18	*	5.3	*	9.3

Maximum concentration in test: 66.67 mg/ml, lowest concentration in test: 2.08 mg/ml, ± S9 − Metabolic activation using rat liver homogenate of the S9 fraction in the liver centrifugate, * − NOEC > 66.67 mg SPM equivalent/ml test medium and no IF_{max} determined

6.5 Discussion

6.5.1 Chemical loads of crude extracts

Chemical analysis showed that PCBs were detectable at minor rates in SPM sampled in 2006 and less conspicuous in the flood of August 2007. Concentrations in 2006 gave a mean of 11.7 ± 8.6 µg/kg and in the August flood a mean of 16.9 ± 16.5 µg/kg. Thus, PCB concentrations were comparable to SPM of a flood in January 2004 at the Rhine (recurrence interval of 2 years) and maximum concentrations of 32 µg/kg (Wölz et al. 2008). Ranking these findings with other studies that investigated river sediments (0 to 339 µg/kg) indicated concentrations in SPM to be comparably low (Mai et al. 2002, Zhang et al. 2004, Samara et al. 2006, Zhang et al. 2007).

In contrast, HCB showed concentrations of 4.8 to 85 µg/kg (median = 16 µg/kg) in SPM of 2006 and 24 to 110 µg/kg (median = 53 µg/kg) in SPM sampled in the August flood, thus, being 3.3 fold increased in the flood comparing medians. Ulrich et al. (2002) determined HCB concentrations with a maximum of 203 µg/kg in SPM sampled in the fish ladder at the barrage of Iffezheim, thus, being twofold increased compared to the present study. Other studies measured HCB concentrations of 220 µg/kg in sediments at the barrage of Iffezheim at a depth of 0.2 to 1.2 m and about 40 µg/kg near to surface (Alcock et al. 2003). Using the Chemistry-Toxicity Test (CTT) approach (Heise et al. 2004, Heise & Foerstner 2006), the action level for HCB (= 20 µg/kg) is clearly exceeded. Thus, e.g., dumping of Rotterdam port sediment at sea would no longer be allowed and deposition at specified dumps causing considerably increased costs would be necessary (Netzband 2007). However, HCB in detected concentrations is perturbing as this compound is classified as 'substance of concern', since it frequently exceeds regulatory criteria for suspended matter (Heise & Foerstner 2006). Further, due to its persistence, HCB is listed as one of the 'dirty dozen' in the Stockholm Treaty on Persistent Organic Pollutants (POPs; SC 2004) and as a priority hazardous substance in the Water Framework Directive (WFD; EC 2008). Thus, HCB is a major hazardous compound and has to be included in measure strategies, since a successful management will take influence on the decision whether a good chemical status is obtained in water, sediment and biota according to the WFD aims (Coquery et al. 2005, Förstner 2008). Elevated compound loads indicate a risk of compound introduction in retention basins and contamination of flooded soils.

6.5.2 Biological hazard potential in crude extracts

Ah receptor agonists were elevated and strongly varying in 2006 with comparable inductions in the August flood. Whereas, the reasons of the increased effects in 2006 remain unclear, so far, it is evident that elevated EROD inductions can be caused by incidents other than flood events. However, the clear difference between both elevated EROD inductions is the time frame. Floods cause among others rapidly increasing contaminant (re-)mobilization and exposure, whereas in 2006 effects seemed to increase more slowly, beginning with SPM sampled in May, but lasted for month and, thus, show elevated long-term contamination.

In a previous study SPM sampled in a flood in January 2004 with a recurrence interval of 2 years showed highest bio-TEQ = 2,300 pg/g, whereas SPM sampled in the winter month (November 2003 to February 2004) induced bio-TEQs = 3,700 pg/g (Wölz et al. 2008). In the present study, bio TEQs were about 2.7-fold increased compared to theses maximum values. Thus, higher impacts are indicated through more intensive floods. Koh et al. (2004) determined maximum bio-TEQs of 1,500 pg/g in sediment of the Hyeongsan River, Korea, using H4IIE-luc cells. Hilscherova et al. (2003) used the same cell line and found bio-TEQ of 1,860 pg/g in sediment of the Tittabawassea River, Michigan, USA. Further, Hollert et al. (2002) used RTL-W1 cells and the EROD induction assay to assess sediments of the catchment area of the river Neckar, Germany, and determined bio-TEQs of about 1,000 pg/g. Comparing these bio-TEQs to SPM sampled in the present study underlines increased AhR-inducing potentials and, thus, elevated hazard potentials. In accordance to the detailed results on chemicals, AhR-agonists indicate an increased load of inducing particle-bound compounds, and, accordingly, an impact to inundated sites, such as retention basins.

6.5.3 AhR-agonists and mutagenic potential in fractions

Whereas increased EROD inductions were determined with SPM crude extracts, active compounds were not identified so far. Thus, an automated EDA method was used to reduce the complexity of each sample and to identify inducing fractions and target compounds. Itemized biotests and chemical target analysis showed that fractions containing PAH caused increased effects. However, fractions containing more polar to polar compounds were identified to be inducing highest. Chemical analysis was performed with respect to so called priority EPA-PAHs (EPA, Laboratory Test Protocol Number 610) with more than four aromatic rings and gave minor EPA-PAH concentrations of 5.2 to 50.9 µg/kg. Chem-TEQs were calculated as products of compound concentrations and cell line specific toxicity factors that were determined relative to the reference (cf. Olsman et al. 2007). Chem-TEQ values equaled far less than 1 % of the bio TEQs (therefore, data not shown in detail). Thus, other non-priority chemicals were causing effects in PAH fractions. In general, these findings are in accordance with other studies that worked on PAH contaminations in sediments (Brack et al. 2002, Barron et al. 2004, Brack et al. 2005) and flood SPM (Wölz et al. 2008).

Next to AhR-agonists mutagenic potentials of SPM crude extracts and fractions were assessed. Significantly elevated bacterial reversions were not determined with crude SPM extracts but flood SPM fractions were mutagenic with SPM sampled after the flood peak. Sediment and SPM extracts of a flood with a recurrence interval of 1 year at the Neckar, Germany, were also not inducing as determined with the Ames Plate Incorporation assay in another study (Hollert et al. 2000). However, SPM sampled in a flood with a recurrence interval of 15 to 20 years at the Neckar was shown to cause IF_{max} = 3.2 (Hollert et al. 2003). In the present study, significant inductions were only detected in fractions of SPM sampled after the flood peak and highest inductions were caused in fraction F14 containing more polar compounds (IF_{max} = 14.7). Elevated mutagenic potencies of compounds eluted in these fractions had been shown before (Schuetzle et al. 1981, Kataoka et al. 2000, Eisentraeger et al. 2008).

Further, crude SPM extracts of 2006 were also investigated using the Comet assay as detailed by Singh et al. (1988) in the modification of Schnurstein & Braunbeck (2001; details not shown). These SPM extracts showed up to 13-fold increased IF_{max}. Kosmehl et al. (2004) assessed sediment cores from the river Rhine, Germany, and gave IF_{max} = 90.5 with RTG 2 cells and IF_{max} = 47.0 with RTL W1 cells. Ranking these findings with the present study indicates lower, but nevertheless elevated mutagenic potentials with the SPM assessed. Since in a subsequent study Kosmehl et al. (2006) showed that mutagenically active compounds of the sediments were bioavailable in principle, using a novel contact assay with zebrafish (*Danio rerio*). With respect to the assumed conflict of interests, these findings indicate that translocation of particles of floods into retention basin may result in deposition of highly toxic compounds on soil.

6.6 Conclusions

The investigation of SPM sampled continuously over months and, with higher frequency, in times of flood events, allows evaluating dioxin-like and Ah receptor agonist activities as well as mutagenic potentials. Investigation of chemical loads and biological activities over months allows detection of variations in activity throughout the year and should be taken into consideration in the evaluation of activities that are determined on small temporal scales in floods.

Whereas AhR-mediated activities can be assumed to be highly increased in floods, further influences might lead to comparably elevated hazard potentials. However, AhR-agonists are highly active throughout the year and, thus, particle-bound contaminants have to be addressed for evaluation.

In particular, particle-bound tracer compounds typical of each catchment area may be used to evaluate contaminant loads as mentioned recently by Brack et al. (2009). At the Rhine, HCB is a known and omnipresent pollutant that acts as such a compound. Since HCB is a particle-bound compound in sediments, elevated concentrations in flood SPM act as indicator for sediment deposition. However, lower concentrations are bound to SPM and detectable throughout the year. In contrast, PCB contaminations seem to be less correlated with sediment remobilization in floods; concentrations are constant over long observation periods.

Automated fractionation methods can be used to identify classes of effective compounds in highly inducing samples. Further, applied target analysis allows identifying concentrations and shares of analyzed compounds to the overall biological activity. Percentages of priority compounds, even when minor, provide valuable information since low shares indicate that other novel compounds are more relevant. Thus, more polar to polar compounds should be investigated with elevated emphasis in future studies that work on hazard potentials of contaminant loads in floods, in particular with respect to floods with high recurrence intervals. Dioxin-like and AhR-mediated, as well as mutagenic activity are valuable endpoints to determine hazard potentials, since concerning activities in crude extracts and fractions with more polar compounds have repeatedly often been shown recently.

With respect to conflict of interests between flood management and drinking water supply, pollution of flooded areas as e.g. retention basin can be assumed (Hilscherova et al. 2007). Even more so, since the addressed retention basins will be operated only for floods with recurrence intervals of 100 years and higher. A further hazard to the aquifer and drinking water resources cannot be derived from these results. Thus, further investigations of RIMAX-HoT focused on floodplain soil and groundwater contamination at a site designated for the operation of a retention basin but also protected as a drinking water protection area.

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Chapter 7

Pollution of riparian areas in consequence of inundation by extreme flooding

J. Woelz[1], T. Schulze[2], M. Fleig[3], G. Reifferscheid[4], U. Lübcke von-Varel[2],
W. Brack[2], H. Hollert[1]

[1] Department of Ecosystem Analysis, Institute for Environmental Research, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

[2] UFZ Helmholtz Centre for Environmental Research, Department of Effect-Directed Analyses, Permoserstrasse 15, 04318 Leipzig, Germany

[3] DVGW-Water Technology Center (TZW), Chemical Analysis Department, Karlsruher Strasse 84, 76139 Karlsruhe, Germany

[4] German Federal Institute for Hydrology, Am Mainzer Tor 1, D-56068 Koblenz, Germany

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7.1 Abstract

In this study, soil was sampled at inundated and non-inundated sites within a projected retention basin that is planned to be operated with floods of recurrence intervals greater than or equal to 100 years. This basin overlaps with a water protection area that is essential for a projected nearby waterworks. The detailed investigations are part of the RIMAX-HoT joint research project (2005 - 2009) that assessed the conflict of interests between flood management and drinking water supply.

Sampled soil cores were cut into distinct layers and investigated using *in vitro* biotests. Dioxin-like and AhR-mediated EROD enzyme inductions (Cytochrome P450 monooxygenase) were assessed using the fibroblast-like RTL-W1 cell line from rainbow trout (*Oncorhynchus mykiss*). Mutagenic potentials were assessed with the Ames Fluctuation assay and the tester strains TA 98 and TA 100 of *Salmonella typhimurium* bacteria. While mutagenic activity was not detected in soil layers, elevated EROD inductions were measured in topsoil, that were decreasing in deeper layers. However, one site - a ground swale - was determined to be highly inducing as reflected by a biological equivalent concentration (bio-TEQ) of about 41,000 pg/g. Chemical analysis with respect to HCB (0.049 mg/kg), EPA-PAHs (39 mg/kg) and selected PCBs (0.19 mg/kg) gave relative increases at this site. Further, chemical loads and biological activities were determined to be increased at least down to 90 cm subsurface.

The highly polluted topsoil layer was chosen for fractionation using a recently developed automated effect-directed fractionation method that was used to identify effective compound categories. Fractions containing PAHs were determined to cause the bulk of EROD-induction as reflected by an added fraction bio-TEQ of 32,000 pg/g (of a total bio-TEQ = 43,000 pg/g of all added fractions). Further, fractions containing moderately polar and polar compounds caused elevated inductions (bio-TEQ ≈ 8,200 pg/g). Although crude extracts were not mutagenic single fractions showed heterogeneous and elevated potentials that were computed as NOEC values (No observed effect concentration) and maximum induction factors (IF_{max}). With most fractions, tester strain TA 98 (frameshift mutation) showed significantly reduced NOECs independent of S9 (rat liver homogenate) metabolic activation. TA 100 indicated only some few fractions to cause base pair substitution. In accordance to the EROD assay, fractions containing PAHs, moderately polar and polar compounds caused elevated mutagenic activity. However, the latter compounds were more toxic and showed NOECs down to 0.03 mg/ml and $IF_{max} = 29$.

Relating these findings to the assumed conflict of interests, an impact to the aquifer and, thus, drinking water resources cannot be excluded. Since ground sampled in a swale was shown to be highly polluted far below surface, compounds may be less retained and passage to the aquifer is facilitated at those sites. Further, the elevated load of mutagenic moderately polar and polar compounds may easily pass the unsaturated soil zone. Introduced to the aquifer, these compounds may represent a threat to groundwater quality. Thus, further research with

respect to groundwater contamination and hazardous compounds will assist to evaluate the risk of aquifer pollution.

7.2 Introduction

Floodplain soil often is loaded with many contaminants as a consequence of inundation during flood events (Zonta et al. 2005). In general, floods cause increasing sediment erosion in accordance with water discharge. Erosion can reach deep and remobilize in particular highly loaded (older) sediment layers (Hollert et al. 2007a, Stronkhorst & van Hattum 2003). Following erosion sediment can be translocated as suspended particulate matter (SPM) and, thus, be displaced at any inundated site. Therefore, next to downstream river sections, eroded matter primarily affects floodplains, depositing in a considerable amount since currents are lower at flat river banks that are usually abundantly covered with vegetation (Jeffries et al. 2003).

SPM are initial matter for soil genesis and appear to be an important nutrient source (Wassen et al. 2002). However, remobilized sediments are also hazardous since in the river they act as both sinks and important secondary sources of contaminants introduced into the aquatic environment (Foerstner 2004, Kosmehl et al. 2004). Thus, matter deposited on floodplains provides a potential to (highly) contaminate affected sites, in particular with intensified erosion during extreme floods (Weber et al. 2008b). Typical contaminants at the river Rhine are polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and as a special case of the Rhine catchment hexachlorobenzene (HCB; Heise & Foerstner 2006, Klok & Kraak 2008, Wölz et al. 2008).

Deposited matter preferably accumulates in surface depressions and water basins that, thus, often contain the highest contaminant loads (Asselman & Middelkoop 1995). Following floods, these contaminants can impact adjacent areas by wind drift but significant amounts remain on floodplains (Baborowski et al. 2007). These may be retained in the topsoil layers that provide humic compounds and clay minerals. However, floodplain soils are not a uniform matrix, but are highly heterogeneous geosorbents composed of various sized grains, which have different origin, formation, and physicochemical properties (Yang et al. 2008).

The findings presented in this study are part of the joint research project 'Flood retention and drinking water supply − Preventing conflicts of interests' (RIMAX-HoT, Maier et al. 2006, Kühlers 2009). This project aimed to identify potential conflicts of interests at the projected retention basin Bellenkopf-Rappenwoert and a nearby planned waterworks Kastenwoert, both located next to Karlsruhe, Germany. Chemical loads and hazard potentials of SPM were previously detailed by Woelz et al. (2009a) and elevated PAH and HCB concentrations as well as elevated AhR-agonist activities and mutagenic potencies were determined in a flood in August 2007. Thus, the present study aimed to assess whether elevated compound concentrations and biological activities can be determined in soil cores sampled at inundated sites compared to non-inundated sites that are located behind a levee. Chemical analysis was used to identify loads of PAHs, PCBs and HCB. 7-ethoxyresorufin-*o*-deethylase (EROD) induction assay and Ames Fluctuation assay showed biological hazard potentials with respect to *in vitro* biotest systems. Since elevated compound concentrations and Ah-mediated activities (computed as bio-TEQs) were measured and an automated fractionation procedure (Lübcke-von Varel et al. 2008) was used to identify effective compound classes. Target analysis showed shares of so-called priority EPA-PAHs (defined by the United States Environmental Protection Agency, US-EPA) to the overall EROD induction. Accordingly, fractions were assessed with the Ames Fluctuation assay and mutagenic potencies were detected as caused by compounds in each fraction.

Thus, the present study aimed to

(a) measure chemical loads and biological responses in soil core layers from inundated and non inundated sites,

(b) to use an automated fractionation procedure to identify effective fractions and shares of target analytes, and

(c) to determine whether inundated sites have an impact in contrast to non-inundated sites.

7.3 Materials and methods

7.3.1 Chemicals used

Provider of chemicals used in this study will only be listed if other than Sigma-Aldrich, Deisenhofen, Germany. Chemicals were at least reagent grade and LiChrosolv grade for fractionation.

7.3.2 Soil sampling

In this study soil was sampled at August 22/23, 2006, at the projected retention basin Bellenkopf-Rappenwoert. Soil was sampled at six locations in the basin area (north, middle, south), with three of them sampled at inundated sites close to the river and three at sites behind a levee that were, thus, not influenced by flooding (Fig. 1).

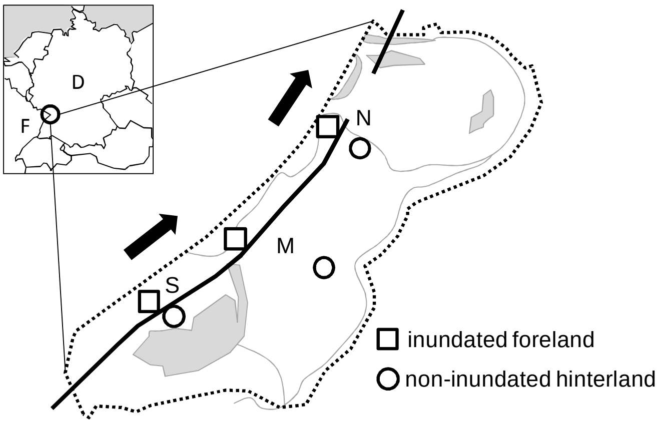

Fig. 1 Location of the projected retention basin Bellenkopf-Rappenwoert near Karlsruhe, Germany. Inundated foreland and non-inundated hinterland are separated by a levee (straight black line). Soil was sampled in the north (N), middle (M) and south (S). Grey lines and filled areas give water courses and basins in the basin. Black arrows show the river Rhine flow direction. D − Germany, F − France

Soil was sampled from surface down to a depth of 90 cm using a viscoplastic standard stainless steel soil corer according to Dr. Pürckhauer (diameter: 28 mm; Schierholz et al. 2000) and a maximum drilling depth of 1,000 mm. Each sample was further separated into three sub-samples of 30 cm (0 - 30 cm, 30 - 60 cm, 60 - 90 cm). Samples were transferred to glass bottles, transported at 4 °C and protected from light. Samples were shock-frozen at -30 °C and freeze-dried on an Alpha 1 - 4 freeze-drier (Christ, Osterode, Germany) at -40 °C and 0.1 mbar as fast as possible and stored at 4 °C in darkness until extraction.

7.3.3 Soil extraction for assessment of total samples

Soil extraction for assessment of total samples

Total soil samples were treated according to DIN 38414, part 22. Samples were freeze-dried in two steps using a BETA 2 - 16 (Christ, Osterode, Germany). Initially, soil was dried for two days at 0.6 to 1 mbar and a temperature of 20 °C to 25 °C. Subsequently, SPM were post-dried for two days and at least 0.001 mbar to lower the residual moisture below 0.5 %. Soil was than sieved at a mesh size of 600 µm for 15 min using an ultrasound bath type Bandelin Sonorex RK 255 H (Schalltech GmbH, Mörfelden-Walldorf, Germany).

10 g of each freeze-dried soil sample were weighed in 200 ml extraction thimbles (Schleicher & Schuell, Dassel, Germany) stoppered with glass wool, placed in 400 ml Soxhlet extractors and extracted with 250 ml dichloromethane (Sigma-Aldrich, Deisenhofen, Germany) for 14 h at 8 - 10 cycles per hour according to the method given by Hollert et al. (2000). The solvent was reduced in volume and residues were evaporated under a gentle N_2-stream. Residues were re-dissolved in 1 ml *n*-hexane and stored at -20 °C until fractionation. Empty extraction thimbles were subjected to extraction and processed in two parallel experiments to serve as process controls.

7.3.4 Soil extraction and clean-up for fractionation

10 g of each freeze-dried soil layer was Soxhlet-extracted as detailed above using dichloromethane (DCM):acetone (3:1; v/v) solvent mixture, reduced in volume, evaporated under gentle N_2-stream and re-dissolved in *n*-hexane:acetone (7:3; v/v). Further, a recently developed membrane-assisted clean-up step (AMAC) technique was used for purification of soil extracts according to the protocol by Streck et al. (2008). For this end, 1 ml extract with a concentration of 10 g soil equivalent/ml was transferred to polyethylene dialysis membranes and extracted using an ASE 200 device (Dionex, Sunnyvale, CA). Detailed extraction conditions are provided by Lübcke-von Varel (2008). Extracts were sampled in ASE glass vials closed by PTFE-coated screw caps. Extracts were reduced in volume, evaporated under a gentle N_2-stream and re-dissolved in *n*-hexane:DCM (9:1; v/v) to a final concentration of 10 g/ml for subsequent fractionation.

7.3.5 Automated fractionation procedure

Method according to chapter 6.3.4

7.3.6 GC-MS analysis of fractions

GC-MS analysis were carried out on a HP 6890 GC coupled to a HP MSD 5973 (Agilent, Palo Alto, USA), equipped with a 30 m x 0.25 mm I.D. x 0.25 µm film HP-5 MS fused capillary silica column, a 5 m pre-column (Agilent J&W, Folsom, USA) and a splitless injector with deactivated glass wool. Chromatographic conditions were as follows: 280 °C injector temperature, 1 µl pulsed splitless injection at oven temperature of 60 °C (1 min isotherm), then programmed at 30 K/min to 150 °C, at 6 K/min to 186 °C and finally at 4 K/min to 280 °C (16.5 min isotherm). Carrier gas velocity (Helium 5.0, Air Liquide, Boehlen, Germany) was 1.3 ml/min at constant flow. The MS was operated in electron impact ionization mode (EI+, 70 eV) with a source temperature of 230 °C scanning from 30 to 500 amu (full-scan mode) or single ion monitoring (SIM) for quantification. Target analytes were quantified using an external calibration in single ion monitoring (SIM). The results were corrected with an internal standard containing deutered PAH (Mix 35, Promochem, Wesel, Germany).

7.3.7 EROD induction assay

Method according to chapter 6.3.7.

7.3.8 Bio-TEQ values

Computing of bio-TEQs according to chapter 6.3.8.

7.3.9 Ames Fluctuation assay

Method according to chapter 6.3.9.

7.4 Results

7.4.1 AhR-mediated activities and identified compounds

Soil sampled at recently inundated sites and at non-inundated sites was assessed in 30 cm layers down to a depth of 90 cm with respect to PAHs, PCBs and HCB EROD induction (Fig. 2). At each location, the highest concentrations of EPA-PAHs could be identified in the top soil layer (0 - 30 cm). Highest load was measured with 39 mg/kg in the topsoil of the northern inundated foreland (NF). Further, PCBs (0.19 mg/kg) as well as HCB (0.049 mg/kg) indicated this sample to be highest contaminated site. Deeper soil layers at this site gave decreasing compound concentrations and only EPA-PAH concentrations were elevated below 60 cm depth. At each other site, PAHs were the only detectable substances and measured in topsoil above a depth of 30 cm. Concentrations were equal and ranged between 0.083 and 0.127 mg/kg in the inundated foreland and 0.07 and 0.142 mg/kg in the non-inundated area behind the levee.

In accordance to the chemical analysis, EROD inductions indicated the NF soil extract as sample with the highest Ah-receptor inducing potencies and maximum induction was determined in the topsoil layer with a bio-TEQ of 43,000 pg/g. In contrast to the NF site, AhR-agonists were less active at the other inundated sites shown with mean concentrations among all of 153 ± 0.7 pg/g and at sites behind the levee with means of 129 ± 77 pg/g. Lower soil layers showed decreasing Bio-TEQs, at least compared to the topsoil at each site investigated.

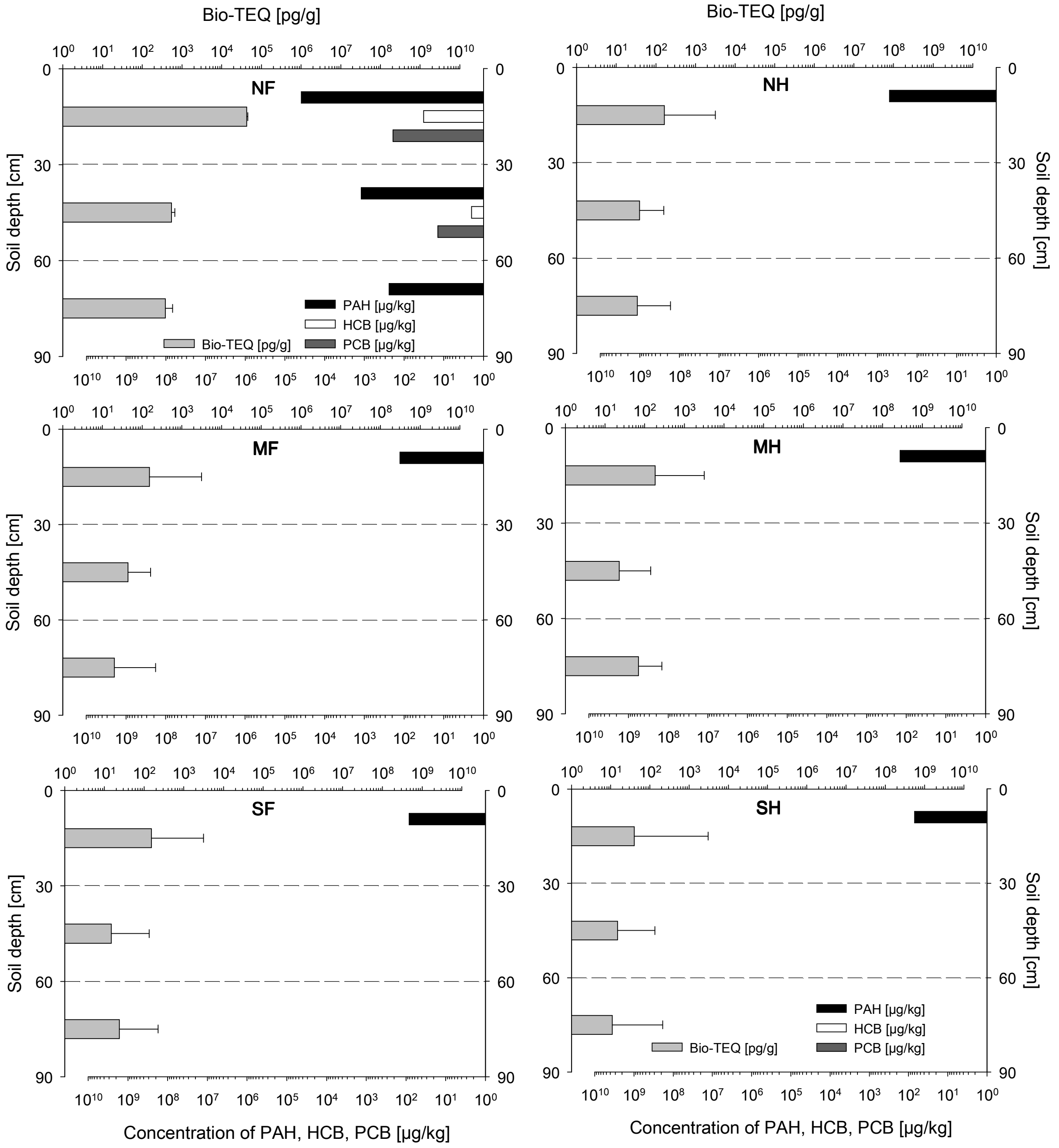

Fig. 2 Concentrations of chemically analyzed HCB, PCBs and PAHs as well as bionalytically determined bio TEQs are shown for distinct soil layers (0 - 30, 30 - 60, 60 - 90 cm), allowing the comparison of samples from the north (N), middle (M) and south (S) of the inundated foreland (F) and the non-inundated hinterland (H) which are separated by a levee.

7.4.2 EROD inducing potential by soil fractions

In order to identify active compound categories in the EROD assay, the highest inducing soil layer (0 - 30 cm at the inundated north site) was selected for effect-directed analysis (Fig. 3). Fractions F1 to F4 containing PCBs and PCDD/Fs indicated no or minor activities. In contrast, PAH fractions (F5 to F11) were highly inducing and showed highest bio TEQ (= 13 x 10^3 pg/g) in fraction 10, containing PAHs with 6 aromatic rings (e.g. indeno[1,2,3-*cd*] pyrene). Fraction 12 containing mainly mononitro-PAHs gave negligible inductions. In contrast, F14 to F17 with more polar compounds indicated elevated activities, but about 4-fold lower than PAH fractions. Induction of F18 with most polar compounds induced negligible.

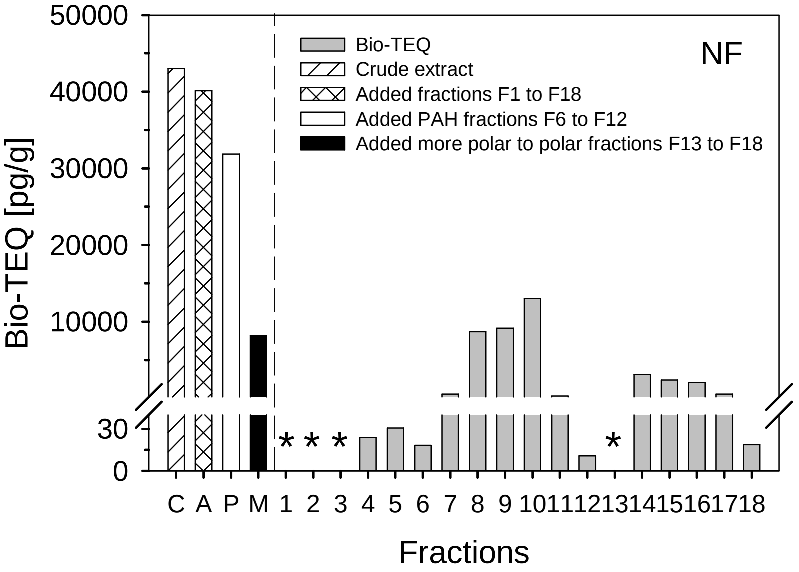

Fig. 3 EROD induction given as bio-TEQs determined with HPLC fractions of the topsoil layer sampled at the NF site. n.d. − no bio-TEQ determined

7.4.3 Mutagenic potential of individual fractions

Total soil extracts of each site caused no mutagenic activity with the Ames Fluctuation assay. In contrast, fractions of the NF sample were investigated and showed elevated potentials. Significantly decreased NOECs and increased IF_{max} were measured in all soil fractions except for fraction F8 containing PAHs with four aromatic rings (Tab. 1). Highest potentials were determined in fraction F17 and TA 98 and S9 metabolism (0.03 mg dry soil equivalent per ml test medium) containing more polar compounds (e.g. 2-hydroxyanthraquinone). For all fractions, highest potentials were determined in the Ames strain TA98 without S9 metabolism. However, fractions treated with TA98 and metabolic activation showed higher mutagenicity. Tester strain TA 100 indicated fractions to be less active. Lower activities were determined in the approach without exogenous S9 supplementation. Fractions were non-active in the Ames tests with S9, except F16 that was the highest inducing fraction with TA100 as well as one of the highest inducing fractions at all.

Tab. 1 Mutagenic activity of HPLC factions determined in the Ames Fluctuation assay with the bacterial tester strains TA 98 and TA 100 with and without adding exogenous S9 supplement for metabolic activation of the NF soil sample. IF_{max} were only computed for fractions with significantly reduced NOECs. Data are given as no observed effect concentration in mg soil equivalent per ml test medium and as maximum induction factor.

Fraction No.	NOEC [mg/ml]				Maximum induction factor (IF_{max})			
	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	TA 98 -S9	TA 98 + S9	TA100 -S9	TA100 +S9
1	*	1.04	*	*	*	4.3	*	*
2	*	16.67	*	*	*	6.7	*	*
3	8.34	8.34	*	*	6.7	3.9	*	*
4	8.34	*	*	*	5.7	*	*	*
5	*	2.08	*	*	*	7.1	*	*
6	4.17	4.17	16.67	*	3.6	5.7	8.0	*
7	*	16.68	*	*	*	3.3	*	*
8	*	*	*	*	*	*	*	*
9	1.04	1.04	*	*	16.1	13.3	*	*
10	4.17	2.08	*	*	8.6	19.0	*	*
11	2.08	2.08	16.67	*	4.3	12.3	3.3	*
12	2.08	2.08	16.67	*	4.3	1.9	2.4	*
13	*	2.08	*	*	*	2.9	*	*
14	2.08	*	*	*	6.7	*	*	*
15	*	*	16.67	*	*	*	4.3	*
16	2.08	*	*	1.04	16.7	*	*	*
17	0.07	0.03	*	*	19.0	29.0	*	*
18	0.13	0.26	*	*	19.0	14.3	*	*

* − NOEC > 33.33 mg SEQ/ml

7.5 Discussion

7.5.1 Chemical contamination of crude extracts

In order to determine contaminations in soil of inundated and non-inundated sites soil layers were assessed at different locations of the projected retention area. Chemical analysis indicated that soil sampled at the inundated area in the north was highest polluted with respect to EPA-PAHs, PCBs and HCB. Since that site is a ground swale it acts as an accumulation basin for SPM, especially during flooding and may explain elevated compound concentrations in the soil core.

EPA-PAHs were determined in each top soil layer at inundated and non-inundated sites. However, the NF soil core showed elevated concentrations down to a depth of 90 cm, which was comparable to topsoil layer concentrations at any other site. Along with PCBs and HCB, these findings show the high pollution at site and indicate that contaminants were translocated deep into the unsaturated zone. EPA-PAH concentrations measured of about 40 mg/kg were comparable to maximum concentrations of 20 mg/kg that were measured by Hilscherova et al.

(2007) in floodplain soil of the rivers Morava and DÍevnice, Czech Republic, and its tributaries after an extreme flood event with a recurrence interval of 100 years. In contrast, concentrations measured were minor, if compared to 2,600 mg/kg EPA-PAHs as determined by Eom et al. (2007) in soil of a highly PAH contaminated site at a former coke oven plant.

PCBs were detectable at NF down to a depth of 60 cm with highest concentrations (0.19 mg/kg) in the topsoil. Measured concentrations were in accordance to the above-named study of Hilscherova et al. (2007) that gave about 0.1 mg/kg in the floodplain soil measured before the flood event at one site. Thus, soil of both catchment areas, the Rhine and the Morava and DÍevnice rivers indicated comparable EPA-PAH loads following an extreme flood, whereas PCB concentrations were usually lower, if compared to NF, but were increased compared to any other site assessed in the present study.

HCB concentrations can be used as a tracer to indicate SPM deposition following inundation, since this compound is a specific contaminant of the river Rhine basin and, thus, elevated concentrations should not be detectable at non-inundated sites. Whereas HCB was highest concentrated in topsoil and 0.05 mg/kg were measured, SPM sampled in a flood in August 2007 with a recurrence interval of 10 years gave a maximum of 0.11 mg/kg at the peak of the flood (Wölz et al. 2009). Thus, HCB was equally concentrated in the topsoil layer sampled of the swale and with flood SPM. This may indicate that HCB concentration in the soil layer originate from inundation, since HCB is less degradable and, therefore, tends to accumulate (Heise & Foerstner 2006, Isensee et al. 1976). Further, HCB measured in sediments sampled at a depth of 0.2 to 1.2 m at the river Rhine barrage of Iffezheim showed concentrations of 0.22 µg/kg and 0.04 µg/kg in surface sediments (Alœck et al. 1998). Thus, concentrations of HCB were comparable to sediments of the barrage, SPM sampled at the peak of a flood with a recurrence interval of 10 years and soil from the projected retention basin that is located downstream the barrage. This may furthermore indicate that soil concentrations are due to inundation and deposition of HCB loaded SPM. With respect to regulatory thresholds, HCB concentrations at this highest contaminated site among all assessed sites are still below, e.g., children playgrounds activity levels of 4 mg/kg as mentioned in the German Soil Conservation Act (1999). Nevertheless, these concentrations highlight the hazard of deposited particle-bound contaminants at inundated sites.

In the pilot study of this project, Ulrich et al. (2002) assessed some topsoil layers with respect to EPA-PAHs and HCB that had been influenced by inundation of some weeks prior to sampling. EPA-PAH concentrations varied between 0.19 to 0.76 mg/kg at rarely inundated sites and 0.37 to 1.64 mg/kg at frequently inundated sites. Accordingly, HCB concentrations were determined and were measured with < 0.001 to 0.002 mg/kg (rarely inundated sites) and 0.015 to 0.053 mg/kg (frequently inundated sites). Presence of HCB in soil of the pilot study that was not detected in the present study might be due to the previous inundation. However, HCB concentrations were equal to the highly polluted NF site in this study. In contrast, EPA-PAHs were about 50-fold higher concentrated compared to the pilot study. These findings show elevated differences of contamination levels in time and space that might also be due to the high soil heterogeneity at the projected retention basin. Thus, an evaluation of pollution levels may only be secure with measurements from many carefully selected sites.

It can be stated that HCB and EPA-PAH concentrations can be elevated at defined sites in floodplains and retention basins. HCB and higher molecular PAHs might be minor dissolved or translocated due to water passage through the soil. However, frequent translocation processes at highly polluted sites with deep reaching contaminations may pose a hazard to the aquifer and drinking water resources over time.

7.5.2 Biological hazard potentials by crude extracts

Each sampling site indicated elevated AhR-agonist activities at least with topsoil layers and the maximum EROD induction, in accordance to chemical analysis, was determined with the foreland site NF. Maximum inductions were determined with bio-TEQ of 43,000 pg/g. Anderson et al. (2009) investigated soil sampled at a PAH contaminated site and determined a maximum bio-TEQ of about 45,000 pg/g using the luciferase gene expression (CALUX) assay. Since different test systems and cell lines were used, that TEQ may not directly be compared to the maximum TEQ determined in the present study. However, a comparison indicates the high pollution at the NF site. Lower bio-TEQ of about 10,000 pg/g were determined by Keiter et al. (2008) assessing sediment from the Danube river. Further, SPM sampled in a flood with a recurrence interval of two years at the river Rhine showed bio-TEQ of about 2,300 pg/g and SPM of a flood at the river Neckar with the same recurrence interval showed maximum bio-TEQ of 8,300 pg/g (Wölz et al. 2008). TEQ values at the NF site are elevated compared to the specified sediment and SPM concentrations and, thus, cannot be explained completely by particle deposition on floodplain soils. However, published studies providing data on EROD inducing sediment and SPM samples are rare and detailed bio-TEQs may potentially act as snap-shots that do not provide information on effective TEQ ranges.

Fractions containing PAHs were determined to contribute highest to the overall biological activity of the sample, in particular the fractions containing compounds of four to six aromatic rings (F8 to F10). Target analysis to determine contributions of EPA-PAHs to the overall biological effect showed that far less than 1 % of the overall biological activity could be explained with theses priority compounds (therefore, data not detailed). These contributions were surprisingly low since other studies showed that EPA-PAHs contributed at least to an extent of some percent to the overall biological effect. Further, these compounds were once prioritized since they were highly concentrated in environmental compartments. Although the NF site showed the relatively highest EPA-PAH concentrations of all assessed soils, total concentrations were low and contributions to EROD induction were minor. Thus, other so far unknown and non-priority PAHs were responsible for the detected EROD inductions and mutagenic potentials in PAH fractions (Brack et al. 2005, Wölz et al. 2008).

7.5.3 Identification of active fractions

Since EPA-PAHs concentrations and bio-TEQs were found to be highest concentrated with the NF site topsoil layer, this sample was used for an automated fractionation procedure to identify EROD inducing fractions and compound classes.

Pattern of EROD-inducing fractions were in line with previously published investigations of suspended particulate matter in the RIMAX-HoT project, using the same automated fractionation method (Wölz et al. RIMAX-SPM 2009). With respect to PAH containing fractions, findings were further in accordance to SPM sampled at the Rhine in another study (Wölz et al. 2009b) and fractionated using a precursor fractionation method (Brack et al. 2003a). Effect pattern comparable to other studies at the river Rhine may advice that the highly polluted NF site soil is influenced by (frequent) inundation and, further, that particle-bound contaminants were deposited at site. However, inducing potencies among the fractions varied between the different investigations. The highest bio-TEQ (= 6.500 pg/g) was determined by Woelz et al. (2009b) at the flood peak, being about 7-fold less concentrated than maximum NF soil concentrations in this study. Thus, this site is relatively high contaminated what might be due to the accumulative item since the site is a ground swale.

Further, more polar compounds (e.g. (hydroxyl-)quinones, keto-, dinitro-, hydroxy-PAHs, and N-heterocycles with rising polarity, 2-hydroxyanthraquinone) were determined to cause elevated EROD inductions. More polar to polar compounds were determined to cause elevated effects in some studies investigating sediments and SPM in the recent years (Keiter et al. 2008, Wölz et al. 2009b). Thus, these compounds which are often given less attenuation should be set in the focus of upcoming research into environmental pollution. Even more so, since higher polarity indicates that compounds are more likely to be dissolved, and, thus, are better bioavailable in the aquatic environment than non-polar PCBs, PCDD/Fs and most PAHs. Investigations of more polar and polar compounds were not in the focus of this study, and, thus, effective compounds may only be discussed as model compounds. Likewise, Petrovi et al. (2003) discussed emerging contaminants such as surfactant degradates, pharmaceuticals and polar pesticides.

Next to EROD induction mutagenic potentials of crude extracts from each sediment site and layer as well as of the fractions from the NF site were assessed. However, in contrast to fractions crude extracts showed no significant effects. This indicates the removal of masking or inhibiting compounds during the fractionation procedure. Following fractionation, masking compounds might be separated in parts or completely in (therefore) non-active fractions (Brack et al. 2005). Although cytotoxicity was not quantified by photometrical means, optical inspection advised at least no viewable cytotoxic effects that would turn out as less turbid well bottoms compared to the negative control. Thus, cytotoxicity of the crude extract *versus* the bacteria strains may be excluded as a reason of masking (Chenon et al. 2003). Using the example of the NF site, the soil layers showed no significant reversion, but most fractions indicated elevated or highly increased mutagenic activity at least with the tester strain TA 98. Thus, in the crude extract antagonistic processes – inhibiting frameshift mutations – may have inhibited DNA interferences, since cytotoxic effects were not observed. Mutagenically active fractions containing PAHs, more polar and polar compounds were shown to be mutagenic in other studies before (Fernandez et al. 1992, Thomas et al. 2002).

Fractions were shown to commonly cause increased reversion rates and highest maximum induction factors with tester strain TA 98, whereas TA 100 indicated only some active fractions and minor induction factors. Thus, most fractions caused frameshift mutations.

Further, most fractions showed significant mutagenic activity with TA98 and direct as well as with indirect S9 treatment. For these fractions, mutagenic potentials were comparable with respect to NOECs and IF_{max}. However, there is no clear trend towards direct or indirect mutagenic activity. It may only be stated that mutagenic potentials were increased in fractions containing PAHs that are well known inducers of mutagenic effects (Brack et al. 2005, Perez et al. 2003, White 2002) as well as in fractions with moderately polar and polar substances (Marvin & Hewitt 2007, Villalobos-Pietrini et al. 2007).

At least elevated mutagenic potentials in the latter fractions might be hazardous in the context of the dispute between flood retention and drinking water supply. Compounds with polar characteristics are more likely to be solved and transported in water and, thus, might more easily pass the unsaturated zone and reach the aquifer. However, the investigations of total soil and fractions were carried out using organic extracts of soil matter and, thus, e.g., bioavailability has not been addressed. Nevertheless, independent of availability to organisms high contaminations were determined and these have to be considered in risk evaluation in the named conflict of interests.

7.6 Conclusions

Remobilized and highly contaminated sediments may be translocated to floodplains and preferably deposit in ground swales that act as accumulation basins for pollutants. Thus, topsoil layers of inundated sites can show elevated contaminant loads and biological responses with *in vitro* biotests. Typical contaminants of rivers and pattern of biological responses in fractions can be used as tracers to indicate pollution as a consequence of flooding.

To evaluate contamination and contaminant translocation at site, soil should be investigated as a core with distinct layers since topsoil samples provide only limited information of surface pollution. This is of relevance since soils may be addressed as stable long-term memories of contamination levels and patterns. Thus, contaminated soils should be considered as important secondary sources of pollutants, in particular in floodplains with facilitated compound translocation due to flooding. In this context, elevated chemical loads and biological responses, in particular in deeper soil layers, indicate that contaminants may more easily pass the unsaturated zone with its retardation mechanisms in the topsoil.

Elevated EROD inductions and maximum mutagenic inductions were determined at least with fractions containing PAHs, moderately polar and polar compounds. At least, PAHs are more likely to be dissolved and can more easily be translocated through the unsaturated zone into the aquifer. Thus, with respect to the assumed conflict of interests between retention basins and water protection areas, impacts through contaminant translocation to the unsaturated zone of the aquifer cannot be excluded with present investigations. Since the addressed retention basins will be operated in floods with recurrence intervals of greater than or equal to 100 years, considerably more sediment erosion and contaminant remobilization will be likely to take place. Elevated deposition or transfer of contaminants might impacts soils and aquifers due to stored and akinetic water. This instance should be addressed carefully in planning the morphology and operation of retention basins. Furthermore, with respect to (eco-)toxicological impacts, the hazard potential of flood events and inundation to the aquifer may assist to evaluate risks towards water quality and, thus, were in the focus of another part of the RIMAX-HoT project.

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Chapter 8

Contaminant entry into and transport in the saturated groundwater zone subsequent to extreme flood events

J. Wölz[1], K. Grosshans[1], M. Fleig[2], G. Streck[3], T. Schulze[3], A. Rastall[4], L. Erdinger[4],
W. Brack[3], D. Kühlers[5], T. Braunbeck[6], H. Hollert[1]

[1] Department of Ecosystem Analysis, Institute for Environmental Research, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

[2] DVGW-Water Technology Center (TZW), Chemical Analysis Department, Karlsruher Strasse 84, 76139, Karlsruhe, Germany

[3] UFZ Helmholtz Centre for Environmental Research, Department of Effect-Directed Analyses, Permoserstrasse 15, 04318 Leipzig, Germany

[4] Institute of Hygiene and Medical Microbiology, University of Heidelberg, INF 324, 69120 Heidelberg,

[5] Stadtwerke Karlsruhe GmbH (SWK), Karlsruhe, Germany

[6] Department of Zoology, Aquatic Toxicology and Ecology Section, University of Heidelberg, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany

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8.1 Abstract

In this study, the yeast estrogen screen (YES) assay with *Saccharomyces cerevisiae* was used to determine the estrogenic potential of solid phase-extracted water samples from three groundwater wells in a projected retention basin near Karlsruhe, Germany. Further, fractions derived from a recently developed fractionation procedure of a highly polluted soil as well as of suspended particulate matter (SPM), sampled during a flood event at the river Rhine at the barrage of Iffezheim, Germany, was assessed for endocrine activity. Target analysis was applied to identify effective compounds. Estrogenic activities of each sample were expressed as 17ß-estradiol equivalent concentrations (E2-EQ). Groundwater was sampled between June 2006 and January 2008 and more frequently following a flood event with a recurrence interval of 10 years in August 2007. Well no. 1 located closest to the river Rhine showed elevated concentrations of the river trace compound carbamazepine (CBZ) at all sampling times. Subsequent to the August flood, concentrations were also elevated in well no. 2. Further, E2-EQ indicated a flood dependent increase of estrogenic activity in the month following the flood at well no. 1. Groundwater sampled at well no. 2 showed increasing E2-EQ with a delay of some weeks. Heterogeneous but also highest inductions (6.7 ng E2-EQ/L) were detected at well no. 3 with the longest distance to the Rhine.

Since elevated YES activities were determined in groundwater, and since the hypothesis has been stated that translocation of particle bound pollutants may influence the ground water, an automated fractionation method and target analysis were used to identify effective compound classes and single compounds of SPM and soil extracts. Fractions F13 to F18 containing more polar compounds (e.g. (hydroxyl-)quinones, keto- , dinitro- , hydroxy-PAHs, N-heterocycles, hydroxyanthraquinone), caused elevated endocrine activities in the soil sample and the SPM. Further, fractions F6 to F12 containing PAHs showed minor effects for the fractionated soil sample and F4 induced significantly with SPM 2 sampled after the flood peak. Added fractions E2-EQ gave comparable activities for soil (2 ng E2-EQ/g) and SPM (0.9 and 2.3 ng E2-EQ/g). Target analysis identified minor concentrations of active compounds and, thus, other non-analyzed substances were effective. This indicates the need of further investigations with respect to more polar and polar compounds and their potential hazard to drinking water.

This study was part of a project (RIMAX-HoT) that aimed at the identification/ characterization of the possible conflict of interests between flood management (retention basins) and drinking water supply (waterworks close to the basin). With respect to the question, whether the operation of retention basins increases the risk of contaminant introduction into the aquifer (drinking water resource), the results presented document a significant hazard potential.

8.2 Introduction

Ground water contamination in riparian areas is (still) of increasing concern in many regions worldwide, since they often are used as drinking water resources (Levin et al. 2002). Once the aquifer is contaminated, residues may remain for long time. Further, the movement of groundwater is difficult to monitor and there are substantial time lags between emissions and detection of chemicals (Finch et al. 2007). Whereas groundwater contamination may result from mineralization or other natural processes, it is usually attributed to waste disposal practices and industrial and agricultural activities (Böhlke 2002, Naik et al. 2007). These activities may continuously impact the groundwater quality. Nevertheless, further events such as floods have the potential to heavily pollute the aquifer in riparian and inundated areas. At least in floods with higher recurrence intervals, thus being more hazardous, considerable amounts of sediments may be eroded (Hollert et al. 2007a). These suspended sediments contribute to suspended particulate matter (SPM), which is translocated downstream or to flooded sites along the rivers. Remobilization of sediments impacts inundated areas since sediment acts as sink and important secondary source of contaminants (Brils 2008, Kosmehl et al. 2004). Whereas particle-bound compounds deposit and consolidate most time of the year in rivers, they may become available by erosion during flood events. Thus, they can be translocated and deposited at inundated sites. Further, groundwater is recharged by inundating flood water that infiltrates the soil at floodplains and indicates the potential of mass transfer through the unsaturated zone in the saturated zone of the aquifer (Brouyere et al. 2004, Kazamaa et al. 2007). Contaminant introduction into the aquifer is of elevated interest since major streams as the river Rhine and its aquifer are important in terms of drinking water abstraction and, thus, are in the focus of scientific investigations (Schwarzbauer & Heim 2005). In the European Union, the Water Framework Directive (WFD; 2000/60/EC) constituted a general set of subjects of protection and objectives to achieve a 'good water status' for all waters, by 2015. This includes the protection of groundwater resources and, thus, the WFD demands measures to ensure the progressive reduction of groundwater pollution and to prevent its further pollution (Borja et al. 2004).

The presented study details results of the joint research project 'Flood retention and drinking water supply – Preventing conflicts of interests (RIMAX-HoT)' as introduced by Maier et al. (2006). The project aimed to characterize the possible conflict of interests at the planned retention area Bellenkopf-Rappenwoert and a projected nearby waterworks Kastenwoert, both located next to Karlsruhe, Germany. This part of the study focused on the assessment of endocrine effects in groundwater and soil as well as suspended particulate matter (SPM) samples, which were taken during the project. Estrogenic activity was determined using the Yeast Estrogen Screen (YES) assay with transgenic bakery yeast *Saccharomyces cerevisiae*, containing the human estrogen receptor (hER).

Groundwater was sampled over a period of 2 years at three groundwater wells in the projected retention basin. Since elevated YES activities were determined in groundwater, and since the hypothesis has been stated that translocation of particle-bound pollutants may influence the ground water, an automated fractionation method and target analysis were used to identify effective compound classes and single compounds of SPM and soil extracts. The soil originated from a site which proved highly contaminated in a previous study (Woelz et al. 2009a). Furthermore, SPM sampled in the course of a flood with a recurrence of 10 years, showing elevated loads and toxic effects, was assessed (Woelz et al. 2009b).

This last study within the RIMAX-HoT project aimed

- to assess endocrine inducing potentials in groundwater samples of the model retention basin,
- to investigate agonist activities in fractions of soil (sampled at the basin) and SPM (sampled at the barrage of Iffezheim close to the basin),
- to use target analysis to possibly identify effective compounds and
- to conclude how these findings can assist to resolve the conflict between floods to groundwater quality and drinking water safety.

8.3 Materials and methods

8.3.1 Chemicals used

Subsequent provider of chemicals will only be listed if other than from Sigma-Aldrich, Deisenhofen, Germany. Chemicals were at least reagent grade.

8.3.2 Sampling and Preparation

Groundwater was sampled at three wells situated at a diagonal within the projected retention basin Bellenkopf-Rappenwoert and located behind a levee (Fig. 1) at several times over a period of 2 years (Tab. 1). Well 1 is situated directly behind a levee and in a distance of 250 m to the Rhine. Distances between well no. 1 and no. 2 as well as between well no. 2 and no. 3 were about 500 m. Wells had a depth of about 30 m. Before sampling groundwater, each well was pumped dry (MP1; Grundfos, Grödig, Austria) and allowed to refill again to update the sampled groundwater with 2.5 m^3/h. Water was pumped into 2 L brown glass bottles, transported to a cooling chamber and stored at 4 °C until extraction.

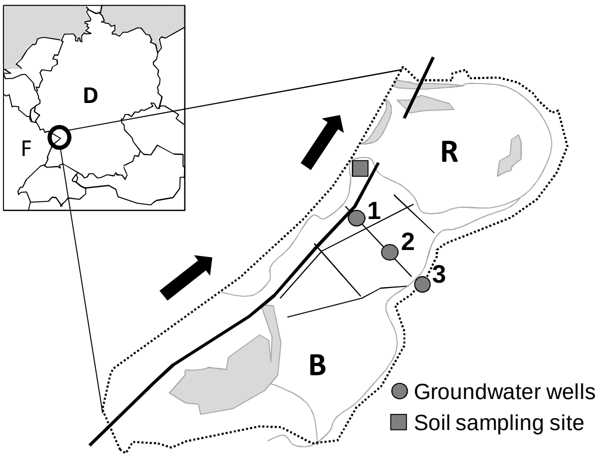

Fig. 1 Location and scheme of the projected retention basin Bellenkopf-Rappenwoert near Karlsruhe, Germany. Grey lines and filled areas give water courses/resources in the basin. F − France, D − Germany, B − Bellenkopf, R − Rappenwoert

In order to investigate whether elevated estrogenic activities in groundwater samples were caused by contaminants of flood water, SPM that was sampled in the course of a flood with a recurrence interval of 10 years as well as a soil sample of an inundated site were additionally assessed as detailed by Wölz et al. (2009 a,b). SPM 1 was sampled between 10.40 a.m. and 12.00 a.m. at August 10, 2007 before the flood peak and SPM 2 was sampled between 12.00 a.m. at August 10, 2007, and 14.50 p.m. at August 11, 2007 after the flood peak. The soil was sampled at August 22/23, 2006 in the inundated foreland of the projected retention basin (shown in Fig. 1).

Tab. 1 Sampling schedule for ground water collection at wells numbered according to fig. 1.

Date	Groundwater wells		
	1	2	3
26.06.2006	x	x	x
27.08.2006	x	x	x
30.05.2007	x	x	x
13.08.2007	x	x	x
16.08.2007	x	n.a.	n.a.
22.08.2007	x	x	x
31.08.2007	x	n.a.	n.a.
10.09.2007	x	n.a.	n.a.
11.10.2007	x	x	x
19.11.2007	x	x	x
17.12.2007	x	x	x
14.01.2008	x	x	x

n.a. − not assessed

8.3.3 Water extraction

For extraction, water samples were filtered over 0.1 mm glass fibre filters (type C5, MembraPure, Bodenheim, Germany), acidified with concentrated H_2SO_4 to pH 2.0, divided into two 1 L samples and extracted using reverse phase C18 solid phase extraction columns (RP-C18 SPE; 1 g; Bakerbond, J.T. Baker, Deventer, The Netherlands), which had been conditioned with 3 x 3 ml *n*-hexane, 3 x 3 ml acetone as well as 1 x 3 ml deionised water according to a protocol by Spengler et al. (2001) and Rastall et al. (2004). After extraction, columns were centrifuged for 10 min at 2000 g and dried under a nitrogen stream. Elution was carried out with 2 x 5 ml acetone. Eluted samples were blown close to dryness under a nitrogen stream, reconstituted in 2.5 ml dimethylsulfoxide (DMSO) and stored at 4 °C until chemical and biological analysis. Deionised water and tap water were treated and extracted according to the same protocol and were used as process controls.

An automated fractionation method, bioanalytical investigations and target analysis were used to identify effective compound classes and single compounds of SPM and soil extracts. SPM 1 was sampled at the peak of a flood event (August 10, 2007, 12 p.m.) and SPM 2 after the flood peak (August 11, 2007, 14:50 p.m.). The soil was sampled at an inundated and highly contaminated site in the north of the projected retention basin in order to serve as a worst case scenario in respect to hazard potential to the ground water (see fig. 1).

8.3.4 Automated fractionation of SPM and soil samples

The method according to chapter 6.3.4.

8.3.5 Chemical analysis – Carbamazepine (CBZ)

The HPLC (HP-1100, Agilent Technologies, Palo Alto, CA, USA) was equipped with a mass spectrometer API 2000 (PE-SCIEX; Waltham, Massachusetts, USA), separation column (250 x 2 mm, 5 µm C18 Luna; Phenomenex, Torrance, CA, USA), solid phase material SDB1 Bakerbond (JT Baker, Devender, The Netherlands) and a UV detector (UV-2201, Shanghai). The analysis conditions were: initial column temperature 60 °C (1 min), increased at 20 °C/min to 180 °C, then increased at 3 °C/min to 207 °C and at 1.5 °C to 260 °C that were finally hold for 5 min. The carrier gas was helium. The injector temperature was 50 °C, 300 °C/min to 270 °C and the volume injected in splitless mode was 4 µl. The detector temperature was 310°C. Detection limit was 10 ng/L.

8.3.6 Method for the instrumental analysis of estrogenic compounds

Chemical analysis included four compound classes: Natural and synthetic steroids, nonyl- and octylphenol, bisphenol A and musk compounds. Prior to analysis a derivatization of steroidal compounds was inevitable for a proper determination with gas chromatography (Streck 2009). All other compounds were processed without derivatization. Derivatization was done modifying a method proposed by Labadie and Budzinski (2005). Briefly, all samples were dried under a gentle stream of N_2 and then re-dissolved in 50 µl of a mixture of MSTFA (N-methyl-N-(trimethylsilyl)-trifluoroacetamide; purity > 97 %), 0.6 % 2-mercaptoethanol (Merck, Darmstadt, Germany) and 1 mg ammonia iodide (Riedel-de Haën, Seelze, Germany). The samples were heated for 40 minutes at 65 °C. After cooling to room temperature, the samples were dried again with N_2 to dryness and finally re-dissolved in 500 µL toluene.

Analysis was achieved using an Agilent HP6890 capillary column gas chromatograph equipped with a HP5973 mass selective detector used in electron impact mode (70 eV). The compounds were separated on an HP5-MS capillary column (length 30 m; inner diameter 0.32 mm; stationary phase thickness 0.25 µm). Helium was the carrier gas. The injector was kept at a temperature of 250 °C, the interface between the gas chromatograph and the ion source at 280 °C, while the ion source itself obtained a temperature of 250 °C. All injections were done in splitless mode with a volume of 1 µl. The oven was programmed as follows: 60 °C for one minute, increasing at 30 K/min to 150 °C, continuing with a gradient of 6 K/min to 186 °C, then increasing at 4 K/min to the final temperature of 280 °C, which was kept for 6.5 minutes. The analysis was conducted in SIM mode. Concentrations of the target analytes were calculated using an external calibration and corrected by means of the injection standards. Limit of detection was 7.5 mg/L.

8.3.7 Yeast Estogen Screen (YES) assay

Recombinant yeast cells (*Saccharomyces cerevisiae*) stably transfected with the gene for the human estrogen receptor (hER) and containing expression plasmids carrying strong promoter sequences and the lac-Z (β-galactosidase) reporter gene (Routledge & Sumpter 1996) were used to investigate estrogen-like activity of suspended SPM, soil and groundwater. The YEAST Screen assay was carried out according to Routledge and Sumpter (1996) using a slightly modified version of the procedure described by Rastall et al. (2004) and Keiter et al. (2006). Briefly, 100 µl aliquots of extracted groundwater, soil and SPM were serially diluted along alternate rows of a 96 well microtitre plate. A 17β-estradiol (E2) positive control was added to a separate row and accordingly serially diluted to give a final concentration range of 1.0 x 10^{-8} to 4.8 x 10^{-12} M. 100 µl of the ethanol vehicle were then added to each vacant well and the ethanol in all 96 wells allowed to evaporate. 50 ml of YES assay medium containing 500 µl of a 1.65 x 10^{-2} M aqueous solution of the chromogenic substrate chlorophenol-red-β-D-galactopyranoside (CPRG) and 4.0 x 10^{7} recombinant yeast cells were then prepared and 200 µl transferred to each well. The plates were sealed and incubated at 32 °C for 72 h. Estrogenic potentials were subsequently determined photometrically at 540 nm following the conversion of the CPRG from yellow to red by β-galactosidase secreted into the growth medium in response to the presence of hER agonists in the sample. With each test distilled water was used as a negative control and, further, process controls were tested accordingly. Significant activities compared to the negative control were determined using the 99 % confidence interval.

Activities were computed as estrogen equivalent concentrations (E2-EQ) by normalizing estrogen activities of samples or fractions to the natural estrogen 17ß-estradiol (Hollert et al. 2005, Tan et al. 2007). E2-EQs are calculated as the quotient of the EC_{50} (effective concentration that produces 50% of the maximum effect level) and is given either in the unit ng E2/L (subsequent ng/L) of groundwater or as ng E2/g Soil or SPM (subsequent ng/g):

$$\text{Eq. 1 E2-EQ} = EC_{50}\,[17\text{ß-estradiol}] / EC_{50}\,[\text{sample}]$$

8.4 Results

8.4.1 Investigation of groundwater samples

The groundwater sampled at three sites in a diagonal to the river Rhine indicated elevated concentrations of river key contaminants such as the pharmaceutical CBZ (Fig. 2). Increased but heterogeneous concentrations were measured at well no. 1, which is closest to the river Rhine (distance: 250 m). Well no. 2 showed elevated concentrations, in particular following August 2007. In contrast, CBZ was not detectable in water of well no. 3 with the longest distance to the river Rhine.

With respect to endocrine activity, statistical significant but nevertheless negligible low effects were determined with water sampled at well no. 1 and no. 2, whereas well no. 3 showed moderately elevated endocrine effects in 2006 (Fig. 2). Effects were low during the following samplings, but showed elevated endocrine activities following the flood event of August 2007. In particular, well no. 1 indicated increasing endocrine effects following the flood and showed a decreasing tendency after August. The maximum E2-EQ was determined with 2.9 ng/L (August 10, 2007). Likewise, well no. 2 showed a clearly increasing activity following the flood, however, with a delay and a maximum of E2-EQ = 2.6 ng/L (September 10, 2007). Groundwater of well no. 3 caused endocrine activities in the YES assay, which showed approximately the activity pattern of well no. 2 ($r_{\text{Pearson}} = 0.80$). However, endocrine effects at well no. 3 were heterogeneous and not clearly increased in the time after the flood. Well no. 3 showed the maximum E2-EQ of all samples assessed (= 6.67 ng/L).

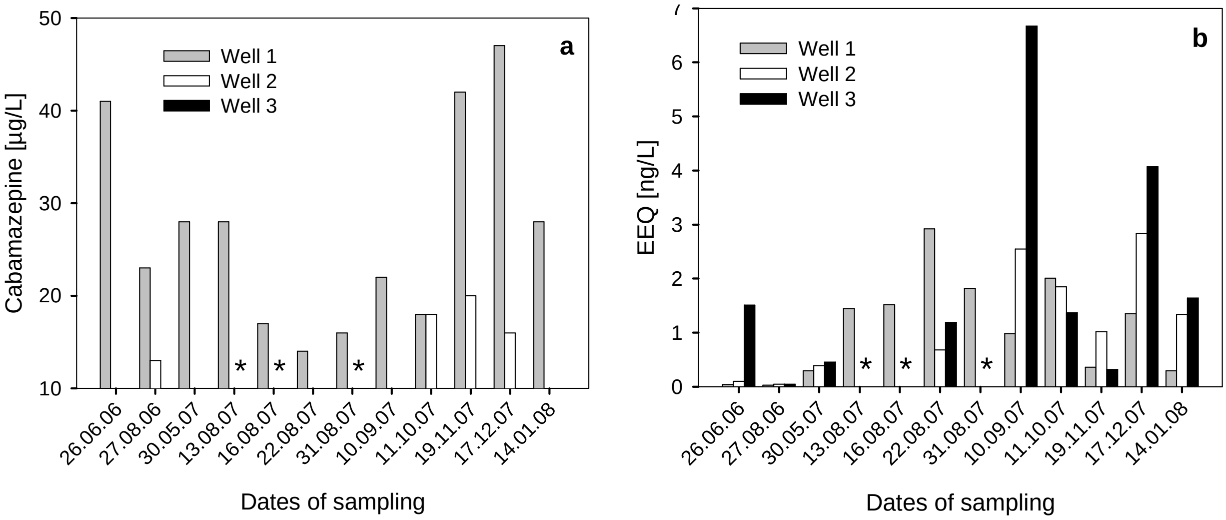

Fig. 2 (a) Carbamazepine (CBZ) concentration and (b) estrogenic activities at each well and sampling time with groundwater of the projected retention basin Bellenkopf-Rappenwoert. Endocrine activities are given as E2-EQs in ng/L and were calculated from one assay with two measurements. Significant endocrine activity was determined using the 99% confidence interval. * − wells no. 2 and 3 not assessed

8.4.2 Estrogenic activity in individual fractions and target analysis

Suspended particulate matter no. 1 (SPM 1) caused endocrine activities in fractions F14 to F17 containing more polar compounds (such as N-heterocycles) and maximum activities were detected in F15 giving E2-EQ = 1.44 ng/L (Fig. 3). Addition of activities in single fractions equaled E2-EQ = 2.3 ng/L. Extracts of SPM 2 caused lower activities in fractions with more polar compounds. Nevertheless, highest endocrine effectiveness was determined in fraction F15 (E2-EQ = 0.5 ng/L). Further, in contrast to SPM 2, fraction F4 caused (minor) endocrine activity (E2-EQ = 0.31 ng/L). Added single fractions of SPM 2 equaled E2-EQ = 0.9 ng/L. The soil surface layer (0 - 30 cm depth) indicated lower endocrine activities in F6 to F8 (Fig. 3). Fractions containing the more polar compounds (F13 to 17) caused elevated endocrine activities and maximum induction was determined in fraction F15 (E2-EQ = 0.7 ng/L). The total equivalent concentration of all fractions was E2-EQ = 2 ng/L.

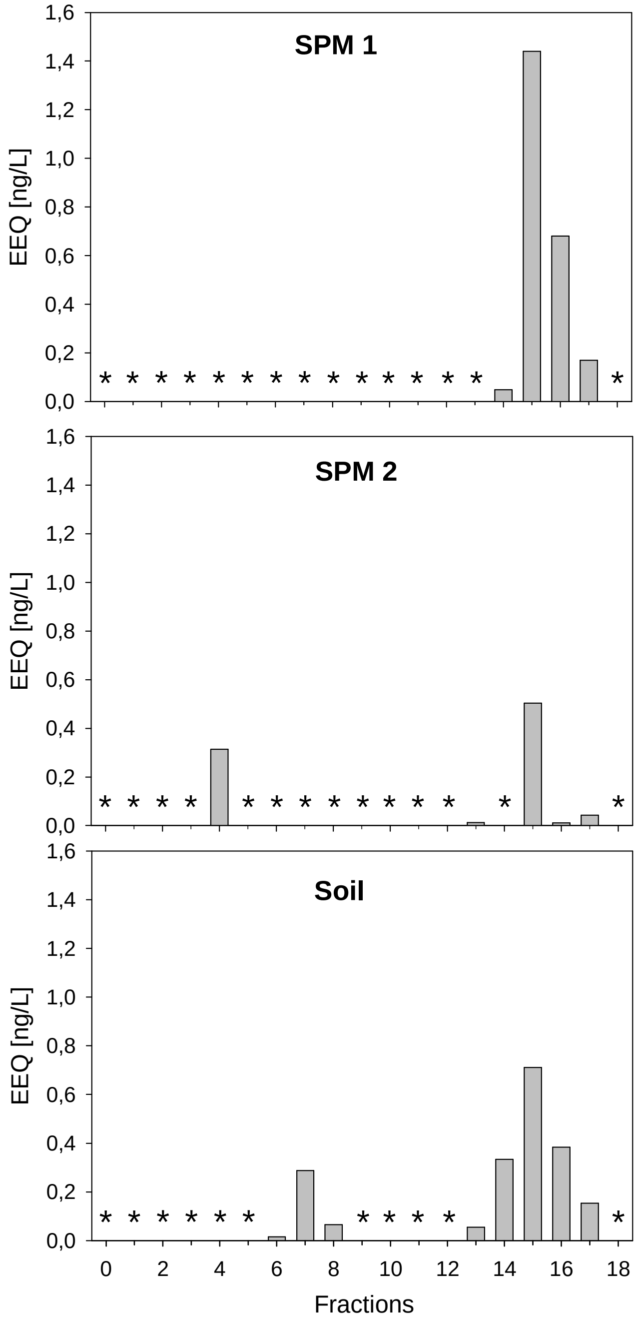

Fig. 2 Endocrine activities in HPLC-fractions of SPM sampled during a flood event in August 2007 (recurrence interval of 10 years) as well as of a topsoil layer (0-30 cm) of a site with elevated contamination. Endocrine activity is given as E2-EQ in ng/L and was calculated from one assay with two measurements. Significant endocrine activity was determined using the 99% confidence interval. * − fractions not inducing

8.4.3 Target analysis in fractions

In order to identify effective compounds, target analysis was conducted; data are provided in tab. 2. Further compounds were evaluated but not detected above detection limit (such as estrone, nonylphenol, triclosan). Concentrations of each compound were lower than 1 ng/kg. Highest concentrations were determined for amberonne in fraction F14 (0.86 ng/kg; SPM 1) and octylphenol in F17 (0.8 ng/kg; SPM 2). Amberonne and galaxoide were highest concentrated in F14 of the soil sample. However, 2,6-diisopropylnaphthaline and octylphenol were present in each fraction, and, thus total concentrations, at least of octylphenol were highest concentrated. Further, fractions F14 showed the highest added compound concentrations (maximum of 2.2 ng/kg in SPM 1). REP values are shown when available from literature and were used to compute chem-TEQ values (chem-TEQ [ng/g] = compound concentration [ng/g] x REP; detailed by Woelz et al. 2008, ESPR). However, chem-TEQs explained far less than 1 % contribution to the overall endocrine activity.

Tab 2 Concentrations of endocrine compounds in HPLC fractions of suspended particulate matter (SPM 1 and 2) and of a soil layer sample from the northern inundated foreland (0 - 30 cm). REP values are shown when provided in other studies. Different REPs are given for bisphenol A, thus, the lowest[2] and highest values[3] are shown.

ng/kg	REP	SPM 1				SPM 2				Soil			
		F14	F15	F16	F17	F14	F15	F16	F17	F14	F15	F16	F17
Benzophenone[1]	$2*10^{-6}$	0.18	n.d.	n.d.	n.d.	0.21	n.d.	n.d.	n.d.	0.10	n.d.	n.d.	n.d.
OTNE (Amberonne)	n.a.	0.86	n.d.	n.d.	n.d.	0.53	n.d.	n.d.	n.d.	0.27	n.d.	n.d.	n.d.
2,6-Diisopropylnaphthaline	n.a.	0.02	0.04	0.02	0.02	0.04	0.02	n.d.	0.02	0.03	0.02	0.01	0.03
Galaxolide (HHCB)	n.a.	0.62	n.d.	n.d.	n.d.	0.36	n.d.	n.d.	n.d.	0.18	n.d.	n.d.	n.d.
Tonalide (AHTN)	n.a.	0.21	n.d.	n.d.	n.d.	0.14	n.d.	n.d.	n.d.	0.05	n.d.	n.d.	n.d.
Diphenylsulfone	n.a.	n.d.	0.27	n.d.	n.d.	n.d.	0.34	n.d.	n.d.	n.d.	0.15	n.d.	n.d.
Octylphenol		0.31	0.66	0.55	0.54	0.52	0.24	0.65	0.80	0.09	0.12	0.05	0.07
Bisphenol A (2,3)	$2.5*10^{-5}$ - $2.63*10^{-4}$	n.d.	n.d.	n.d.	0.56	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Added concentrations	/	2.20	0.97	0.57	1.12	1.80	0.60	0.65	0.82	0.72	0.29	0.06	0.10

[1] Han et al. 2002, [2] Vinggaard et al. (2000), [3] Routledge and Sumpter (1996), n.a. − not available

8.5 Discussion

8.5.1 Carbamazepine as a tracer for riverine contamination

In order to determine groundwater contamination by compounds of the river Rhine CBZ was determined since this compound is a typical trace contaminant at the river Rhine for anthropogenic impact (Schwarzbauer & Heim 2005). Groundwater sampled at three wells in the projected retention area was shown to cause elevated endocrine activity independent of the distance to the river. However, estrogenic activities were was quite heterogeneous among the wells. Oellers et al. (2001) determined CBZ concentrations between 30 and 250 ng/L in water samples from the Lake Greifen (Greifensee) and its main tributaries Aa and Aabach, Switzerland. Liebig et al. (2006) measured 454 ng/L in German surface waters and Herberer et al. (2002) detected 25 to 1,075 ng/L in waterway samples of Berlin, Germany. Thus, ranking the CBZ concentration of this study (14 to 47 µg/L) indicates minor concentrations in groundwater of the aquifer than detected in many surface waters in Central Europe. Further, CBZ was shown to be a drinking water contaminant that was measured with maximum concentrations of 24 and 258 ng/L in finished drinking water of Canada and the United States, respectively (Jones et al. 2005, Stackelberg et al. 2004).

Since the pharmaceutical carbamazepine is an anthropogenic contaminant, in particular of the aquatic environment, detection in groundwater indicates an interaction of river water with aquifer layers. In this study, CBZ indicates the interaction of Rhine water with the aquifer throughout the year and changes in consequence of the August flood.

Total concentrations at the sites varied two- to threefold over the sampling time frame. Nevertheless, concentrations were lowest in August 2007 and highest inductions were measured in the weeks and month after the flood event in August 2007. These findings indicate that groundwater velocity, at least in this time, was directed to the groundwater well 1 and compounds were transported with a lack of time due to slow groundwater velocity. However, CBZ concentrations with about 40 ng/L were also measured at the first sampling in June 2006. Thus, CBZ contents seem to be also under the control of factors other than flood. Since the water level was high in June and precipitation was minor in this month, it may be assumed that river water was intruding into the aquifer, causing the measured concentrations.

Further, well no. 2 showed elevated CBZ concentrations, in particular from October to December 2007. Although this well is located at a distance of about 750 m to the Rhine, mass transfer through the aquifer seems to be possible, in particular, since CBZ may only be introduced in the groundwater *via* groundwater velocity. This assumption is further supported by the findings of another subproject in RIMAX-HoT indicated a hydraulic conductivity of 1.5 x 10^{-3} m/s (Kühlers et al. 2009). Well no. 3 seemed not to be influenced by river Rhine water, since CBZ was not detected.

8.5.2 Estrogenic activities in the groundwater

Endocrine activities over time at each well advised that CBZ may be used as a tracer compound for river water interaction with the groundwater. However, CBZ is no endocrine active compound and its specific distribution does not necessarily indicate how far other active compounds may be translocated in the aquifer. In particular, the pattern of endocrine effects at each well over the time and among the wells is independent of the presence of the tracer compound. Well no. 1 showed low activities before the flood, but were immediately increased following the flood. Subsequent samples showed steadily decreasing activities. This well that is closest to the river was, thus, intensively interfering or reset by river Rhine water, at least within days. This finding is in accordance to the CBZ analysis results.

Further, endocrine activities were increasing in well no. 2 with a delay of days. However, the exact time of beginning increase might have been missed, since this well was first sampled about 10 days after the flood. A flood dependent increase of activity can be assumed, since CBZ concentrations increased, but showed a longer delay. CBZ with a log K_{OW} of 2.45 (Wiegel et al. 2004) might be translocated more slowly compared to compounds with lower K_{OW} being more polar and more easily and faster introduced in backland aquifer. Water sampled at well no. 3 was conspicuous and gave elevated effects with most samples. Further, highest inductions were determined with groundwater extracts of this well even though it is the farthest away from to the river water. CBZ concentrations were not significantly increased at any sampling time at this well, at least until the end of sampling. Estrogenic effects were very heterogeneous and, in addition, with the distance and CBT results, it may be assumed that activities at this well were not influenced by river water or flooding, but by another contaminant source. In fact, the Federbach creek, which flows close to well no. 3, is known to be highly polluted by organic compounds (Obrdlik & Fuchs 1991), and can, therefore, be expected to be the source of contamination.

The increased estrogenic activity at the projected retention basin was obvious comparing E2-EQs with groundwater that was sampled at further drinking water protection areas in the region of Karlsruhe (E2-EQ = 0.18 to 0.38 ng/L; details not shown). Ranking the endocrine activities of the present study indicates that effects were in the lower range of river water with 0.3 to 19.4 ng E2-EQ/L (Pawlowski et al. 2004, Vermeirssen et al. 2005) and sewage treatment plant effluents showing 1 to 68 ng E2-EQ/L (Peck et al. 2004, Tan et al. 2007). Further, effects can be discussed with other studies investigating estrogenic activity of groundwater samples. Ancke-Hahn et al. (2009) determined E2-EQ between 0.63 and 2.48 ng/L in the area areas of communities in South Africa. Braeken and van der Bruggen (2009) reported of E2-EQ = 19.85 ng/L in groundwater. Thus, E2-EQs of the present study were comparable to the first study and lower compared to the latter one; nevertheless, indicating an elevated endocrine activity in the groundwater sample that was comparable to other sites. Further, a flood event with a recurrence interval of 10 years was shown to have the potential for groundwater contamination until far (some hundred meters) in the non-inundated hinterland. And additionally, according to the objectives of the WFD groundwater and drinking water resources should not be endocrine active. Thus, the recent outcomes indicate a potential conflict with this European directive.

8.5.3 Active fractions and target analysis

Automated fractionation clearly showed that more polar compounds were major contributors to the estrogenic activity of SPM and soil extract fractions. Fraction 15 was shown to cause the maximum effect with each sample. Schlenk et al. (2005) used EDA for investigation of marine sediments and also found fractions containing more polar compounds to cause the bulk of estrogen activity. SPM 1 sampled before the flood peak of the August flood 2007 (recurrence interval of 10 years) showed more than twofold increased estrogenic effects compared to SPM 2 sampled shortly after the flood peak. Thus, estrogenic active compounds seem to be more concentrated with increasing discharge but quickly decreased after maximum discharge. However, equivalent concentrations of this study are relatively low compared to sediment E2-EQs determined in other studies investigating endocrine effectiveness of sediments, SPM and soils using *in vitro* assays.

The added activities of single fractions in this study (0.9 to 2.3 ng/L) were minor compared to E2-EQ values reported in the literature as shown by Oh et al. (2000) and Hashimoto et al. (2005) with E2-EQ between 3.4 and 70 ng/L.

Total inductions of SPM 1 and the frequently inundated soil sample showed comparable E2-EQ values that might indicate equal effective compounds. In order to identify endocrine active effective compounds target analysis was applied. Measured compounds and computed chem-TEQs only minor explained a small portion of E2-EQs determined in the fractions. However, part of the endocrine compounds known show activities at concentrations of nanogram per liter, thus, close to the detection limit of many compounds. Highly active compounds such as estrone could not be quantified in this target analysis, but compounds like estrone are very potent inducers and E2-EQs determined with concentrations just below detection limits could explain most of determined activities determined. Since measurements may simply not be sensitive enough to detect the addressed compounds, the possibility of concentrations below detection limit should at least be kept in mind (Hollert et al. 2005).

8.6 Conclusions

The YES assay is suitable to identify estrogenic activities in groundwater samples as well as in fractions of soil and SPM samples. Chemical analyses of tracer compounds and biological activity can be used to provide information on the range of interactions between river and aquifers and on changes over time and space. Significant compound introduction into the aquifer can be expected at sampling sites that are very close to inundated areas. Elevated effects may also be caused with delay at sites with some hundred meters distance to the river. However, elevated tracer compound concentrations or endocrine activities caused by floods may not be expected in the distant hinterland.

Automated fractionation procedures can be used to identify compound categories of effective substances and allows to focus further research. Since more polar compounds are meanwhile known to be important inducers in different biotests, they are of high relevance for endocrine activity. However, further compound classes such as PAHs need to be given more emphasis. Target chemical analysis may assist in identifying effective compounds. Nevertheless, YES-inducing compounds can be numerous and analysis needs a broad adjustment to cover these substances.

Focusing on areas of conflict between retention basins to manage future flood impacts and waterworks to manage the provision of drinking water, a definite advice cannot be provided. However, bank filtrate was shown to influence groundwater far into the hinterland not only during floods, but also in times with average discharge. Thus, flooding of retention areas may intensify the risk of compound introduction into the saturated zone. Further research is needed to elucidate on the particular question if compounds are more easily translocated to the aquifer at inundated sites.

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Chapter 9

Contaminant transport to public water supply wells via flood water retention areas

D. Kühlers[1], E. Bethge[2], G. Hillebrand[3], H. Hollert[4], M. Fleig[5], B. Lehmann[3],
D. Maier[6], M. Maier[1], U. Mohrlok[2] and J. Wölz[4]

[1] Stadtwerke Karlsruhe GmbH (SWK), Karlsruhe, Germany

[2] Institute for Hydromechanics (IfH), Karlsruhe, Germany

[3] Institute for Water and River Basin Management (IWG), Karlsruhe, Germany

[4] Institute for Environmental Research (IfU), Aachen, Germany

[5] DVGW-Technologiezentrum Wasser (TZW), Karlsruhe, Germany

[6] Heinrich-Sontheimer-Laboratorium (HSL), Karlsruhe, Germany

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9.1 Abstract

The essential processes and mechanisms of the transport of contaminants from a river to a well field via a flood water retention area are presented. The transport is conceptualized as a succession of three phases:

(1) Contaminant entry into the retention area
(2) Passage through the soil zone and
(3) Transport with the groundwater flow

Depending on the conditions of a given location and on the properties of the contaminants of interest, processes within each transport phase may reduce the concentration of the contaminants at the well field. For the Kastenwoert-Rappenwoert study area, the results of the described processes are shown by chemical and ecotoxicological analyses as well as by numerical modeling. Based on the results of the analyses, it is predicted that some contaminants in the study area will be completely detained along the transport path, while others will be transported as far as the well field, although in significantly reduced concentrations.

9.2 Introduction

Along many rivers, flood retention areas have to be built to protect downstream settlements against the impacts of extreme flooding. In these floodplains, riparian aquifers are often used for drinking water production. Consequently, the proximity of retention areas to drinking water production wells may lead to conflicts of interest. Drinking water providers are concerned that river water, which often bears elevated loads of inorganic and organic particle-bound and dissolved contaminants, could be directed through the retention areas toward production wells, decreasing the groundwater quality at the municipal well fields. To predict the magnitude of the effect, an in-depth understanding of the processes and mechanisms active along the transport path of the contaminants is crucial.

9.2.1 Contaminant transport

The transport of the contaminants from the river to a water department's well field via a retention area occurs in three consecutive phases:

(1) entry into the retention area
(2) passage through the soil zone and
(3) transport with the groundwater flow

For these phases, corresponding conceptual "compartments" can be identified, all of which are depicted schematically in fig. 1. Depending on the conditions at a given site and on the properties of the contaminants of interest, each of the compartments, the retention area, the soil zone and the aquifer, may act as a barrier, reducing the concentration of the contaminants.

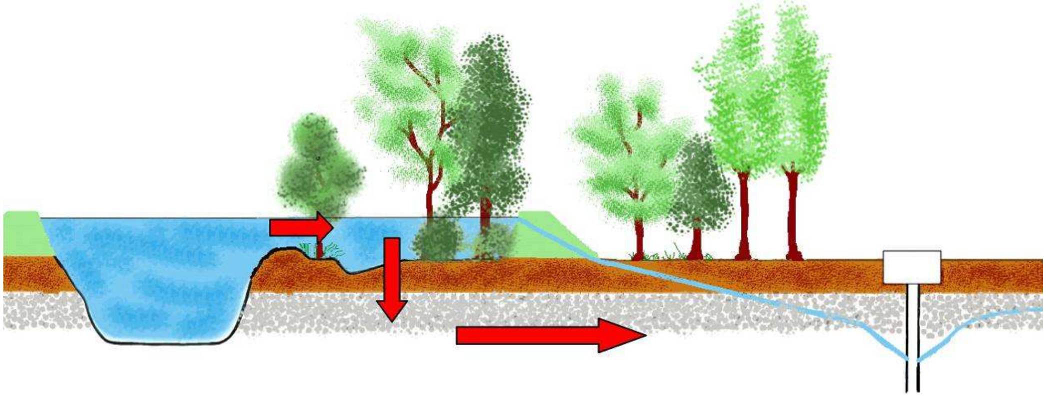

Fig 1: Contaminant transport from a river to a well field via a flood water retention area

In the first transportation phase, contaminants are conveyed into the retention area and retained within. The transport of dissolved substances may be assumed to be identical to that of conservative tracer compounds for which no reduction in concentration occurs at this stage. Significant concentration effects, however, are observed for contaminants adsorbed to suspended sediments. The transport of sorbed contaminants is strongly dependent on the highly complex depositional processes of sediments within the retention area, which lead to an accumulation at particular locations. During the second transport phase, the contaminants pass from the surface of the retention area through the soil zone and into the aquifer. The soil zone reduces the contaminant mass entering the groundwater primarily through the following mechanisms: reduction of the percolation rate, retardation of the contaminants in the soil matrix and microbiological degradation.

The third phase consists of the transport of the contaminants with the groundwater flow. The concentration of contaminants at the production wells can be reduced during transport through the aquifer by the processes of advective transport, dilution, retardation and degradation.

9.2.2 Kastenwoert-Rappenwoert study area

A site-specific examination of the contaminant transport processes and mechanisms presented in the previous section was carried out at the Kastenwoert-Rappenwoert study area, located south of Karlsruhe, Germany. The investigation area is situated on the eastern bank of the Rhine River close to the point where the German Federal States of Baden-Wuerttemberg and Rhineland Palatinate border each other and France. It is located within the Upper Rhine Graben, a sedimentary rift basin that is bounded by the raised shoulders of the Vosges Uplands of France to the west and the Black Forest to the east. The study area consists primarily of forest with limited agricultural use and small expanses of open water. The soil at the site is the product of changing flow and sedimentation conditions during the development of the floodplain. There are significant differences between more elevated areas, where sandy soil predominates and the lower-lying beds of infilled abandoned river channels with their finer sediments. Due to the topography of the retention area, the soil zone thickness varies between approximately 1 and 4 m. In general, the clay and organic carbon content of the soil decreases with depth along the soil profiles (Bechler and Hofmann, 1996). The aquifer is composed entirely of fluvial sediments of varying thickness. In the study area, the uppermost aquifer, from which the groundwater withdrawal is planned, extends to a depth of about 30 m and is composed of highly permeable sediments with an average hydraulic conductivity of about 1.5 x 10^{-3} m/s. The Rhine River has an average annual discharge of 1,250 m^3/s near the study area (HVZ, 2008) and its watershed encompasses the entire surrounding region. However, water levels in the Rhine are largely independent of local precipitation because the Rhine derives its water primarily from the Alps. When water levels in the Rhine are high, they have a damming effect on groundwater draining to the river from the surrounding aquifer. Under the influence of the local reversal in the groundwater gradient, the groundwater flows northward along secondary streams until it eventually empties into the Rhine further downstream. Contaminants found in significant concentrations in the Rhine near the study area include highly adsorptive organic compounds like HCB, PAH and PCB (Maier et al., 1998, Kosmehl et al. 2004, 2007), as well as highly mobile organic compounds like EDTA, pharmaceutical residues and X-ray contrast agents (Fleig et al., 2008). The study area, depicted in Fig. 2, contains the planned “Bellenkopf/Rappenwoert” flood water retention area. With an areal extent of 5.1 x 10^6 m^2 the retention area will provide a flood water storage volume of 14 x 10^6 m^3. The retention area is one component of the Federal State Baden-Wuerttemberg 'Integrated Rhine Program (IRP)', which includes 13 flood water retention areas along the Rhine River between Basel, Switzerland, and Mannheim, Germany. The Bellenkopf/Rappenwoert area has, however, also been designated as part of the wellhead protection zone for the Kastenwoert well field (maximum extraction of 7.4 million m^3/a) which the local water department plans to build adjacent to the planned retention area site. Planning for both projects is nearing completion. Extensive chemical and ecotoxicological analyses of the suspended load in the river, soil and groundwater were conducted to determine the status quo in the study area in each of the presented compartments. Using this baseline, numerical models were applied to predict the interactive effect of the projects, neither of which has yet been realized. Together the results of the analyses and the modeling were used to characterize the three compartments of the transport path.

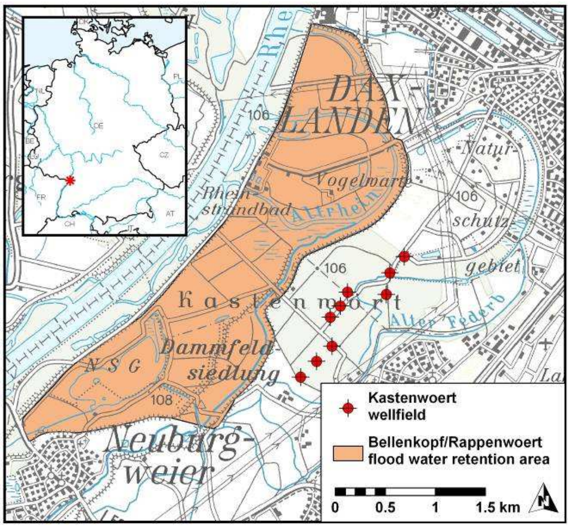

Fig 2: The Kastenwoert-Rappenwoert study area

9.3 Phase 1: Entry of contaminants into the retention area

9.3.1 Characterization

The transport of contaminants into the retention area and their retention constitute the first phase of the contaminant transport. The contaminants reach the retention areas either in dissolved form or adsorbed to suspended sediments. Substances dissolved in the water of the flood wave are transported like a conservative tracer compound. The suspended sediments, that often hold adsorbed contaminants, behave differently depending on their size and weight. Therefore, to study and evaluate the first phase of the contaminant transport, in-depth knowledge about the advective and diffusive transport mechanisms as well as the deposition of suspended sediments within the retention area is crucial.

A series of field measurements were conducted near the study area to get an impression of deposition patterns on the inundated floodplains. Sediment traps consisting of artificial turf mats were used to determine the amount of sediments deposited during two flood events, a smaller one in September 2006 and a larger one in August 2007. As an example, Fig. 3 shows a cross-section of the floodplain with the approximated peak water levels for both flood events and the average amounts of sediment deposited at three locations of increasing distance from the river main channel. Firstly, Fig. 3 illustrates that higher flood events yield higher sediment deposits, but no direct proportionality is found. Furthermore, comparing different values of the same flood event, higher inundation heights increase the amount of deposited sediments. This effect becomes particularly evident for low relative inundation heights.

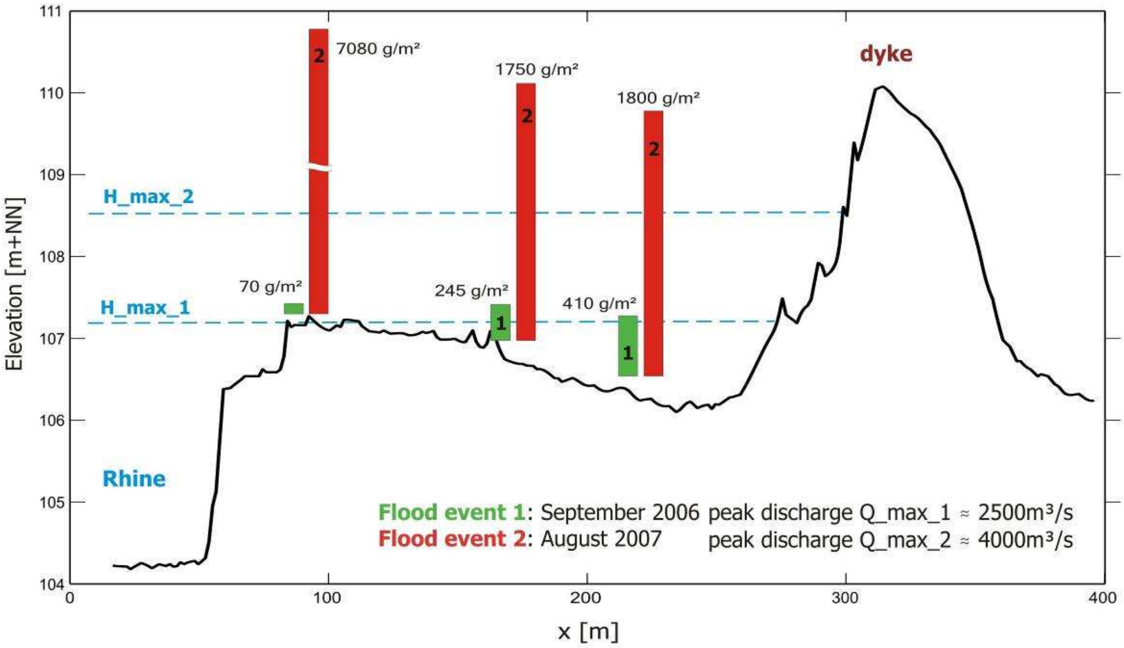

Fig 3: Deposited sediments of two flood events at a cross-section of the floodplain of the Rhine River near Neuburgweier, Germany. The peak flood height is depicted for both events.

The corresponding grain size distribution of the deposits is depicted in fig. 4. The average grain size decreases with increasing distance from the river main channel. This is due to the reduced transport capacity of the overbank flow. Coarser sediment is deposited soon after reaching the overbank area. Finer sediments are more uniformly distributed throughout the floodplain. (This is supported by additional data which are not presented here.) On the other hand, the comparison of the two flood events shows that the grain size distribution at one location does not vary strongly for different flood events. The main difference can be found with the clayey fraction. For the 2007 flood event, the proportion of deposited clay sediment is higher than for the 2006 flood event, probably due to the longer duration of floodplain inundation (about 7 days in 2007 instead of about 2 days in 2006) rather than to the higher flood level.

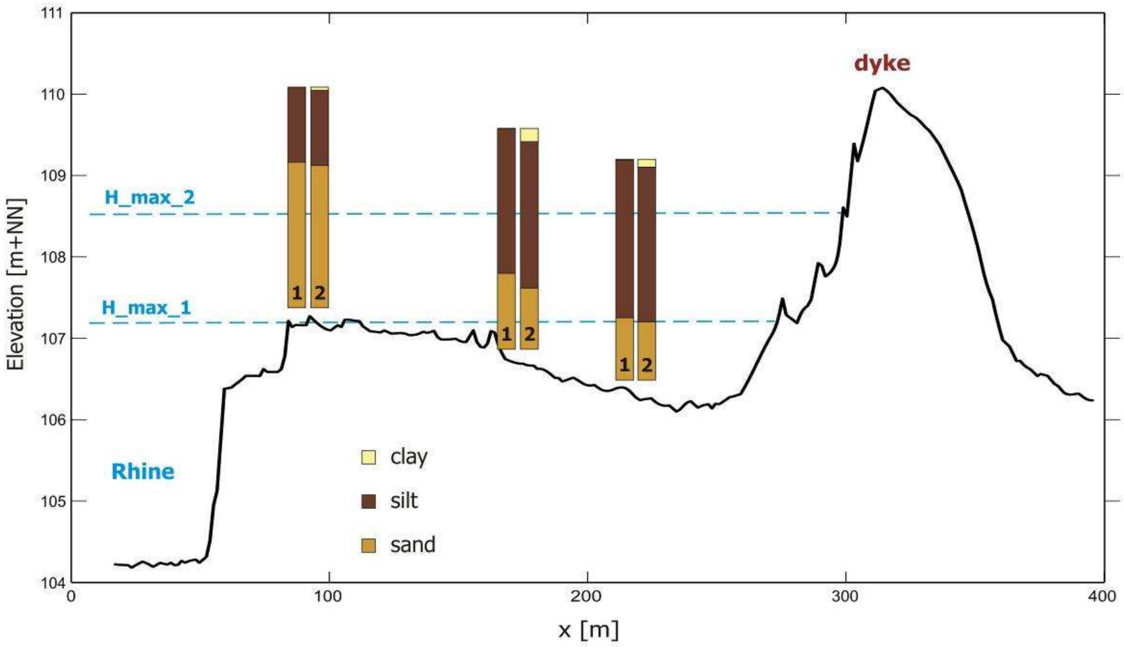

Fig 4: Grain size distribution of deposited sediments of two flood events at a cross-section of the floodplain of the Rhine River near Neuburgweier, Germany. The peak flood height is depicted for both events. The bars show the proportions of the respective grain size classes.

Based on the field study and on an additional literature review (cf. e.g. Asselman and Middelkoop, 1995, He and Walling, 1997, Howard, 1992, Walling and Bradley, 1989), the following conclusions on the most important influencing factors of the deposition patterns of suspended sediments on floodplains can be drawn:

- Local suspended sediment concentrations significantly influence the amount of deposited sediment. High concentrations lead to high deposition rates
- Grain sizes influence suspended load and settling velocities. Coarser sediments deposit on the floodplain close to the river channel. Finer fractions are more uniformly distributed within the suspension across the floodplain
- The total deposition is directly proportional to the duration of the specific flood event for near-steady conditions. For unsteady conditions, high local inundation heights at a

given site generally correspond to long inundation periods. Thus, high local inundation heights usually correlate with increased deposition. Trapping effects of flood water in local depression areas also substantially increase the amount of deposited sediments

- Local flow velocities influence the deposition rates by reducing the probability of sediment deposition for high velocities. However, there is evidence that a definite critical value above which no deposition occurs does not exist in reality as was believed in the past (Haralampides et al., 2003, Krishnappan and Engel, 1997, Krone,1993, Kuijper et al., 1991).

9.3.2 Chemical analysis

To predict the concentration of contaminants that could be extracted at the planned well field, it is important to identify the substances that are present in the river water. A comprehensive data set measured by governmental institutions and water suppliers were analyzed to get an overview of the current water quality situation in the Upper River Rhine. Special attention was given to measurements taken during high flow events. The most important groups of chemical substances regularly found dissolved in water samples from the Upper River Rhine are:

- Total and surrogate parameters with organic carbon (DOC), organic sulphur (AOS) or
- organic halogens (AOX)
- Complexing agents (NTA, EDTA, DTPA)
- Pesticides and their metabolites (N,N-dimethylsulfamide) and
- Xenobiotic organic compounds (chlorinated benzenes, MTBE)

Most of these substances are polar and often quite persistent, so it can be assumed that they potentially infiltrate into the groundwater. Organic substances with a high K_{OW}, like Hexachlorbezene (HCB), polychlorinated biphenyls (PCB) and polycyclic aromatic hydrocarbons (PAH), are almost exclusively found adsorbed to suspended matter. Therefore, suspended sediments were also analyzed. Suspended sediments were collected on deposition with turf mats as described in sect. 2.1 during the flood event in August 2007 at various distances from the Rhine River. After lyophilization the amount of different adsorbed organic compounds were determined.

From the bank of the Rhine River towards the dyke the amount of the indicator compound HCB decreases by more than 90 %. The concentrations of other compounds decrease by half at locations further away from the river. Only AOX and the carbon fraction increase with distance from the Rhine River.

9.3.3 Ecotoxicological analysis

Suspended sediment are known to provide huge surfaces and binding sites for organic and inorganic compounds (Hollert et al., 2007, Kosmehl et al., 2007). Furthermore, most rivers are (highly) loaded with contaminants of various sources, at least as a legacy of the past

(Stronkhorst and van Hattum, 2003). While sediments may act as contaminant sinks under normal hydrological conditions, (extreme) flood events cause sediment erosion and, thus, sediments and contaminants may be released into the water column (Hilscherova et al., 2007, Hollert et al., 2000, 2003, Wölz et al., 2008). Subsequently, they may pose a threat to organisms in the aquatic and inundated terrestrial environment, but also to human interests and health, e.g. regarding drinking water supply (Maier et al., 2006). Thus, the recent ecotoxicological study focused on the evaluation of impacts by particle-bound organic compounds. In order to evaluate the risk of particle translocation from the river on inundated sites, e.g. retention areas, a battery of in vitro biotests comprising several ecotoxicological endpoints was applied. For instance, the acute toxic Neutral Red retention assay (cytotoxicity; Babich and Borenfreund, 1992, Klee at al., 2004, Keiter et al., 2006) and the mechanism specific 7-ethoxyresorufin-*o*-deethylase (EROD) assay (Ah receptor-mediated activity; Behrens et al., 1998, Keiter et al., 2008, Kennedy et al., 1996, Lorenzen and Kennedy, 1993) were used. Under normal hydrological conditions, some samples of suspended sediments indicated temporarily elevated cytotoxic effects, while most samples were only minimally toxic. On the other hand, EROD inductions were mostly increased and some samples clearly indicated significant effects that could not be correlated with any other parameter. The sediments collected with turf mats during the flood event in August 2007 were also examined using effect-directed analysis. Combining fractionation methods (in cooperation with Dr. Werner Brack, Helmholtz Centre for Environmental Research Leipzig), cell-based *in vitro* biotests and chemical analysis were applied to identify effect causing compounds. Fractions containing PAHs and more polar to polar compounds were found to be most toxic (Brack et al, 2005). In conclusion, based on the fact of particle translocation in the retention area, a contamination of the inundated site by primarily bound compounds has to be considered, unless measures may diminish particle entry or guarantee short residence times.

9.3.4 Modelling

In order to determine the deposition patterns within the planned retention area, input data about hydrologic, hydraulic and sediment parameters is necessary. Water levels and local flow velocities were determined by a 2D hydrodynamic numerical model for different flood scenarios. The output data from this model was converted into raster-based datasets. Several deposition models from the literature with different approaches were tested with the field data from the investigated flood events and then applied to the retention area. The results will not be presented in this article, but some essential findings should be mentioned:

- Advection appears to be a significant process for the floodplain in question, mainly due to the dominant flow through topographic depressions, side channels or ditches on the floodplain, which are activated during flood events.
- Pure diffusion models, which do not account for any advection across the floodplain, significantly underestimated the sediment deposited at greater distances from the main river channel (e.g., Pizzuto, 1987). Side channels could be incorporated in the model as additional sediment sources to at least partly compensate for this effect.

- Each of the investigated models uses several empirical parameters, which greatly influence not only the amount of deposited sediments but also the deposition pattern. The determination of those empirical parameters is challenging, a factor which must be taken into account when evaluating the results of the simulations.
- While hydraulic parameters may be determined with sufficient accuracy, reliable information on sediment characteristics during flood events are difficult to obtain. This should be considered, as model results prove to be highly sensitive to variations of model input parameters (especially water levels and sediment concentrations).

9.4 Phase 2: Passage through the soil zone

9.4.1 Characterization

As described in the previous section contaminants can reach the ground surface of the retention area in dissolved form or sorbed to settled flood water sediments. Because of the filtering effect of the soil matrix, particle-bound contaminants are mainly retained at the soil surface. Therefore, a considerable mass flux of dissolved contaminants to the aquifer is more likely. The infiltration of dissolved organic contaminants into the soil zone is determined by the seepage rate. Preferential pathways (macropores) can allow soil water and solutes to bypass the soil matrix and travel quickly into deeper soil regions (Beven and Germann, 1982; Wang and Narasimhan,1985).

Within the soil zone compartment, the contaminant mass flux into the aquifer is reduced by three processes. First, the infiltration of contaminants into the soil zone is decreased. Contaminated sediments are mainly retained at the soil surface, and additionally the infiltration of dissolved contaminants is decreased by the low permeable floodplain soil. Secondly, the transport of infiltrated contaminants within the soil zone is retarded by adsorption to the soil matrix. As a third effect, microbes act to degrade the infiltrated contaminant mass. The mechanisms can influence each other. A low infiltration rate reduces the contaminant input into the soil zone and slows down the transport velocity towards the aquifer. This, in turn increases the travelling time of contaminants, thereby supporting the microbial degradation.

The input of dissolved organic contaminants into the soil zone of the retention area is determined by the infiltration rate of the flood water. This infiltration generates water flow through the soil zone which is affected by several changes in the hydraulic conditions during a flood event. For the soil zone, three consecutive stages with different hydraulic conditions can be identified during a flooding period:

- Stage 1 (S1): Infiltration of flood water into unsaturated soil
- Stage 2 (S2): Infiltration of flood water under saturated conditions
- Stage 3 (S3): Drainage of soil after the flood event

The essential factors controlling the mass input into the soil zone are the hydraulic properties of the soil (average hydraulic conductivity) and the characteristics of the flood event (flooding height and duration). The hydraulic gradient between surface and groundwater plays a major role for these processes. It evolves towards lower values during the flooding period and shows large spatial variability depending on the elevation of the groundwater table and the thickness of the soil zone. Additionally, steep hydraulic gradients often occur at the inland bank of a retention area during a flood. Within a highly conductive soil zone, the resulting seepage rate will yield a strong mass input into the soil zone there.

The mass output to the aquifer is controlled by the transport velocity within the soil and the storage capacity of the soil zone. The transport velocity is controlled by the average seepage velocity and the retardation by sorption to the soil matrix. The sorption of organic contaminants to the soil matrix is related to the soil organic carbon content and the octanol:water partitioning coefficient. The storage capacity of the floodplain soil is a function of those two properties and the soil thickness. The highly variable properties of the floodplain soils and the hydraulic conditions within the retention area have to be taken into account to calculate the contaminant mass flux through the soil zone to the aquifer.

9.4.2 Chemical analysis

Soil was sampled to a depth of up to 90 cm at two locations. The first site, located directly along a Rhine backwater, is periodically inundated. The second, situated 15 m inland is only inundated during extreme flood events. The concentrations of HCB, PCB and PAH at the site near the river were five times higher than the values from the second site and unlike at other sites increase with depth. The backwater site, an in filled oxbow, was filled with contaminated sediment over time. Decreases in sediment contaminant loads in recent decades led to the observed concentration depth profile. The observed increase is therefore clearly not to be explained by transport processes in the soil zone. In conclusion, the repeated deposition of contaminated river sediments at frequently inundated sites is confirmed by the results of the chemical analyses.

The remobilisation of sediment-sorbed pollutants was examined in batch experiments with a soil-to-water-ratio of one to ten. Only a few chemicals (HCB, 1,2,3-trichlorbezene) showed even small reductions.

In another series of experiments, soil from the study area was used to fill small columns through which water containing a known concentration of an organic pollutant was percolated. The outflow concentration was determined at short time intervals. Most contaminants broke through very rapidly, within one to two days, appearing at the column outlet at the same concentration as at the input. The experiments thus demonstrated that not all pollutants could be retained by the soil or degraded by microorganisms.

9.4.3 Ecotoxicological analysis

In sect. 2.3 it was shown that contaminants, once entering the retention area and passing the first compartment, increase the contamination risk of the following compartments soil and groundwater. Thus, the toxic effects and displacement of particle-bound compounds were assessed comparing soil samples of periodically inundated sites, including those described in sect. 3.2, and non-inundated sites at the planned retention area Bellenkopf/Rappenwoert.

The assessment of soil profiles with a depth of 90 cm, separated in sub-samples, allowed the determination of total effects and the effect course in each profile by applying the in vitro biotests mentioned above. While cytotoxic effects were minor at nearly all sites, EROD activities were elevated at some sites and significantly increased in surface depressions, containing accumulated deposited sediments. In addition, the Ames Fluctuation assay (Perez et al., 2003) was applied and indicated considerable mutagenic effects, according to the highly effective samples identified in the EROD assay. At each site with exception of the backwater site described in sect. 3.2, it could be shown that toxic effects decreased from the surface to the lowest soil layer assessed. Commonly, these findings are due to the high contents of humic compounds in the upper soil layer, providing huge binding surfaces. In particular at river banks, lower soil layers consist of large grained compounds as gravel and, thus, provide significantly less binding sites (Fernandez-Galvarez 2007), resulting in a decrease in toxic effects.

Concluding, at least in depressions at periodically inundated sites, toxic effects were comparable to effects detected in the first compartment. Thus, SPMs displaced in (extreme) flood events and deposited on floodplains accumulate and act as contaminant sinks that may subsequently turn into sources, potentially releasing hazardous compounds into the soil. In absence of mechanisms to reduce contaminant deposition on floodplains/retention areas these aspects should be considered.

9.4.4 Modeling

To calculate the transport of dissolved organic contaminants through the soil zone of the retention area to the aquifer, a one-dimensional contaminant transport model (FW_{inf}) was developed (Bethge and Mohrlok, 2008). The transport processes during a flood event were described using a time efficient analytical modeling approach. The relatively short computing time allowed the mass flux to the aquifer to be calculated for a large number of locations, thus accounting for the spatial variability of the floodplain soils.

In the model FW_{inf}, the soil zone was represented with a two-layered soil profile, where a loamy topsoil overlies a sandy subsoil. The presence of macropores is considered for the topsoil only. To calculate the transport of the infiltrated contaminants through the soil zone, advective transport of the contaminants with the soil water movement is considered. The bypass flow through macropores in the topsoil was taken into account by a simple macropore bundle approach (Chen and Wagenet, 1992). The retarding effect of the contaminant sorption to the soil matrix was simulated using a retardation factor, whereas contaminant mass degradation was implemented with a first order kinetic model.

The mass balance model FW_{inf} was applied to the planned flood water retention area “Bellenkopf/Rappenwoert”. Field infiltration experiments have been conducted to estimate the macropore porosity of the topsoil. Soil samples were taken from the field to determine the saturated conductivity and water retention parameters. Additionally, the organic carbon content was determined at different depths. The soil map and land use categories (forestry, agricultural, open water bodies) were used to regionalize these data over the floodplain and to outline simulation units that were used to calculate the one dimensional contaminant transport within the soil zone.

To calculate the mass flux through the soil zone a flooding scenario with a 20 year recurrence interval was chosen. For the properties of the contaminant in the flood water an average sorptivity ($\log K_{OW} = 0.3\ m^3/kg$) and degradation rate ($\lambda = 1.1 \times 10^{-7}$ L/s) were chosen (similar to the pharmaceutical carbamazepine). The concentration in the flood water was set to $1.0 \times 10^{-3}\ kg/m^3$.

In fig. 5 the areal distribution of the contaminant mass flux to the aquifer is shown for the retention area Bellenkopf/Rappenwoert. Near the inland embankment drainage measures to control the inland groundwater level establish a large hydraulic gradient between surface water and groundwater head. As a result almost 99 % of the mass output from the soil zone into the groundwater occurs within 200 m of the inland dykes. The highest contaminant mass fluxes are found where high seepage rates coincide with small contaminant storage capacity of the soil zone (small thickness of the soil zone, low organic carbon content).

In fig. 6 the calculated overall mass balance for the soil zone of the study area is shown for the different flow stages of the seepage rate (Sect. 3.1). Mass input (M_{in}) is observed during the flow stages S1 and S2, mass flux into the groundwater (M_{out}) takes place during flow stages S2 and S3. The highest mass input and output are observed in flow stages S2. For the given contaminant properties the mass storage in the soil (M_{soil}) and mass degradation (M_{deg}) are small compared to the mass inflow and outflow.

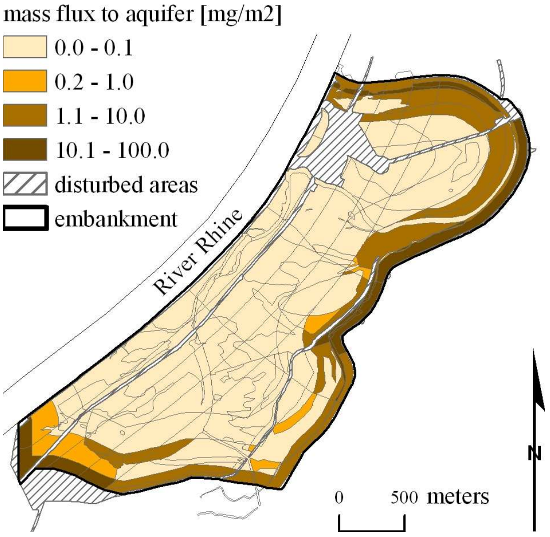

Fig 5: Areal distribution of the calculated contaminant

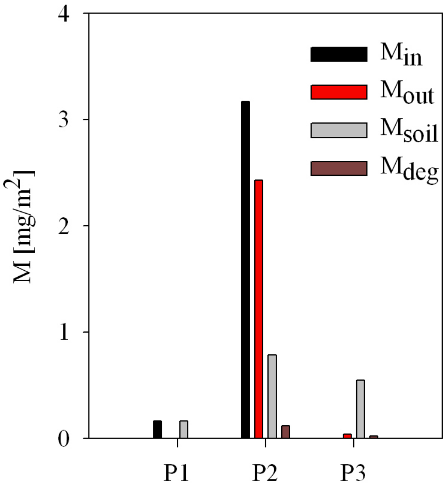

Fig 6: Calculated average contaminant mass flux to the aquifer mass fluxes during the infiltration phases.

9.5 Phase 3: Groundwater flow

9.5.1 Characterization

The third phase of the contaminant transport to the well field of a water department is the transport with the groundwater flow. A reduction of concentrations in the production wells occurs as the result of mechanisms of advective transport, dilution, retardation and degradation.

The contaminants are transported primarily advectively with the groundwater flow. Therefore, even if they enter the groundwater, they can only reach the production wells if the groundwater gradient in the extremely transient flow field is oriented towards the production wells for a sufficiently long time. If instead the groundwater gradient is directed primarily towards the river, the appearance of a significant concentration of contaminants in the production wells can be excluded. Therefore, water that infiltrated into the aquifer during flooding of the retention area, can, in most cases, only reach the well field if the well field's zone of contribution overlaps the retention area under average hydrologic conditions. If there is no overlap, the groundwater quality at the well field will be unaffected by the retention area in most cases.

Fig. 7 depicts an idealized well field with a catchment area which overlaps with the flood water retention area and even extends to the river at average hydraulic conditions. In such a case, even under average hydraulic conditions, the groundwater extracted at some production wells will contain a significant percentage of infiltrated river water. When the retention area is flooded with river water, the gradient between the retention area and the well field will increase and thereby directly increase the percentage of river water at the municipal wells.

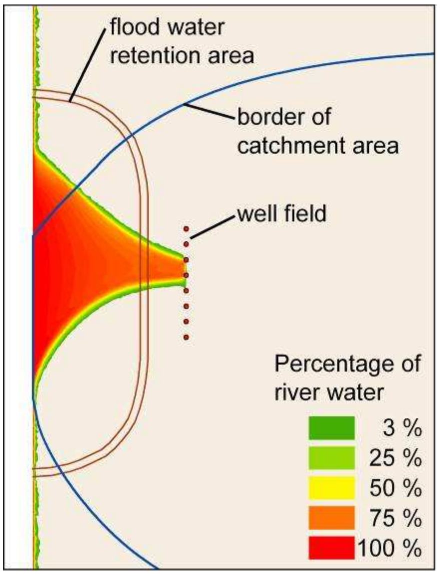

Fig. 7: River water in the aquifer before flooding of the retention area. of the retention area.

Furthermore, during flooding, river water will infiltrate in the aquifer mainly at the edge of the retention area, as discussed in sect. 3.4 and depicted in fig. 8. After flooding, this infiltrated water will be transported by the normal groundwater flow to the production wells and will eventually reach them, as much as years after the flooding event, depending on the velocity of the groundwater flow.

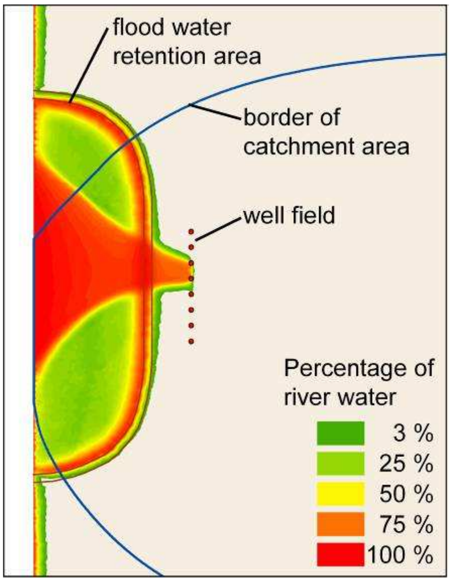

Fig. 8: River water in the aquifer after flooding

The concentration of substances from the river that reach the pumping wells will be reduced by dilution. First, it must be noted that the volume of the river water entering the aquifer during flooding of the retention area will generally be much smaller than the volume of groundwater already in the aquifer. On its way towards the extraction well, the water and the substances it carries with it will be mixed with the groundwater by dispersive processes, reducing the concentrations of contaminants significantly. An additional dilution process takes place in the withdrawal wells and in the water treatment plant. The pumping wells of the water works draw groundwater from both the retention area side and the inland side. Under average hydraulic conditions, when the retentions area is not flooded, considerably more water from inland reaches the pumping wells because of the low groundwater gradient between the well field and the stagnation point, which lies in direction of the retention area. Furthermore, several of the withdrawal wells often extract no infiltrated river water (Fig. 7 and fig. 8). Both mechanisms strongly reduce the concentrations of infiltrated substances from the retention area at the water works.

Retardation and degradation can further reduce contaminant concentrations at the well field, but both mechanisms are highly dependent on the properties of the substance of interest. Certain substances, such as many X-ray contrast agents, will neither be retarded nor degraded in an aquifer.

9.5.2 Chemical analysis

The analysis of ground water samples collected following the flood event in August 2007 revealed changes in concentrations of chemical compounds directly associated with river water. In particular, increases in the concentrations of complexing agents and fuel additives as well as dissolved oxygen, pharmaceutical products and a pesticide metabolite were observed.

The analyses confirmed that inputs of river water to the aquifer occurred not only through infiltration at the river bank, but also at a backwater site. The tests established that dissolved organic contaminants were able to pass through the soil zone and enter the aquifer. The ability of microbial organisms to degrade organic compounds strongly influences the amount of pollutants that reach the drinking water wells. Because the planned well field will substantially alter the groundwater flow regime, it was impossible to examine changes in contaminant concentrations in field studies. Therefore laboratory experiments were carried out.

In a series of tests, defined amounts of typical pollutants were added to Rhine water which was then circulated in a closed experimental plant. The tests showed that the concentrations of some pharmaceuticals and organic pollutants were reduced by less than 50 % even after thirty days and were therefore defined as substances of concern for drinking water suppliers. It must be concluded, that transport through the aquifer cannot protect the well field against some persistent organic pollutants.

9.5.3 Ecotoxicological analysis

While sec. 2.3 and 3.3 introduced the background and outcomes regarding contaminant entry with deposition/displacement through the soil, the focus here is to determine whether contaminants may pass through the soil layer and enter the drinking water aquifer.

Neither Ah receptor agonist (EROD assay) nor mutagenic activity (Ames Fluctuation assay) indicated significantly increased activities, nor could cytotoxicity be determined. However, endocrine activity mediated through the human estrogen receptor (ER) in the YES assay (Routledge and Sumpter, 1996) could be measured, showing elevated endocrine effectiveness for some of the samples and considerable fluctuations at different sampling times.

Nevertheless, the data reflected a time-effect correlation, at least, with groundwater sampled at short intervals following the more intense Rhine flood (recurrence interval approximately10 years) in August 2007. So far, there are no data available which would indicate a flood dependent increase; and maybe only a temporal coherence is given.

As endocrine active compounds seemed to be the most relevant groundwater contaminants at the site, effect-directed analysis (Lübcke-von Varel et al., 2008) was applied to identify relevant inducers. Furthermore, fractionation was performed with flood SPMs and highly effective soil. Endocrine activity was measured in each sample, at least constituting to a considerable extent to effects in groundwater. Additional chemical analysis to identify effective compounds is underway.

In conclusion, previously effective compound categories and ecotoxicological endpoints were no longer relevant in the groundwater, while endocrine activity was detectable. Furthermore, activities were measured in sample fractions of the other compartments. Thus, although the origin of the measured ER agonist activities remains so far unclear, considering the complete ecotoxicological assessment an impact of particle-bound compounds to drinking water supply cannot be excluded. Further research has to focus on the identification of effective compounds and will be completed following this project.

9.5.4 Modeling

For the Kastenwoert study area a numerical groundwater flow and transport model was constructed to predict the influence of the planned retention area on the percentage of Rhine water in the groundwater extracted at the well field. The 3-dimensional finite element model was constructed using the groundwater modeling program FEFLOW from DHI-WASY. The model area covers an 11 km stretch of the eastern Upper Rhine valley (a total of approx. 120 km^2). The large model area was necessary in order to include the whole catchment area of the water work. The spatial discretization of the model, in particular near the Rhine, had to be very fine in order to ensure stable mass transport calculations. In total, the groundwater model contained 529,060 elements (75,580 per layer) and 304,904 nodes (38,113 per slice). A simulation time of 46 years was chosen (from January 1960 to December 2005), the longest time period for which data were available. This made it possible to examine long-term developments. The lengths of the time steps, which varied between a few minutes to 7 days, were automatically selected by the program system by the predictor-corrector scheme (AB/TR).

In order to prepare the model for transport simulation, the Dirichlet boundary condition simulating the Rhine River was assigned a mass concentration of 100 mg/L. The water infiltrated through the retention area was given the same value. Thereby the percentage of the Rhine water in the aquifer during the transport simulation could very simply be observed and illustrated. As some of the substances in the river water, for example X-ray contrast agents, are neither degraded nor retarded in the groundwater flow, these processes were not included in the transport simulation. On a standard PC, the calculation of a complete transport scenario took about 3 weeks.

Fig. 9 illustrates the ratio of Rhine water to groundwater in the planned well field as calculated with the numerical groundwater model described here. The red line illustrates the percentage of Rhine water in a scenario in which the municipal wells start pumping in 1960 and pump consistently and steadily for the next 46 years at a combined rate of 7.4 million m^3/a. The retention area is not active in this scenario. The percentage of Rhine water in the extracted groundwater lies between 5 and 10 % in this scenario. The green line shows the fraction of Rhine water in the planned water works in a scenario in which both the wells and the retention area are brought online in 1960. Consistent with historical Rhine water levels measured during that period, about 50 flood events, some of them quite small, were simulated across the retention area. The repeated flooding of the retention area over the course of the simulation period raises the percentage of Rhine water in the extracted groundwater by about 5 to 10 %. Following the most significant flood event of the observation period, which occurred in 1999, the percentage of Rhine water rises by as much as 15 % to compose about one quarter of the extracted groundwater.

The simulation results were entirely consistent with the mechanisms described in sect. 4.1. During the flood events, peaks in Rhine water input are observed and repeated flooding of the retention area leads to an overall increase of the percentage of Rhine water in the pumping wells of the water works in the long term. Due to dilution effects, the probability is very high that the percentage of Rhine water extracted at the water work will remain far less than 50 %, despite the proximity of the retention area.

9.6 Discussion and conclusion

The transport of contaminants from a river to a well field via a flood water retention area is affected by the processes and mechanisms occurring in the three compartments: Retention area, soil zone and aquifer.

The first compartment is chiefly characterized by the sedimentation of suspended matter within the flood water retention area. Generally, the concentrations of dissolved contaminants are unaltered by the processes in the first compartment. Contaminants sorbed to suspended matter, on the other hand, are significantly affected, as they will be deposited with the suspended matter.

The chemical and ecotoxicological analysis of suspended matter in the Rhine River show significant presence of contaminants. The same contaminants and ecotoxicological effects could be detected in the upper part of the soil layer of several periodically inundated sites, with intensities varying from site to site. Thereby, the result of the variations in the highly complex depositional processes of the sediment within the retention area could be demonstrated. Numerical models can be applied successfully to improve the prediction of deposition patterns, which in turn define locations within the retention area where contaminants will accumulate.

The second compartment is the soil zone. Its effect on the contaminant mass flux to the aquifer is mainly determined by the properties of the soil and the contaminant as well as the flood event characteristics. The variability of soil properties and hydraulic conditions within the retention area leads to distinct differences of the soil zone’s ability to prevent a contaminant input into the aquifer. Applying the contaminant transport model FW_{inf} on the Kastenwoert-Rappenwoert study area, the highest contaminant mass fluxes of dissolved organic contaminants into the aquifer are calculated for low lying soils in the near dyke area with a low organic carbon fraction.

Presently, only a small section of the floodplains directly adjacent to the Rhine River is inundated during floods. The chemical and ecotoxicological analysis of the areas subject to inundation have revealed high concentrations of sorptive contaminants and significant ecotoxicological effects in the upper part of the soil layer, while the lower part of the soil zone and the groundwater were free of sorptive contaminants and any significant ecotoxicological effects. It may, therefore, be concluded that the storage capacity of the floodplain soil is sufficient for the current mass input to completely retain sorptive contaminants. The contaminants still found in the groundwater are known to be highly persistent and mobile and thereby had evidently been able to pass through the soil zone or river bank without being significantly affected. However, these substances were found to show no ecotoxicological effects.

In the third compartment, the contaminants are transported with the groundwater to the well field. In most cases contaminants from the river can only reach the production wells if the catchment area of the well field at average hydraulic conditions overlaps with the flood water retention area. In this case, the concentrations of the contaminants are significantly reduced on their way in the aquifer by dilution, and sometimes additionally by microbiologic degradation and retardation.

Regarding the low flow velocities of the groundwater in the study area, it has to be concluded that the organic contaminants recently found in the groundwater have partially been present in the aquifer for several years. Therefore it must be assumed that, given the groundwater flow calculated by the numerical aquifer simulation, these substances would be able to reach the planned well field even after very long transit times.

Using numerical modeling of the study area, it was predicted that some contaminants found in the Rhine River, including HCB, PAHs or PCBs, which are mostly adsorbed to suspended matter, will probably be completely retained within the transport path, while others, like EDTA or X-ray contrast agents, which are highly mobile and persistent, will most likely travel to the well field, although in significantly reduced concentrations.

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Chapter 10

Conclusions of the study

In the light of the preceding chapters, the entire study has been successful in investigating the repercussions of flooding events on the degree of hazard potential of water-borne and sediment-borne contaminants in river systems. The study broadly employed effect-directed analysis (EDA) to determine which contaminants are bound to SPM and are responsible for the toxicological effects. This methodology uses a combined approach of *in vitro* biotests, chemical analysis and fractionation methods. Used biotests demonstrated that cytotoxicity, dioxin-like/Ah receptor (AhR)-mediated and endocrine activities, as well as mutagenic potentials, were increased with many samples assessed. Furthermore, the combination of fractionation methods to separate persistent, less persistent and more polar compounds, respectively, with chemical analysis and biotesting were shown to be adequate tools to identify effective compound classes in distinct fractions.

The first part of the study investigated on contaminants bound to suspended particulate matter (SPM) sampled in flood events. Results revealed that less persistent polycyclic aromatic hydrocarbons (PAHs) and more polar compounds are the main inducers of biological responses. These results allow to direct future investigations to more relevant and higher effective compounds.

The appraisal of environmental contamination is normally limited to *a priori* selected compounds. This is considered a comprehensible strategy since chemical analysis cannot account for any possible contaminant. However, such scheme of preselecting toxicants poses the risk of oversighting hazard potentials that can be caused by non-prioritized compounds, as shown with most samples and fractions in this study. Evaluation is considered a failure when the analyzed contaminants only reflect a few percent of the total biological activities while disregarding the majority of effective compounds. In order to avoid false evaluation, investigations should be designed on a broader perspective. When chemical analysis cannot explain biological effects, extended fractionation methods can assist to identify responsible compounds or at least compound classes. Accordingly, biological analysis has to include different significant endpoints to reflect given impacts of contaminant loads.

However, flood SPM indicated elevated chemical loads and biological hazard potentials. Thus, the question arises whether river contaminants impact, e.g. inundated sites. In particular, areas that are especially chosen as retention basins and which are built temporarily to protect downstream sites from extreme floods with recurrence intervals of more than or equal 100 years should be given crucial attention. When these retention basins are operated and flood water is kept over periods of days to weeks, particles and bound contaminants can deposite on large scale on the basin ground. Furthermore, flood water can infiltrate the unsaturated zone and infiltrate the aquifer that often is used as drinking water resource. Thus, conflict of interests is expected. This aspect was investigated in the second part of the present study at a model site where conflict ensues.

Investigations showed that flood SPM is highly loaded with contaminants and causes maximum effects at the peak of discharge. Further research was carried out on soil sampled at inundated and non-inundated sites and was shown to reveal contamination of sites due to particle deposition. Results indicated that soil has to be sampled at several sites; different geomorphological structures such as ground swales have to be included in the assessment since they accumulate river pollutants. Likewise, soil has to be sampled both at the surface and deeper layers since proximity of contaminants to aquifer provides important information on potential pollution hazards to drinking water resources.

Accomplishment of EDA, again, provided useful information on the classes of effective compounds and the relative contributions to the total biological effects. In particular PAHs of higher molecular weight can be expected to be very effective. Furthermore, more polar compounds can be expected to be highly effective. Chemical analysis of priority compounds, again, was less appropriate to explain biological effects. Both results underline the need to broadly analyze complex environmental samples, and, in particular, to include further and recently determined substances of concern, e.g. heterocycles. Thus, hazard identification and assessment of contaminants (e.g. in drinking water resources) should also be directed towards these compounds.

Another aspect of the study was to assess if river contaminants infiltrate the aquifer during a flood event. Typical tracer compounds such as the pharmaceutical carbamazepine can be analyzed in order to determine how deep river water encroaches in the aquifer. In parallel, biological effects have to be measured since tracer concentration and effects are not necessarily connected to each other, in particular, since infiltration depth differs between (effective) compounds. Thus, test design, applied biotests and chemical analysis have to be selected carefully and should be angled broader to competently identify the effective toxicants.

Evaluation of SPM during flood events and the consequent particle deposition at inundated sites revealed an elevated contamination load and a concomitant increase of biological effects. Since even aquifer is infiltrated far distant in the hinterland, impacts to drinking water resources appear to be possible. In this study, investigations were accomplished with samples of a flood with a recurrence interval of up to 10 years. Underlying conflict of interests will be intensified when retention basins are operated with floods and with a recurrence interval of greater than or equal to 100 years. Thus, inundated sites, retention basins, and aquifers will be impacted by even higher contaminant loads. Recent and future flood management strategies have to cope with factors other than just the physical impact of extreme floods.

Chapter 11

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