Screening, quantitative analysis and toxicology of organic compounds in sediments and suspended particulate matter of the Saar and the Rhine (Germany)

Screening, quantitative Analyse und Toxikologie organischer Verbindungen in Sedimenten und Schwebstoffen der Saar und des Rheins (Deutschland)

Dissertation

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Die Wasser, wie lieblich sie brennen und glühn!

Sie spielen in grünendem Feuer;

Es geisten die Nebel am Ufer dahin,

Zum Meere verzieht sich der Weiher –

Nur still!

Ob dort sich nichts rühren will?

Eduard Mörike «Die Geister am Mummelsee»

Chemie brauchbar gemacht

für jede Extremität des menschlichen Körpers

von Stimmen begleitet hinter klangvollen Botschaften.

Anonymer *Tag* Westwerk Leipzig (Oktober 2012)

It's like a jungle sometimes it makes me wonder!

How I keep from going under?

Grandmaster Flash «The Message»

Preface

This dissertation thesis is based on several studies regarding the assessment of chemical burdens and toxicological effect potentials of sediments, soils and suspended particulate matter in river systems and riparian lands due to human activities. The individual studies were (including funding institutions and grand numbers):

- «Ecotoxicological assessment of the Rhine sediments and suspended particulate matter in inundated areas» (German title: «Ökotoxikologische Bewertung von Rheinsedimenten und Schwebstoffen in Überflutungsgebieten») funded by the Stadtwerke Karlsruhe GmbH (SWK; 2002–2003)
- «Development of a standard operation procedure 'sediments and suspended particulate matter'» (German title: «Entwicklung einer Verfahrensrichtlinie 'Sedimente und Schwebstoffe'; FKZ 301 02 013) and «Validation of the SOPs 'sediments and suspended particulate matter' under routine conditions» (German title: «Validierung der SOPs 'Sedimente und Schwebstoffe' unter Routinebedinungen»; FKZ 301 02 018) funded by the German Federal Environmental Agency (UBA; 2003–2006).
- «Models for assessing and forecasting the impact of environmental key pollutants on freshwater and marine ecosystems and biodiversity (MODELKEY)» funded by the European Community (511237 GOCE, FP6; 2005–2010)
- «LPDA (Low Pressure Dialytic Analysis)-device and method using special semi-permeable dialysis membranes for clean-up of solid phase extracts» (German title: « LPDA (Low Pressure Dialytik Analysis)-Gerät und -verfahren mit Hilfe spezieller semipermeabler Dialysemembranen zur Aufreinigung matrixbeeinflusster Substanzextrakte») funded by German Federation of Industrial Research Associations – AiF (Contract-No. KF 0011005 UL6; 2007–2009)
- «Risk management of extreme flood events — Flood retention and drinking water supply – Preventing conflicts of interest (RIMAX-HoT)» funded by the German Federal Ministry of Education and Research (BMBF; No. 02WH0691; 2005–2009)

The scientifc contributions of Tobias Schulze in planning, proposing and performance of the mentioned studies as well as data evaluation/assessment, reporting and (co-)authoring of scientific papers were as follows:

- The study funded by SWK was proposed by the University Heidelberg (Germany; Prof. Dr. Thomas Braunbeck, Prof. Dr. Henner Hollert). The particulate contributions were the planning and proposing, co-ordination and performance of the chemical-analytical sub-project, data evaluation/assessment and reporting (together with Prof. Dr. mult. Dr. h.c. Konstantin Terytze, FU Berlin) as well as co-authoring of one published journal paper and authoring of one paper in preparation for publication.
- The study funded by UBA was in charge planned, proposed, co-ordinated, and performed by Tobias Schulze and Mr. Mathias Ricking (FU Berlin) including sampling, chemical analysis, data evaluation/assessment and reporting. The particulate study regarding the extractability and effect potentials of Saar sediments was planned and performed by Tobias Schulze with assistance of Dr. Thomas-Benjamin Seiler (RWTH Aachen) performing the bioassays and Dr. Georg Streck (Leipzig) providing artifical mixtures for effects confirmation. One journal paper was authored and published as outcomes from UBA project. A second paper was prepared and published during this thesis.
- The sub-study within the projects MODELKEY and LPDA regarding the comparison of different extraction methods was planned and performed by Tobias Schulze (FU Berlin / UFZ Leipzig), Dr. Thomas-Benjamin Seiler (RWTH Aachen), Dr. Katrin Schwab (Currenta GmbH & Co. OHG, Leverkusen) and Dr. Georg Streck (UFZ Leipzig). MODELKEY was proposed and co-ordinated by Dr. Werner Brack (UFZ Leipzig). LPDA was proposed by Dr. Werner Brack and Dr. Georg Streck (UFZ Leipzig). The particularly contribution was the planning, the performance of different extraction methods, the data evaluation and assessment and the co-authoring of two journal papers. These papers are in preparation and considered for publication.
- The contribution within RIMAX-HoT was the consulting of Dr. Jan Wölz (RWTH Aachen) regarding effect-directed analysis (EDA), planning of the particulate EDA-study and co-ordination of the EDA laboratory work (together with Urte Lübcke-von Varel and Dr. Werner Brack), partly performance of chemical analysis, data evaluation/assessment and co-authoring of two published journal papers.

This thesis contains the following contributions of scientists, technicians and students:

- The laboratory work and help in fieldwork in the UBA projects in section 2 were particularly performed by Muna Al-Samir, Joachim Bartels, Anja Löhe, Silke Meier, Christian Menz, Kerstin Mittelhaus, Kathleen Müller, Mr. M. Ricking, Jeannette Rümmler, Dr. Anne Seebach and Sarah Zapf at FU Berlin. Prof. Dr. Augusto Mangini and Dr. Clemens Woda (Heidelberg Academy of Science) analysed instable isotopes in two sediment cores for estimation of sedimentation rates and sediment age.
- The laboratory work in section 3 was particularly done by the co-workers in the UBA project (see above). The biological samples were processed by Dr. Thomas-Benjamin Seiler and co-workers (University Heidelberg and RWTH Aachen). Dr. Thomas-Benjamin Seiler further provided advices on the biological data and reviewed this section. Dr. Georg Streck (UFZ Leipzig) provided the artificial standard mixtures for the confirmation experiments.
- The laboratory work in the sub-study of MODELKEY in section 4 was partly performed by the technicians Joachim Bartels and Silke Meier at FU Berlin as well as Thomas Anger, Marion Heinrich and Ines Rein at UFZ Leipzig. Dr. Thomas-Benjamin Seiler and co-workers at University Heidelberg and RWTH Aachen performed the biological analyses. The data evaluation and assessment was done together with Dr. Katrin Schwab (Currenta Leverkusen), Dr. Werner Brack and Dr. Georg Streck (UFZ Leipzig) as well as Prof. Dr. Henner Hollert, Dr. Thomas Benjamin Seiler (RWTH Aachen) and Prof. Dr. Thomas Braunbeck (University Heidelberg).
- The grain size and carbon analysis within the SWK project in section 5 was performed by Dr. Anne Seebach and the trace elements analysis by Manuela Scholz (both FU Berlin). The polychlorinated dioxins and furanes were analysed by the commercial laboratory «Analysen Service GmbH Berlin» (assessment report No. 531-03-1). Prof. Dr. Henner Hollert (RWTH Aachen) and co-workers at University Heidelberg performed the sampling, sample preparation and biological analyses. Particularly, data and results of the diploma thesis of Dr. Markus Ulrich and the state examination thesis of Volker Garke were included.
- The sampling, sample processing, chemical/biological analysis and data evaluation/assessment in section 6 and 7 was performed by the RIMAX-HoT project partners (Prof. Henner Hollert and co-workers, RWTH Aachen; Prof. Dr. Thomas Braunbeck and co-workers, University Heidelberg; Dirk Kühlers, Stadtwerke

Karlsruhe GmbH; Michael Fleig and co-workers, DVWG-Water Technology Centre (TZW) Karlsruhe; Dr. Georg Reifferscheid, German Federal Institute for Hydrology Koblenz).

Parts of this thesis have been published or are in preparation for publication:

Schulze, T.; Seiler, T.-B.; Streck, G.; Braunbeck, T.; Hollert, H.: Comparison of different exhaustive and biomimetic extraction techniques for chemical and biological analysis of polycyclic aromatic compounds in river sediments; Journal of Soils and Sediments 9, 1419-1434.

Schulze, T.; Ulrich, M.; Garke, V.; Maier, M.; Terytze, K.; Braunbeck, T.; Hollert, H.: Risk assessment of river suspended particulate matter and floodplain soils in the Rhine catchment using chemical analysis and in vitro bioassays (in preparation for submission to «Environmental Science and Pollution Research»).

Seiler, T.-B.; Streck, G., **Schulze, T.**; Schwab, K.; Brack, W.; Braunbeck, T.; Hollert, H.: On the comparability of procedures for sediment extraction in environmental assessment. Part A: Bioanalytical investigations (in preparation for submission to «The Science of the Total Environment»).

Streck, G.; **Schulze, T.**; Seiler, T.-B.; Schwab, K.; Brack, W.; Braunbeck, T.; Hollert, H.: On the comparability of procedures for sediment extraction in environmental assessment. Part B: Chemical analytical investigations (in preparation for submission to «The Science of the Total Environment»).

Ulrich, M.; **Schulze, T.**; Leist, E.; Glaß, B.; Maier, M.; Maier, D.; Braunbeck, T.; Hollert, H. (2002): Abschätzung des Gefährdungspotenzials für Trinkwasser und Korrelation verschiedener Expositionspfade (Acetonischer Extrakt, Natives Sediment) im Bakterienkontakttest und Fischeitest; Umweltwissenschaften und Schadstoff-Forschung - Zeitschrift für Umweltchemie und Ökotoxikologie 14, 132-137.

Wölz, J.; Fleig, M.; **Schulze, T.**; Maletz, S.; Lübcke-von Varel, U.; Reifferscheid, G.; Kühlers, D.; Braunbeck, T.; Brack, W.; Hollert, H. (2010): Impact of contaminants bound to suspended particulate matter in the context of flood events; Journal of Soils and Sediments 10, 1174-1185.

Wölz, J.; **Schulze, T.**; Lübcke-von Varel, U.; Fleig, M.; Reifferscheid, G.; Brack, W.; Kühlers, D.; Braunbeck, T.; Hollert, H. (2011): Investigation on soil contamination at recently inundated and non-inundated sites; Journal of Soils and Sediments 11, 82-92.

Summary

River sediments and suspended particulate matter (SPM) are sinks as well as secondary sources for nonpolar organic compounds that might pose a risk to aquatic ecosystems' goods and services. Sources of these pollutants are for example wastewater treatment plants (WWTP) effluents or direct discharges as well as dry and wet atmospheric deposition. Surface run-off of contaminated soils and dust from streets are other important origins. The particularly bound substances with different physical and chemical properties are constrained to partitioning processes between the solids and the water phase. Hence, they might get bioavailable and thus pose an ecotoxicological risk for water organisms. During flood events, contaminated sediments could be remobilized and transported to riparian lands. Hence, there are concerns regarding risks for terrestrial ecosystems' goods and services.

Thus, the objectives of this thesis were:

1. Which organic compounds are present in sediments and suspended particulate matter of the Saar and the Rhine?
2. How is the spatial and temporal distribution of these substances and their historical record in sediment cores?
3. Are the particularly bound compounds permanently sequestered in the sediments and suspended particulate matter? Alternatively, is there a possibility of desorption and bioaccessibility of these compounds?
4. Which extraction approach is appropriate to map a certain fraction of particularly bound substances?
5. How is the ecotoxicological impact of the sediments, soils and suspended particulate matter investigated in this study?

In the first part, sediments and SPM samples of the Saar and the Rhine were investigated regarding organic compounds using target and suspect screening analysis with gas chromatography – mass spectrometry (GC-MS). Sediment core samples were collected at representative sampling sites along the both rivers. The cores were radiometrically analyzed to estimate sedimentation rates and sediment ages using the instable isotopes ^{210}Pb and ^{137}Cs. However, only two of five cores yielded valid results showing continuous sedimentation. Furthermore, a time series of monthly composite SPM samples was collected

during the year 2005 at four sampling sites of the Rhine (Weil am Rhein, Iffezheim, Koblenz, Bimmen) and two sampling sites of the Saar (Güdingen, Rehlingen). This study was conducted, among other things, to improve information regarding nonprioritized organic compounds in sediments and suspended particulate matter (SPM) of the Saar and the Rhine.. Moreover, the source appointment and the assessment of the ecotoxicological potential of the target- and nontarget compounds were in the focus of this study. Additionally, the spatiotemporal occurrence and distribution of the compounds in SPM and sediments was investigated. Target analysis revealed elevated concentrations of polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls in the Saar and at the sampling site Bimmen at the Lower Rhine due to the activities in the Saar and the Ruhr industrialized and coal mining districts. However, the concentrations of PAHs and PCBs in samples from radiometrically dated sediment cores decreased since the 1980s due to effects of pollution control programs such as the Convention on Pollution Control of Rhine River. Hexachlorobenzene (HCB) was confirmed as one of the main organic contaminant in the Rhine. It was released majorly by a former chemical plant in Rheinfelden due to pentachlorophenol production. The combination of univariate and multivariate analysis such as analysis of variance and self-organizing maps revealed a general picture of the spatiotemporal occurrence and distribution as well possible sources of the target analytes the Rhine and the Saar. The compounds identified by suspect and nontarget screening occurred in most SPM and sediment samples. The potential adverse effects of those compounds were discussed comprehensively. As a nontarget compound, α-tocopherol acetate (a derivative of «vitamin E») was elucidated. It is a marker for the input of municipal WWTPs. Furthermore, the antioxidant dioctyldiphenylamine as well as the heat transfer agents methyldiphenylmethane and methylbisdiphenylmethane were identified. The identity of these compounds were confirmed using search in mass spectral libraries (Wiley 9 and NIST 08) and virtual (*in silico*) fragmentation by means of the online software MetFrag as well as comparison with published mass spectra.

In the second part, the extractability and potential toxicity of particularly bound organic compounds was investigated. Sediment samples from the Saar as well as the Elbe and the Bílina in Czech Republic was extracted with different exhaustive and biomimetic extraction approaches. Soxhlet, ultrasonic and accelerated solvent (ASE) extraction as well as membrane dialysis extraction (MDE) were used as exhaustive methods. Extractions with Tenax®-TA as well as mixtures of water with methanol or 2-hydroxypropyl-β-cyclodextrin

(HCBD) were applied as biomimetic approaches. The extracts were analyzed for the contents of different organic compounds (e.g., polycyclic aromatic hydrocarbons (PAHs) and organochlorines) and their toxicological potential using three different bioassays (i.e. the 7-ethoxyresorufin-O-deethylase induction assay (EROD) for dioxin-like effects, the neutral red assay for cytotoxicity and the fish egg assay with *Danio rerio*). The main objective of these investigations was the generation of comprehensive knowledge concerning the relationship between the physical-chemical properties of compounds and sediments as well as extractability and resulting toxicity. MDE yielded a similar or better extraction power compared to the established Soxhlet and ASE approaches. Thus, MDE was proven as method for the exhaustive extraction of nonpolar compounds from sediments, SPM and soils. With respect to the biotests, extracts from ASE, MDE and Soxhlet extraction showed similar results regarding cytoxicity and dioxin-like effects. However, Soxhlet extracts were more effective in the fish egg assay. Biomimetic extraction with HCBD and Tenax®-TA yielded variable results. Recommendations for the integrated risk assessment of sediments including exhaustive extraction as well as biomimetic methods and dosing were compiled.

In the third part, potentially adverse effects of contaminated sediments and SPM for riparian lands were investigated. In a proof-of-concept study, soils from the flood retention area Bellenkopf-Rappenwört (Karlsruhe, Germany) and suspended particulate matter from the Rhine near Iffezheim barrage (Germany) were analyzed using biological and chemical analyses. Two sediment contact assays (fish egg test with *Danio rerio* and dehydrogenase assay with *Arthrobacter globiformis*) were used with native samples and acetonic extracts. Additionally, acetonic extracts were tested for dioxin-like effects and cytotoxicity in the EROD and neutral red assay, respectively. Furthermore, the samples were analyzed for organic compounds (i.e. PAHs, hexachlorobenzene (HCB), polychlorinated biphenyls and polychlorinated dibenzodioxines/-furanes), grain sizes, contents of organic carbon and trace elements. A main aim was the investigations of two exposition pathways in the contact tests – native sediment versus acetonic extract. It was shown that acetonic extracts did not significantly overestimate effects comparing to native samples. However, 92% of the effects in the EROD assay were not explained by the PAHs and the PCDD/Fs. A second, long-term study was performed with frequently sampling of composite SPM samples at Iffezheim barrage and soils from inundated and noninundated riparian land at Bellenkopf-Rappenwört area. Effect-directed analysis (EDA) was used to examine selected soil and SPM samples regarding effect potentials *in vitro* and to unravel responsible compound classes or

substances, respectively. All samples were pre-analyzed using the using the EROD assay and the Ames fluctuation assay to test for dioxin-like potency and mutagenicity, respectively. Effective samples were selected for automatic fractionation according aromaticity, planarity and polarity. Each sub-fraction was reanalyzed using both biotests. Active sub-fractions were analyzed regarding PAHs, PCBs and HCB using GC-MS. The SPM samples were not mutagenic at all. In the case of EROD assay, only 1% of effects were explained by the measured PAHs. Thus, even if PCDD/Fs are included (as in the previous study), the analysis of priority compounds is not sufficient to derive real cause-effect relationships and to unravel the responsible substances. Another objective of the study was the investigation of the impact of potential hazardous particle-bound contaminants to riparian lands. The effect-potentials of soils from frequently inundated floodplains were similarly to those of the SPM samples. This is maybe caused by the observed accumulation of organic compounds in the floodplain soils. In contrast, significant effect potentials were only found in soil samples collected in the zone of a depression in the noninundated area behind the levee.

Zusammenfassung

Fluviale Sedimente und Schwebstoffe bilden sowohl eine Senke als auch eine sekundäre Quelle für hydrophobe organische Verbindungen mit ökotoxikologischem Potential. Der Eintrag dieser Substanzen in die Gewässer erfolgt beispielsweise durch Klär- und Direkteinleitung, trockene und nasse atmosphärische Deposition, oder den Eintrag von Bodenpartikeln und Oberflächenabfluss bei Niederschlagsereignissen. Die partikulär gebundenen Stoffe mit unterschiedlichen physiko-chemischen Eigenschaften können aufgrund von Verteilungsprozessen zwischen Sediment bzw. Schwebstoff und der Wasserphase für Wasserorganismen verfügbar werden und bilden damit ein ökotoxikologisches Gefährdungspotential. Während Flutereignissen besteht die Gefahr, dass belastete Sedimente remobilisiert und diese in Auengebiete eingetragen werden, somit die Besorgnis einer Gefährdung terrestrischer Schutzgüter besteht.

Das Ziel dieser Dissertation war daher die Beantwortung folgender Fragen:

1. Welche organischen Verbindungen sind in den Sedimenten und Schwebstoffen der Flüsse Saar und Rhein zu finden?
2. Wie sind diese räumlich und zeitlich verteilt und wie ist ihr historischer Rekord in Sedimentkernen?
3. Sind diese Verbindungen dauerhaft an die Feststoffe gebunden? Oder besteht die Möglichkeit der Desorption und damit Bioverfügbarkeit?
4. Welches Verfahren ist geeignet eine bestimmte Fraktion partikulär gebundener Schadstoffe zu extrahieren?
5. Wie ist das ökotoxikologische Gefährdungspotential der untersuchten Sediment, Schwebstoffe und Böden?

Der erste Teil der vorliegenden Arbeit befasst sich mit der chemischen Analyse und Identifizierung von organischen Verbindungen in Sedimenten und Schwebstoffen des Rheins und der Saar. An verschiedenen repräsentativen Standorten des Rheins und der Saar wurden Sedimentkerne mit einem Gefrierkernverfahren bzw. einem Kernstechverfahren gewonnen. An zwei Standorten in Koblenz (Rhein) und Güdingen (Saar) konnte mit einer radiometrischen Analyse instabiler Isotopen (^{210}Pb, ^{137}Cs) eine Bestimmung der Sedimentationsraten und des Sedimentalters durchgeführt werden. Diese beiden Kerne

sowie eine Zeitreihe von Schwebstoffmonatsmischproben von vier Standorten am Rhein (Weil am Rhein, Iffezheim, Koblenz, Bimmen) und zwei Standorten an der Saar (Güdingen, Rehlingen) aus dem Jahr 2005 wurden mit Gaschromatographie-Massenspektrometrie (GC-MS) quantitativ und qualitativ auf organische Verbindungen untersucht. Diese Studie wurde unter anderem durchgeführt, um die Datenlage hinsichtlich nichtregulierter organischer Substanzen in Sedimenten und Schwebstoffen der Saar und des Rheins zu verbessern. Weiterhin sollte die die Eintragsquellen identifiziert und das ökotoxikologische Potential dieser Verbindungen sowie deren räumliches und zeitliches Auftreten bzw. Verteilung untersucht werden. Die quantitative Analyse erbrachte erhöhte Konzentrationen von polyzyklischen aromatischen Kohlenwasserstoffen (PAH) und polychlorierten Biphenylen (PCB) in den Schwebstoffen der Saar und vom Standort Bimmen am Niederrhein, die dem Einfluss der Reviere an Saar und Ruhr zuzuschreiben sind. Die Analyse je eines datierten Sedimentkerns aus Rhein und Saar zeigte aber sinkende Konzentrationen seit den 1980er Jahren und damit die Wirksamkeit der unterschiedlichen Umweltschutzprogramme, wie beispielsweise das Abkommen zur Rheinhaltung des Rheins. Als Hauptkontaminant in den Schwebstoffen des Rheins wurde Hexachlorbenzol (HCB) bestätigt, das hauptsächlich von einer ehemaligen Chemiefabrik in Rheinfelden zur Produktion von Pentachlorphenol emittiert wurde. Die im Rahmen eines Suspekt- bzw. Nontargetscreenings identifizierten Substanzen aus anthropogenen, industriellen, technischen und natürlichen Quellen wurden in den meisten Sediment- und Schwebstoffproben gefunden. Die möglichen adversen Effekte dieser Verbindungen auf die aquatischen Ökosysteme bzw. humane Gesundheit wurden umfassend diskutiert. Als Nontarget-Verbindungen wurde das α-Tocopherolacetat (ein Derivat des Vitamin E) identifiziert, das als Marker für den Eintrag von kommunalen Kläranlagen gilt, da es in der Natur nicht vorkommt. Weiterhin wurden das Antioxidans Dioctyldiphenylamin sowie die als Wärmetauscher eingesetzten Verbindungen Methyldiphenylmethan und Methylbisdiphenylmethan gefunden. Die Identität dieser Verbindungen wurde über eine Suche in Massenspektrenbibliotheken (Wiley 9 und NIST08) und über eine Vorhersage der charakteristischen Massen im Massenspektrum durch virtuelle (*in silico*) Fraktionierung mit der Onlinesoftware MetFrag sowie über einen Vergleich mit publizierten Massenspektren bestätigt. Mit einer Kombination aus quantitativer Analyse sowie uni- und multivariater Statistik, wie beispielsweise Varianzanalyse und self-organizing maps, konnte ein umfangreiches Bild der regionalen und zeitlichen Verteilung bzw. Auftretens sowie möglicher Eintragsquellen nichtpolarer organischer Verbindungen in den Rhein und die Saar gezeichnet werden.

Der zweite Teil dieser Arbeit umfasste die Frage nach der Extrahierbarkeit und potentiellen Toxizität partikulär gebundener organischer Verbindungen. Sedimentproben wurden mit unterschiedlichen erschöpfenden und nichterschöpfenden Extraktionsverfahren (Soxhlet-, Ultraschall- und Beschleunigte Lösungsmittelextraktion (ASE), Membran-Dialyse-Extraktion (MDE) sowie Extraktion mit dem Polymer TENAX®-TA und Methanol- bzw. 2-Hydroxypropyl-β-Cyclodextrin-Wassergemischen). Die Extrakte wurden mit GC-MS auf verschiedene Gruppen organischer Verbindungen untersucht (z.B. polyzyklische aromatische Kohlenwasserstoffe und Chlororganika) sowie in unterschiedlichen Biotests auf ihre Wirksamkeit getestet (i.e. 7-Ethoxyresorufin-O-deethylase-Test auf dioxinähnliche Wirkung (EROD), Neutralrot-Test auf Zelltoxizität, Fischembryotest). Das Ziel dieser Untersuchung war es unter der Anwendung chemischer und bioanalytischer Methoden neue Erkenntnisse zum Zusammenhang zwischen Extrahierbarkeit, Stoff- und Sedimenteigenschaften und resultierender Sedimenttoxizität zu erlangen. Die Ergebnisse erbrachten eine ähnliche und teils höhere Extraktionsleistung des MDE-Verfahren verglichen mit den Soxhlet- und ASE-Extraktionsverfahren hinsichtlich der analysierten chemischen Verbindungen. MDE ist damit ein validiertes Verfahren zur erschöpfenden Extraktion hydrophober und nichtpolarer Verbindungen aus Sedimenten, Schwebstoffen und Böden. Die ASE-, MDE- und Soxhlet-Extrakte zeigten ähnliche zytotoxische und Dioxin-ähnliche Effekte, aber nicht im Fischeitest mit einer erhöhten Wirksamkeit der Soxhletextrakte. Die Extraktion mit Cyclodextrin und TENAX®-TA zeigten stark variierende Resultate. Aus den Ergebnissen wurden Empfehlungen für eine umfassende Risikobewertung von Sedimenten unter der Berücksichtigung von Verfahren zur Bestimmung des bioverfügbaren Anteils abgeleitet sowie weiterer Forschungsbedarf aufgezeigt.

Der dritte Teil dieser Abhandlung untersucht potentielle adverse Effekte des Eintrages von Schwebstoffen und remobilierten Sedimenten des Rheins in den geplanten Hochwasserretentionsraum Bellenkopf-Rappenwört (Karlsruhe, Deutschland) auf die dortigen Böden. In einer Pilotstudie wurden native Boden- und Schwebstoffproben aus dem Untersuchungsgebiet mit Sedimentkontakttests (Fischeitest mit *Danio rerio* und Dehydrogenasetest mit *Arthrobacter globiformis*) sowie acetonische Extrakte in Zelltests (Neutralrottest und EROD-Test) untersucht. Ergänzend wurden die Proben auf Schwermetalle und organische Verbindungen (PAHs, PCBs, Hexachlorbenzol (HCB) und polychlorierte Dibenzodioxine/-furane) sowie Korngrößenverteilung und Gehalt an organischem Kohlenstoff analysiert. Hinsichtlich der Sedimentkontakttests und der

Expositionspfade (natives Sediment versus acetonischer Extrakt) ergaben sich keine eindeutigen Unterschiede in den Effekten. Acetonische Extrakte führten hier also nicht notwendigerweise zu einer Überschätzung des Gefährdungspotentials der Proben. Desweiteren konnten Effekte im EROD-Test durch die analysierten prioritären Verbindungen nur zu 8% erklärt werden. In einer vertiefenden Studie wurden über einen längeren Zeitraum Schwebstoffe des Rheins bei Iffezheim beprobt sowie weitere Bodenproben aus den häufig und seltenen überfluteten Bereichen der Aue bei Bellenkopf-Rappenwört gewonnen. Nach einer Voruntersuchung ausgewählte Proben wurden einer wirkungsorientierten Analytik unterzogen um biologische Effekte *in vitro* zu untersuchen sowie für die Effekte verantwortlichen Substanzklassen bzw. Verbindungen zu identifizieren. Die Proben wurden automatisiert nach ihrer Aromazität, Planarität und Polarität fraktioniert und die Unterfraktionen mit dem EROD-Test auf Dioxin-ähnliche Wirksamkeit und dem Ames Fluktuationstest auf Mutagenität getestet. In den Biotests wirksame Fraktionen wurden chemisch-analytisch mit GC-MS auf PAHs, PCBs und HCB untersucht. Die Proben zeigten insgesamt keine Mutagenität. Im EROD-Test konnte nur 1% der Toxizität durch die gemessenen PAHs bestätigt werden. Wie sich schon in der Pilotstudie gezeigt hat, ist die alleinige Analyse von prioritären Verbindungen nicht geeignet reale Ursache-Wirkungsbeziehungen herzustellen und verantwortliche toxische Substanzen zu identifizieren. Ein weiteres Ziel dieser Studie war die Untersuchung potentiell adverser Effekte partikelgebundener toxischer Verbindungen auf Auengebiete. Für regelmäßig überflutete Auenbereiche konnte eine potentielle Gefährdung durch eingetragen Schwebstoffe hinsichtlich der untersuchten biologischen Endpunkte EROD-Induktion und Mutagenität sowie Fischei- und Bakterientoxizität nachgewiesen werden. In diesen Bereichen findet weiterhin eine Akkumulation von organischen Verbindungen wie PAHs, HCB und PCBs statt. Im nichtüberfluteten Areal hinter dem Deich wurden nur in Bodenproben aus dem Bereich einer Senke erhöhte Effekte gefunden.

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α-TA	α-tocopherol acetate
α-TP	α-tocopherol
AhR	aryl hydrocarbon receptor
AMAC	accelerated membrane assisted clean-up
ANOVA	one-way analysis of variance
Ant	anthracene
ASE	accelerated solvent extraction
BaA	benzo[a]anthracene
Bgi	benzo[ghi]perylene
Bio-TEQ	biological toxicity equivalent
BMU	best-matching units
Chem-TEQ	chemical toxicity equivalent
Chry	chrysene
DAMAC	direct accelerated membrane-assisted clean-up
DBI	Davies-Bouldin validity index
DCM	dichloromethane
DMSO	dimethylsulfoxide
DODPA	dioctyldiphenylamine
dw	dry weight
EDA	effect-directed analysis
EROD	7-ethoxyresorufin-O-deethylase induction assay
Flu	fluoranthene
FNU	turbidity
GC-MS	gas chromatography – mass spectrometry
GPC	gel permeation chromatography
HBCD	2-hydroxypropyl-ß-cyclodextrin
HCA	hierarchical cluster analysis
HCB	hexachlorobenzene
Ind	indeno[1,2,3-cd]perylene
K20	grain size fraction <20 µm
K200	grain size fraction <200 µm
K63	grain size fraction <63 µm
K630	grain size fraction <630 µm
LAB	linear alkyl benzenes
LOD	limit of detection

LOQ	limit of quantification
MAE	microwave-assisted extraction
MBDPM	methylbisdiphenylmethane
MDE	membrane dialysis extraction
MDPM	methyldiphenylmethane
MOA	mode of toxic action
NADPH	nicotinamide adenine dinucleotide phosphate
NOEC	no effect concentration
NR	neutral red retention assay
PAH	polycyclic aromatic hydrocarbons
PAC	polycyclic aromatic compounds
PCA	principal components analysis
PCB	polychlorinated biphenyls
PCM	polycyclic musk compounds
PCT	polychlorinated terphenyls
P-gp	P-glycoprotein
Phen	phenanthrene
PLE	pressurized liquid extraction
PNA	N-phenylnaphthylamine
PTFE	polytetrafluorethylene
Pyr	pyrene
Q	discharge
SB	sedimentation box
SEQ	sediment equivalent
SIM	single ion monitoring
SOM	self-organizing map
SOX	Soxhlet extraction
SPM	suspended particulate matter
TC	total carbon
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TIC	total inorganic carbon
TOC	total organic carbon
USE	ultrasonic-assisted extraction
WFD	European Water Framework Directive
WWTP	wastewater treatment plant

1 Introduction

1.1 Objectives

The objectives of this thesis were:

1. Which organic compounds are present in sediments and suspended particulate matter of the Saar and the Rhine?
2. How is the spatial and temporal distribution of these substances and their historical record in sediment cores?
3. Are the particularly bound compounds permanently sequestered in the sediments and suspended particulate matter? Alternatively, is there a possibility of desorption and bioaccessibility of these compounds?
4. Which extraction approach is appropriate to map a certain fraction of particularly bound substances?
5. How is the ecotoxicological impact of the sediments, soils and suspended particulate matter investigated in this study?

1.2 Synopsis of the remaining chapters and sections

The main scientific outcomes in relation to the general objectives of this thesis and the approximate share of the author's scientific contributions were as follows:

The first chapter **«Screening and identification of organic compounds in river sediments and suspended particulate matter»** (p. 7) containing section 2 deals with the question which particulate bound organic compounds can be found in sediments and suspended matter (SPM) of the Saar and the Rhine. The sediment and SPM samples from different representive sampling locations were analysed using gas chromatography – mass spectrometry and evaluated by combined target analysis as well as suspect and nontarget screening. This is the first study that provides an overview on a broad range of solid phase bound organic compounds in SPM of the Rhine and especially of the Saar. The role of the anthropogenic compound α-tocopherol acetate (α-TA) as a marker for municipal wastewater discharge in SPM and sediments was discussed comprehensively. The innovative *in silico* fragmentation program MetFrag was used to confirm the tentative identification of α-TA, dioctyldiphenylamine, methyldiphenylmethane and methylbisdiphenylmethane as only rare reported anthropogenic marker compounds. The combination of multitarget analysis with

univariate and multvariate statistical methods such as analysis of variance and self-organizing maps helped to draw a vital picture of the regional distribution and possible sources of nonpolar organic pollutants in the Rhine and the Saar catchments.

As the sole author of this section, I was responsible for the whole chapter. This included field sampling, gas chromatography – mass spectrometry (GC-MS) analysis, data evaluation and statistical analysis as well as design and preparation of the manuscript. The radiometrical analysis of sediment core samples and interpretation was performed by Prof. Augusto Mangini and Dr. Clemens Woda (Heidelberg Academy of Science, Germany). This section was kindly reviewed by Dr. Christa Schröter-Kermani (Umweltbundesamt, Berlin, Germany) and Dr. Martin Krauss (UFZ Leipzig, Germany).

The second chapter **«On the extractability and effect potentials of organic compounds in river sediments»** containing section 3 and 4 covers the questions of extractability and potential toxicity of organic compounds bound to river sediments. This chapter gives answers to the questions, which fractions of particularly bound organic compounds are extractable using different extraction approaches and how the extractability influences the toxicological potential of river sediments.

The study presented in section 3 **«Comparison of different exhaustive and one biomimetic extraction techniques for chemical and biological analysis of polycyclic aromatic compounds in river sediments»**[1] (p. 58) investigated the extractability and potential toxicity of river sediments of the Saar with procedures representing either partitioning based, nondepletive or virgorous extraction methods. An innovative aspect of this paper is the comparison of the index of confirmation quality (ICQ) approach with the biological and chemical toxicity equivalent approaches. Additionaly, in this paper sediments of the Saar were characterized for the first time for their toxicological effect potential within a scientific context.

As the first author, I was primarily responsible for experimental design, main parts of the field and laboratory work (e.g., sampling, sample preparation, extraction, fractionation, analysis for black carbon), GC-MS analysis, data evaluation, statistical analysis and manuscript

[1] **Schulze, T.**; Seiler, T.-B.; Streck, G.; Braunbeck, T.; Hollert, H.: Comparison of different exhaustive and one biomimetic extraction techniques for chemical and biological analysis of polycyclic aromatic compounds in river sediments; J Soils and Sediments 12, 1419-1434 (DOI: 10.1007/s11368-012-0574-1)

preparation. The second author contributed the bioanalytical results and the third author organized the preparation of the artifical extracts to estimate the ICQ. All (co-)authors were involved in discussion of the results and improvement of the manuscript.

In section 4 **«Excursus: On the comparability of procedures for sediment extraction in environmental assessment»**[2] [3] (p. 84) are shown the results of an «excursus» study regarding the comprehensive investigation of the extraction power, repeatability and applicability of five exhaustive and three nondepletive extraction methods in combination with biotesting to reach out the extractability and remaining effect potential. This study was not performed using sediments from the Rhine catchment but from the the Elbe catchment. However, it was a continuation of the investigations presented in section 3 and thus included in this thesis. In this paper the recently developed membrane dialysis extraction (MDE) and accellerated membrane-assisted clean-up (AMAC) procedures were compared with standard procedures such as ultrasonic and Soxhlet extraction as well as clean-up using gel permeation chromatography (GPC) for the first time. An innovative aspect of this study was the comprehensive ranking of the different approaches in order of the extraction power and related effect potentials from a set of bioassays using multivariate statistics.

The main authors of this section, Thomas-B. Seiler, Georg Streck and Tobias Schulze were equally involved in the experimental design, data evaluation and manuscript preparation. Furthermore, I performed different extraction steps (Soxhlet, ultrasonic, methanolic and HCBD) and extract preparation. Thomas-B. Seiler contributed the biotest results and MDE extraction. Georg Streck performed the sediment sampling as well as AMAC, GPC and GC-MS analysis. Katrin Schwab did the TENAX and ASE extractions. All (co-)authors were involved in discussion of the results and improvement of the manuscript.

[2] This section is a synopsis of two manuscripts that are in preparation for submission to «Science of the Total Environment»: Seiler, T.-B.; Streck, G.; **Schulze, T.**; Schwab, K.; Brack, W.; Braunbeck, T.; Hollert, H.: On the comparability of procedures for sediment extraction in environmental assessment. Part A: Bioanalytical investigations / Streck, G.; **Schulze, T.**; Seiler, T.-B.; Schwab, K.; Brack, W.; Braunbeck, T.; Hollert, H.: On the comparability of procedures for sediment extraction in environmental assessment. Part B: Chemical analytical investigations

[3] The underlying manuscripts are part of the PhD thesis of Dr. Thomas-Benjamin Seiler (2010): Total or biomimetic extracts or direct contact exposure? Comparative research towards a realistic ecotoxicological characterisation of sediments; PhD thesis, Ruperto Carola University of Heidelberg; Heidelberg; 340 pp

The third chapter **«Effect potentials and risk assessment of particle-bound contaminants in floodplain soils and suspended particulate matter in the Rhine catchment»** (p. 113) including the sections 5 to 7 examines the question of the potential ecotoxicological impact of (resuspended) sediments and SPM in the Rhine catchment.

The Section 5 **«Risk assessment of river suspended particulate matter and floodplain soils in the Rhine catchment using chemical analysis and in vitro bioassays»**[4] [5] [6] [7] (p. 114) focuses on the assessment of the hazardous potentials of contaminated suspended particulate mattern and remobilized sediments that might pose a risk for floodplain soils using *in vitro* bioassays and chemical analysis. A main aspect was the testing of acetonic extracts and native samples in the sediment contact tests (fish egg test with *Danio rerio* and bacteria contact test with *Arthrobacter globiformis*) to investigate different exposure scenarios at two trophic levels. Additionally, this investigation has unraveled adverse effects of settling suspended particulate matter and remobilized sediments to floodplain soils.

As the author of this section, I developed the manuscript template and prepared the whole manuscript. The biotest results – provided by Markus Ulrich and Volker Garke – were re-analysed using a consistent concentration-response-model. I did an evaluation of the complete biological and chemical dataset, performed statistical analysis and data interpretation. Furthermore, I analyzed the samples for organic compounds using GC-MS analysis and I was responsible to supervise inorganic analysis. This section was kindly reviewed by Dr. Emma Schymanski (Eawag, Dübendorf, Switzerland).

[4] Parts of this section were published as: Ulrich, M; **Schulze, T.**; Leist, E.; Glaß, B.; Maier, M.; Maier, D.; Braunbeck T.; Hollert, H. (2002) Abschätzung des Gefährdungspotenzials für Trinkwasser und Korrelation verschiedener Expositionspfade (Acetonischer Extrakt, Natives Sediment) im Bakterienkontakttest und Fischeitest; Umweltwissenschaften und Schadstoff-Forschung 14, 132-137 (DOI: 10.1065/uwsf2002.07.036)

[5] This section contains data that was elaborated during the diploma thesis of Dr. Markus Ulrich (2002): Gefährdung von Trinkwasser durch partikulär gebundene Schadstoffe in den Rheinauen: Vergleich nativer und extrahierter Proben in zwei in vitro Biotests; Diplomarbeit, Ruprecht-Karls-Universität; Heidelberg; 78

[6] This section contains data that was elaborated the during the state examination thesis of Volker Garke (2003): Optimierung und Anpassung eines in-vitro Bioassays mit RTL-W1- und RTG-2-Zellen zum Nachweis der cytoxischen und Dioxin-ähnlichen Wirkung von komplexen Umweltproben; Staatsexamensarbeit, Ruprecht-Karls-Universität; Heidelberg; 62 pp

[7] This section is in preparation for submission to «Environmental Science and Pollution Research»; Schulze, T.; Ulrich, M.; Garke, V.; Maier, M.; Terytze, K.; Braunbeck, T.; Hollert H.: Risk assessment of river suspended particulate matter and floodplain soils in the Rhine catchment using chemical analysis and in vitro bioassays

In section 6 **«Impact of contaminants bound to suspended particulate matter in the context of flood events»**[8] [9] (p. 141) the contamination of SPM sampled long-term and with higher frequency during a flood event at the Rhine to determine *in vitro* biological effects as well as to identify and quantify organic compound classes and effective contaminants was examined using effect-directed analysis (EDA). The innovative aspect of this study was the extension of an existing biotest battery with the Ames fluctuation assay and the bacterial tester strains TA98 and TA100 (*Salmonella* typhimurium). Furthermore, the study revealed that priority pollutants such as polycyclic aromatic compounds and polychlorinated biphenyls were only partly responsible for the effect potential rather than unknown more polar compounds.

As the third author, I was responsible to assist the first author performing the automated fractionation procedure and to carry out the GC-MS analysis of PAHs. I discussed the results with the first author, interpreted the chemical data and I was involved in manuscript preparation and improvement. All (co-)authors were involved in discussion of the results and improvement of the manuscript.

Section 7 **«Investigation on soil contamination at recently inundated and noninundated sites»**[9] [10] (p. 160) focuses on the assessment of hazardous potentials of particle-bound contaminations in the Rhine to flood retention areas close to a drinking water plant in the context of flood events. The innovation of this study was the extension of the fractionation procedure specified in section 6 for the EDA of soil samples for the first time. A main outcome was the exceedance of legal thresholds in the soil samples regarding priority pollutants and thus raising concerns of further deposition of particle-bound contaminants during flood events posing a risk to the drinking water plant even by more polar compounds.

[8] Wölz, J.; Fleig, M.; **Schulze, T.**; Maletz, S.; Lübcke-von Varel, U.; Reifferscheid, G.; Kühlers, D.; Braunbeck, T.; Brack, W.; Hollert, H. (2010) Impact of contaminants bound to suspended particulate matter in the context of flood events; Journal of Soils and Sediments 10, 1174-1185 (DOI: 10.1007/s11368-010-0262-y)

[9] This section is part of the PhD thesis of Dr. Jan Wölz (2009): Impact of contaminants on aquatic systems and inundated sites with respect to flood events: *In vitro* biotests, chemical target analysis and fractionation methods; PhD thesis, Ruperto Carola University of Heidelberg; Heidelberg; 194 pp

[10] Wölz, J.; **Schulze, T.**; Lübcke-von Varel, U.; Fleig, M.; Reifferscheid, G.; Brack, W.; Kühlers, D.; Braunbeck, T.; Hollert, H. Investigation on soil contamination at recently inundated and non-inundated sites; Journal of Soils and Sediments 11, 82–92 (DOI: 10.1007/s11368-010-0267-6)

As the second author, I was responsible to assist the first author performing the automated fractionation procedure. I contributed the whole GC-MS analysis and interpretation of chemical results and I was involved in manuscript preparation and improvement. All (co-)authors were involved in discussion of the results and improvement of the manuscript.

Chapter A

Screening and identification of organic compounds in river sediments and suspended particulate matter

2 Screening and identification of organic compounds in sediments and suspended particulate matter of the Saar and the Rhine [11]

2.1 Introduction

In the last two decades, different studies regarding suspect and nontarget screening of river sediments and suspended matter in German rivers were reported. In these investigations, several German river systems and estuaries have been covered. Examples are the Elbe and its tributaries (Franke et al. 1995, Franke et al. 1998, Ricking et al. 2003a, Schwarzbauer 1997, Schwarzbauer and Franke 2003, Schwarzbauer et al. 1999, Schwarzbauer et al. 2000), the Lippe (Dsikowitzky et al. 2004a, Dsikowitzky et al. 2004b, Heim et al. 2003, Heim et al. 2004, Kronimus et al. 2004) and the Lower Rhine (Heim et al. 2006). However, the Upper and Middle Rhine as well as the Saar were not investigated so far. Additionally, in the most cases, sediment grab samples or sediment cores were analyzed and thus only scarce information concerning spatial and seasonal differences of suspended particulate matter (SPM) contamination is available.

Sediments and suspended particulate matter are important structural and functional elements of fluvial ecosystems (Ricking et al. 2005, Schulze et al. 2005a). Sediments and SPM provide goods and services for aquatic ecosystems such as sink, accumulation compartment, carrier and source of contaminants and nutrients as well as habitats for benthic aquatic organisms. Origin and amount as well as chemical and biological composition thereof vary between different rivers and river stretches depending on catchment characteristics (e.g., availability of nutrients, climatic conditions, geology, hydrology, land use, urbanization, and state of wastewater treatment technology; ISO 5667-17, LAWA1999, Ricking et al. 2005, Schulze et al. 2005a, Schwoerbel 1993). Furthermore, SPM holds an important fraction of hydrophobic substances transport in water phase (Pohlert et al. 2011b). Thus, SPM was identified as an inherent part of a risk based sediment management that accounts for the probability of contaminant emissions and their potential impact on the environment and sediments (Brils 2008). In this context, sediments were defined as secondary sources of contaminants and SPM is the carrier of these contaminants (Power and Chapman 1992, Schulze et al. 2007a).

[11] This section is particularly based on the final reports of the research projects FKZ 301 02 013 and FKZ 301 02 018 of German Federal Environmental Agency (Schulze et al. 2005a, 2006)

The European Water Framework Directive (WFD) does not specifically deal with sediments and suspended solids. Nevertheless, 28 priority organic compounds and compounds groups (including, e.g., polycyclic aromatic hydrocarbons (PAH), brominated diphenyl ethers, nonylphenols, octylphenols, pentachlorophenol, organotin compounds, DDT, DDD, DDE, endosulfan, hexachlorobenzene, hexachlorocyclohexane, hexachlorobutadiene, pentachlorobenzene, dieldrin, isodrin, aldrin) were suggested for trend monitoring of sediments in the WFD context (Brils 2008). This subset of compounds may not reflect the complex contamination of sediments and SPM (Brack 2011, Schwarzbauer 1997).

In this context, suspect and nontarget screening is one approach for the identification of so far unknown or neglected compounds with a widespread environmental distribution or a high production volume. Unknowns are substances with so far not elucidated chemical structures and environmental properties such as biotic and abiotic transformations products. In combination with the anthropogenic marker concept (Eganhouse 1997, Takada et al. 1997), nontarget and suspect screening was confirmed as a powerful tool to identify and appoint different anthropogenic sources of environmental contamination with organic compounds (Dsikowitzky et al. 2011, Kronimus et al. 2004, Ricking et al. 2003a, Schwarzbauer 1997).

The anthropogenic marker concept classifies molecular markers in environmental samples in two main anthropogenic sources, (1) natural products released by human activities (e.g., coprostanol, caffeine) and (2) synthetic compounds dispensed by industrial processes, usage and waste disposal (e.g., linear alkylbenzenes, nonylphenols, linear alkylbenzene sulfonates), and in compounds with multiple sources (e.g., polycyclic aromatic hydrocarbons, polychlorinated biphenyls; Takada et al. 1997). The anthropogenic marker concept is part of the molecular marker concept that includes contemporary biogenic markers (i.e. compounds synthesized by biota), fossil biomarkers (i.e. altered or nonaltered substances present in fossil fuels and ancient sediments due to burial of biogenic organic matter), and anthropogenic markers (Eganhouse 1997).

The focus of this study was the screening and identification of suspected and so far not reported organic contaminants in sediments and suspended particulate matter of the Rhine and the Saar. Further aims were the identification of different anthropogenic sources (e.g., municipal, agricultural and industrial sources) using the anthropogenic molecular marker approach. Furthermore, the spatiotemporal appearance of these contaminants in SPM was investigated using self-organizing maps as an advanced statistical technique. The contamination with suspected or unknown organic compounds of sediments and suspended

particulate matter of the Saar and the Rhine was not reported comprehensively up to now. There was only one study on the source identification and risk assessment of polycyclic aromatic hydrocarbons in floodplain soils of the Saar (Pies 2009, Yang and Hofmann 2009, Yang et al. 2008). Heim et al. (2006) investigated one sediment core from Duisburg harbor geochronologically using the nontarget screening approach.

2.2 Materials und methods

2.2.1 Chemicals and solvents

Solvents for analysis were purchased from LGC Promochem (Wesel, Germany) and were of Picograde® quality. Isopropyl alcohol (technical grade) and sodium sulfate (for organic analysis) were obtained from Merck (Darmstadt, Germany). All analytical standards and other chemicals were bought from LGC Promochem (Wesel, Germany), Sigma-Aldrich (Steinheim, Germany) or Dr. Ehrenstorfer (Augsburg, Germany).

2.2.2 Sampling devices

2.2.2.1 Sediment corers

In this study were used two types of sediment corers: a dry ice freeze corer and a piston corer.

Dry ice freeze-corer

The dry ice freeze-coring device is a approach for the quick, low-cost and undisturbed sampling of soft and unconsolidated sediments (e.g., Gyttja, sapropel) within approximately 30 minutes (Ricking and Schulze 2003). The device is applicable from a boat or a small drilling platform (Figure 2-1). The device is an aluminium lance (length: 1780 mm, outer diameter 80 mm, wall thickness: 6 mm) with a stainless steel tip and a water tight screwing cap. The lance is filled with approximately 10 kg of dry ice pellets (Linde, Leuna, Germany) and 0.3 l of isopropyl alcohol, closed and lowered down to the sediment using a robe at beginning of sampling. The mixture of dry ice and alcohol is a so called freezing mixture with a temperature of -78 °C. The endothermic process of evaporation of the alcohol and the sublimation of the dry ice reduces the temperature in the sediment that freezes to the lance. The technique allows the sampling of the interface water-sediment due to freezing of the The produced carbon dioxide is released by a check valve from the lance. The core is obtained from the lance by filling hot water filled or blowing hot air into the lance.

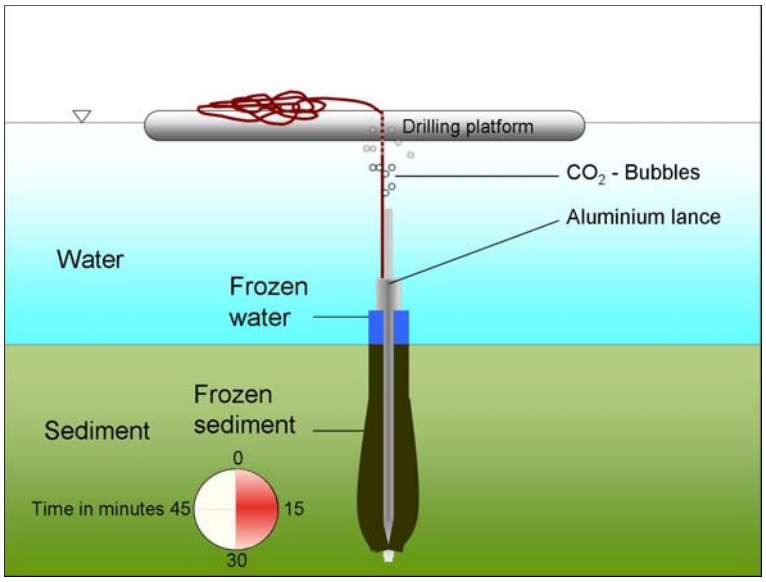


Figure 2-1 Scheme of the dry ice freeze-coring device (adapted from Schulze et al. 2005b)

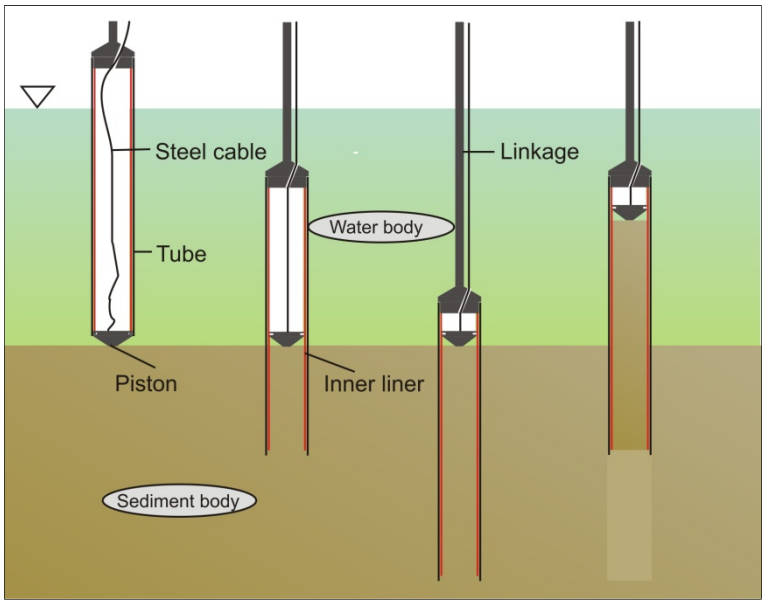


Figure 2-2 Scheme of the piston corer (adapted from Schulze et al. 2005b)

Piston corer

The piston corer device according to Merkt and Streif (Merkt and Streif 1970) in a modification of the Stitz company (Gehrden, Germany) is composed of a stainless steel tube (length: 1 meter, inner diameter: 50 mm) with a cutting head (Figure 2-2). The tube is equipped with a polyethylene tube liner for simple extraction and transport of the core. The corer is closed with a piston. Afterwards it is lowered down to the sediment using linkages from a drilling platform. If the corer is in top position of the core to be obtained, the piston is fixed using a steel cable and the corer is driven down in the sediment using human power, a sledgehammer or a motor hammer. A vacuum develops due to the piston and thus the core is fixed inside the liner to retrieve it without loss of sediments.

2.2.2.2 Suspended particulate matter

Suspended particulate matter was sampled using a mobile sedimentation box (SB; Figure 2-3). The sedimentation box is made of stainless steel (V4A; length x height x width: 400 x 300 x 250 mm; Hollert 2001, Schulze et al. 2007a). The SB can be (1) deployed directly in the water body, for example using a buoy or applied in a monitoring station plugged to the station's piping. The water flows into the SB and the water velocity is slowed down due to the blades such that the SPM deposits in the sedimentation basins.

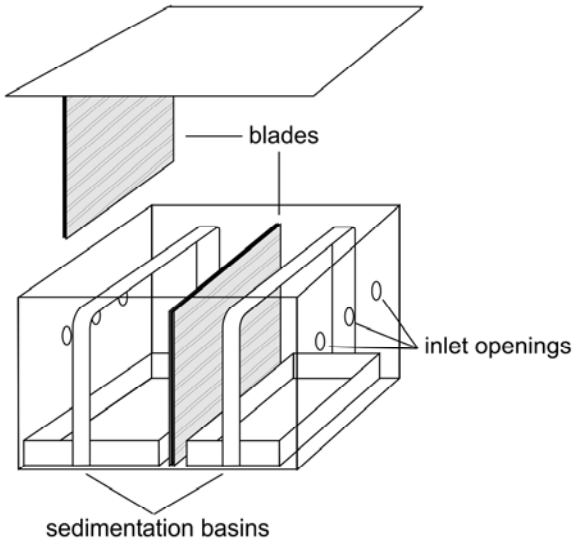


Figure 2-3 Mobile sedimentation box for sampling of suspended particulate matter (cf. Schulze et al. 2007a)

2.2.3 Sampling sites and samples

The sediment and SPM samples were collected at sampling sites of the German Environmental Specimen Bank at the Rhine and the Saar (Figure S2–1 in Appendix; Schulze et al. 2007a, UBA 2012). Table 2-1 lists the sampling dates of sediment cores and collection periods of SPM samples. The sediment cores were sampled using the device described above. After sampling, they were wrapped with alumina foil (Melitta, Minden, Germany) and kept frozen at -18 °C during transport and storage in laboratory. The SPM samples were collected using SBs. They were sieved using an analytical stainless steel sieve with round holes (<2 mm; Retsch GmbH, Haan, Germany) and frozen after collection using a tailor-made special nitrogen freezing device (Schulze et al. 2007a). The frozen SPM was stored in stainless steel containers for transport in the gas phase of liquid nitrogen (-160 °C) using a nitrogen vapor freezer (Chronos 200, Chryotherm, Euskirchen, Germany). In the laboratory, the samples were freeze-dried (Christ, Osterode, Germany) according to DIN 38414-22 and stored in brown glass jars at -18 °C until analysis.

2.2.4 Sample extraction and analysis

2.2.4.1 Ultrasonic assisted extraction of sediment and SPM samples

Freeze dried and sieved (<2 mm) sediment or SPM were were extracted in duplicate at 35 kHz (Sonorex Super RK 514, Bandelin, Berlin, Germany) for 15 min with 40 ml of *n*-hexane:acetone (1:1; v:v) after vortex mixing for 2 min and orbitally shaken (IKA, Stauffen, Germany) for 1 h at room temperature (Schulze et al. 2003, Schwarzbauer et al. 2003a). The solvent was removed by centrifugation at 2000 × G (Heraeus, Hanau, Germany). Resulting extracts were reduced using rotary evaporation at 40 °C and a gentle nitrogen stream to a volume of approximately 0.5 ml. The extract was treated with activated cooper granulate (Merck, Darmstadt, Germany), placed for 3 min in the ultrasonic bath and stored overnight at 4 °C to remove sulfur. The supernatant was removed using a pasteur pipette and the residual cooper was washed two time using *n*-hexane. The solvent was reduced to 0.5 ml using nitrogen.

Table 2-1 Sampling sites of sediment and suspended particulate matter (SPM) samples in the Rhine and the Saar catchments; SB = sedimentation box; SB-M = sedimentation box in monitoring station

Catchment area	Sampling site	Sampling site code	River km	Sediment sampling technique	Sampling date	SPM sampling technique	Sampling periods
Saar	Güdingen	S1	93	Piston corer	24.09.2002	SB	01/05 – 12/05 (monthly)
	Rehlingen	S2	54	-	-	SB	01/05 – 12/05 (monthly)
Rhine	Weil	R1	173	-	-	SB	01/05 – 12/05 (monthly)
	Iffezheim	R2	333	-	-	SB	01/05 – 12/05 (monthly)
	Koblenz	R3	590	Dry ice freeze corer	24.10.2002	SB-M	01/05 – 12/05 (monthly)
	Bimmen	R4	863	-	-	SB-M	01/05 – 12/05 (monthly)

Table 2-2 Silica gel fractionation procedure (with example compounds; PCB = polychlorinated biphenyls, DDE = 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene), LPAC = low-condensed polycyclic aromatic compounds, PAC = polycyclic aromatic compounds; DCM = dichloromethane)

Fraction	Volume (ml)	Solvent	Examples of eluents and compound classes
F1	5	*n*-pentane	Aliphatics, chlorobenzenes, biomarkers
F2	8.5	*n*-pentane/DCM (95/5; v/v)	monocyclic aromatics, PCB, DDE
F3	5.0	*n*-pentane/DCM (90/10; v/v)	dicyclic aromatics, PAC, DDE, PCB
F4	8.0	*n*-pentane/DCM (40/60; v/v)	PAC, organochlorine pesticides
F5	8.0	DCM	heterocycles, plasticizers, fatty acids, O- and N-PAC
F6	8.0	Methanol	polar compounds[a]

[a] as soluble in *n*-hexane

2.2.4.2 Fractionation

The extracts (in 0.5 ml of *n*-hexane) was chromatographically separated in six fractions using silica gel chromatography (Bundt et al. 1991, Franke et al. 1998, Heim et al. 2005). Briefly, the procudure was as follows. The silica gel was activated for 15 h at 200 °C. The solvents, mixing ratios and conditions used for fractionation are shown in Table 2-2. The resulting fractions were reduced to a volume of 200 µl using nitrogen and stored in brown glass vials until analysis at -20 °C.

2.2.4.3 Gas chromatography – mass spectrometry (GC-MS)

Gas chromatography – mass spectrometry (GC-MS) analysis was carried out with a HP 5890 II GC coupled to a HP MSD 5971 (Agilent, Palo Alto, USA), equipped with a 60 m × 0.25 mm I.D. × 0.25-µm film DB-XLB fused capillary silica column (Agilent J&W, Folsom, USA) and a splitless injector. Chromatographic conditions were as follows: 275–300 °C injector temperature, 1–3 µl injection at oven temperature of 60 °C (3 min isotherm), then programmed at 2.5 K/min to 310 °C (20 min isotherm). The velocity of carrier gas (helium 5.0, Air Liquide, Boehlen, Germany) was 1 ml/min at constant flow.

The MS was operated in electron ionization mode (EI, 70 eV) with a source temperature of 180 °C scanning from 50 to 550 amu (full scan mode; scan time: 1.5 sec) or single ion monitoring (SIM) for quantification. Target analytes were quantified using an external calibration in SIM mode. The limit of detection (LOD) was in the range of 0.1–2.9 ng/g dw (PAHs) and of 0.1–0.6 ng/g dw (PCBs) as well as of 0.1 ng/g dw (HCB). The limit of quantification was in the range of 0.2–14.8 ng/g dw (PAHs) and of 0.3–1.7 ng/g dw (PCBs) as well as 0.3 ng/g dw (HCB). The LOD and LOQ were defined as three times the signal-to-noise ratio (S/N) and as ten times the S/N of the analyte peak, respectively.

2.2.4.4 Computer tools for mass spectra analysis

For mass spectra interpretation the software Agilent Chemstation (Agilent, Palo Alto, USA), AMDIS (NIST 2005) and NIST MS Search V 2.0 (NIST 2008) were used. The qualitative mass spectra analysis was performed using a suspect list (including suspected organic contaminants and their characteristic masses as well as retention time information) and mass spectra library search (Wiley 4th, 7th and 9th Edition; NIST 05 / 08), published mass spectra and reference standards. The identity of nontarget compounds that were identified to be

present in different samples were confirmed using library search match values (>80% or >800) in MS Chemstation or NIST MS Search (Schymanski et al. 2011) and *in silico* fragmentation using the software MetFrag (Wolf et al. 2010). MetFrag was recently extended to apply for GC-MS fragmentation (Schymanski et al. 2012) according to the MOLGEN-MS approach (Kerber et al. 2006, Schymanski et al. 2009).

2.2.5 Radiometric dating of sediment cores

The sediment cores of the sites R3 (Koblenz) and S1 (Güdingen) were analyzed using γ-spectrometry for their activities regarding the instable isotopes ^{137}Cs, ^{210}Pb and ^{226}Ra in order to determine the sedimentation rates and to deduce sediment ages. Homogenized and freeze-dried subsamples of the sediment cores of each 10–15 g were stored for three weeks in airtight vessels for equilibration. The γ-emissions of ^{137}Cs, ^{210}Pb and ^{226}Ra were obtained at 662 keV, 46.5 keV and 352 keV, respectively, using a γ-spectrometer with low self-absorption capabilities (Bollhöfer et al. 1994). Correction of the self-absorption of low-energetic γ-spectra was performed according to Appleby et al. (1992). The supported ^{210}Pb activity was related to the ^{226}Ra activity. The unsupported ^{210}Pb activity was subtracted from total ^{210}Pb activity. The time markers «1963» (atom bomb tests) and «1986» (Chernobyl disaster) were estimated by using ^{137}Cs if present in the sediment profiles. Radiometric analysis was performed in the laboratory of Prof. Dr. Augusto Mangini (Heidelberg Academy of Sciences and Humanities, Research Institute for Radiometric Dating of Waters and Sediments).

2.2.6 Total organic carbon content

The analysis of total organic carbon (TOC) contents was performed according to section 3.2.4 (p. 63).

2.2.7 Data analysis

2.2.7.1 Significance testing

Significance testing was performed by one-way analysis of variance (ANOVA) followed by Tukey's posttest to compare each pair of parameters used for analysis using GraphPad Prism® 5.04 (GraphPad 2007). If Kolgomorov-Smirnov test showed inhomogeneity and/or Bartlett's test gave significant differences in variances, the nonparametric Kruskal-Wallis ANOVA followed by Dunn's posttest was used. The significance level was α=0.05.

2.2.7.2 Self-organizing maps

A self-organizing map (SOM) is an unsupervised artificial neural network model consisting of neurons organized on a regular low-dimensional grid that was introduced by Kohonen (1982). Each node is represented by a d-dimensional weight vector $m = [m_1, \dots, m_d]$, where d is equal to the dimension of the input vectors (Vesanto et al. 2000). SOMs are used to project multivariate data from a multi-dimensional array to an usually two-dimensional grid of hexagons each representing one node or map unit. It aims to unravel hidden neighborhood relations between input parameters by unbiased iterative training and organization of the input data. SOMs were confirmed as a convenient tool for the assessment of water quality parameters (Aguilera et al. 2001, Astel et al. 2008), of regional environmental quality elements (Tran et al. 2003) and of sediment quality (Alvarez-Guerra et al. 2008) as well as for the spatiotemporal classification of environmental monitoring data (Jin et al. 2011).

This approach does not utilize predefined association-dependent input values such as number of clusters in hierarchical cluster analysis (HCA) or principal components in principal components analysis (PCA) that may influence and thus bias the results. Furthermore, SOMs are able to visualize multivariate data comparable to HCA and PCA, but there are added advantages of SOMs (Astel et al. 2007): (1) SOM projects similarities variables and semi-quantitative information regarding their distribution between sampling locations, (2) similarities between positive and negative correlations are visualized, and (3) outliers who are not related to well-organized clusters are uncovered.

In each training step, a sample vector x from the input data set is chosen randomly and the distances between it and all weight vectors of the SOM are calculated for the determination of the best-matching unit (BMU), which is the neuron whose weight vector is closest to input vector x according to Vesanto et al. (2000):

$$\|x - m_c\| = \min_i\{\|x - m_i\|\} \quad (2-1)$$

where $\|\ldots\|$ is the distance measure, for example, Euclidian distance. In each training step, averaged weight vectors are calculated from input data vectors of unit i (Vesanto et al. 2000):

$$m_i(t+1) = m_i(t) + \alpha(t)N_{i^*i}(t)[x(t) - m_i(t)] \quad (2-2)$$

where t denotes time and $x(t)$ is an random input vector calculated from input data set at time t. The term $N_{j^*j}(t)$ expresses the neighborhood function of the BMU i^* at time t, i.e. the region of the greatest influence of the input sample on the SOM, and $\alpha(t)$ denotes the learning rate.

The most commonly used neighborhood function is the Gaussian (Kalteh et al. 2008, Vesanto et al. 2000):

$$N_{i^*i}(t) = e^{\frac{\|r_{i^*} - r_i\|^2}{2\sigma^2(r)}} \quad (2-3)$$

where $N_{j^*j}(t)$ is the neighborhood function of the BMU i^* at time t, $\sigma(t)$ denotes the neighborhood radius at time t, and $\|r_{i^*} - r_i\|$ expresses the distance between nodes i^* and i on the map grid. SOM Toolbox 2.0 for Matlab was used for calculation of the SOM (Vesanto et al. 2000).

First, as a prerequisite of Euclidian distance calculation, the input data was transformed using a logistic transformation. Different transformation algorithms were tested and logistic yielded best results regarding quantization error and map topological error (Table S2–2 in Appendix). Second, SOMs were trained using a sheet shaped grid of hexagons with the Gaussian neighborhood function (Equation 2–3) and visualized. The number of nodes (n) is generally dependent of the size of the dataset and determined using the formula: $n = 5 \times \sqrt{number\ of\ samples}$.

Clustering by *k*-means was used for the final classification of the nodes, where the optimal number of clusters was selected by the minimum Davies-Bouldin validity index (DBI; Davies and Bouldin 1979). If the number of clusters is known *a priori*, the *k*-means algorithm clusters

such that each element has a certain probability of being a member of any cluster (Pakhira et al. 2004).

The DBI is defined as (Davies and Bouldin 1979, Pakhira et al. 2004):

$$DBI = \frac{1}{N}\sum_{i=1}^{N} R_{i,kt} \quad (2-4)$$

where $R_{i,qt}$ is a cluster separation measure computed as:

$$R_{i,qt} = \max_{j,j\neq i}\left\{\frac{S_{i,k} + S_{j,k}}{M_{ij,t}}\right\} \quad (2-5)$$

$S_{i,q}$ is a dispersion measure of the points in cluster i and defined by kth root of the kth moment of the points in the ith cluster over their mean:

$$S_{i,k} = \left\{\frac{1}{T_i}\sum_{j=1}^{T_i} |x - z_i|^q\right\}^{1/q} \quad (2-6)$$

where T_i it the number of vectors in cluster i and z_i is its centroid. $M_{ij,t}$ it the Minkowski distance of the centroids of clusters i and j:

$$M_{ij,t} = \left\{\sum_{k=1}^{N} |a_{ki} - a_{kj}|^p\right\}^{1/p} \quad (2-7)$$

where a_{ki} denotes the kth element of the n-dimensional vector a_{kj}, which is the centroid of cluster i. A Matlab script for batch run of the different program parts was used (Script S2–1 in Appendix).

2.3 Results and discussion

2.3.1 Radiometric dating of sediment cores

Figure 2-4 and Figure 2-5 illustrate the results of γ-spectrometric analysis of sediment core sections of core R3 and core S1, respectively. In core R3 (Koblenz, Rhine), the concentration of excess ^{210}Pb (total ^{210}Pb - ^{226}Ra) decreased from 2.7 Bq/g to 0.5 Bq/g from top to bottom. A core age of ~45 years was deducible with average sedimentation rates of 1.95 cm/year in the upper sediment layer 0–35 cm and of 0.8 cm/year below due to a sediment focusing effect at 35 cm depth. The distribution of ^{137}Cs with a maximum activity at 35 cm sediment depth corresponding to the Chernobyl disaster confirmed the age and sediment rates. In core S1 (Güdingen, Saar), the concentration of excess ^{210}Pb declined from 3.3 Bq/g to 1.5 Bq/q from top to bottom. A core age of ~17 years was derivable with an average sedimentation rate of 3.45 cm/year. ^{137}Cs showed a maximum in the bottom sample maybe referring to the Chernobyl accident. However, the sediment core is clipped and thus this observation is tentatively. The interpretation of results is in regard to personal communication with Prof. Dr. Augusto Mangini (Heidelberg Academy of Science).

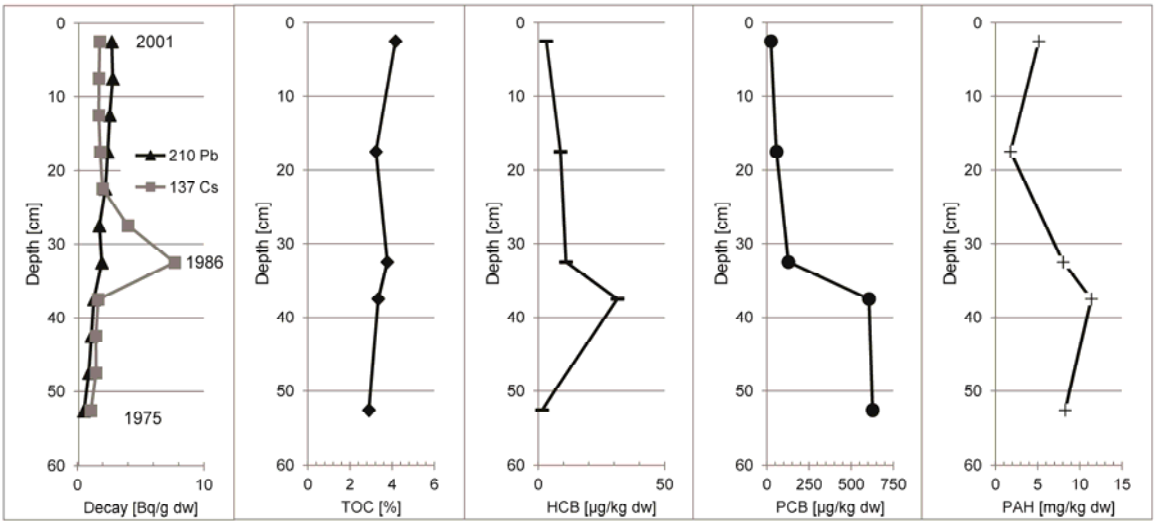


Figure 2-4 Results of radiometric dating of sediment core R3 (Koblenz, Rhine) as well as contents of total organic carbon (TOC,◆), hexachlorobenzene (HCB,_), polychlorinated biphenyls (PCB,●), and polyaromatic hydrocarbons (PAH,+); the data are given as mean depth (cm) of the sediment section, decay of excess ^{210}Pb (▲) and ^{137}Cs (■) as Bq/g dry weight (dw), TOC as percent, HCB and PCB as µg/kg dw, PAH as mg/kg dw (cf. Schulze et al. 2005b)

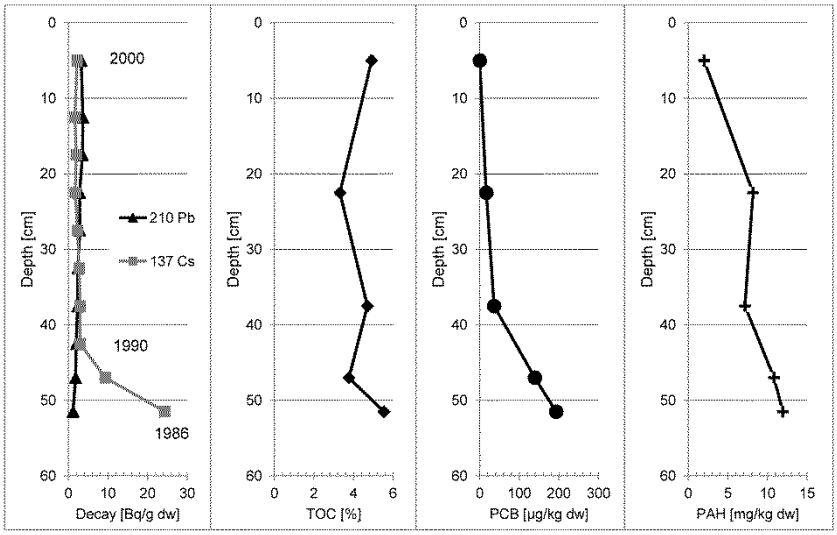


Figure 2-5 Results of radiometric dating of sediment core S1 (Güdingen, Saar) as well as contents of total organic carbon (TOC,◆), polychlorinated biphenyls (PCB,●), and polyaromatic hydrocarbons (PAH,+); the data are given as mean depth (cm) of the sediment section, decay of excess ^{210}Pb (▲) and ^{137}Cs (■) as Bq/g dry weight (dw), TOC as percent, PCB as µg/kg dw, and PAH as mg/kg dw (cf. Schulze et al. 2005b)

2.3.2 Total organic carbon in sediments and suspended particulate matter

2.3.2.1 Sediment cores

The contents of total organic carbon of 3.5% ± 0.4% in sediment core R3 was relatively constant over the whole sediment profile (Figure 2-4). The TOC values declined from top to bottom. The results of TOC in sediment core S1 of 4.4% ± 3.3% showed more variance (Table 2-3). Compared to the TOC contents of different sediments of German rivers (Schulze et al. 2007a), these findings were in the lower to average range.

2.3.2.2 Suspended particulate matter

The contents of total organic carbon in the SPM samples were in the range of 6.3–10.8% in the Saar samples and of 0.1–6.1% in the Rhine samples (Table 2-3). These findings were in the overall range of TOC levels found in German rivers SPM samples (Schulze et al. 2006).

Table 2-3 Contents of total organic carbon (TOC) in the sediment core and suspended particulate matter samples as well as the suspended particulate matter per litre in water at the sampling sites (data shown as mean with standard deviations; cf. Schulze et al. 2005b)

Sampling site code	Sediment core samples	SPM samples	
	TOC (%)	TOC (%)	SPM (mg/l)
S1	4.4 ± 3.2	7.6 ± 2.3	16.7 ± 21.5
S2	-	8.9 ± 1.1	15.8 ± 21.5
R1	-	3.9 ± 2.0	21.4 ± 23.2
R2	-	3.4 ± 1.3	12.6 ± 11.8
R3	3.5 ± 0.5	2.2 ± 0.7	52.6 ± 39.8
R4	-	3.7 ± 1.6	34.7 ± 24.6

2.3.3 PAHs, PCBs and HCB in sediments

2.3.3.1 Sediment core R1 from Koblenz (Rhine)

Figure 2-4 shows the results of target screening analysis of sediment core R3. The concentrations of hexachlorobenzene (HCB) increased from 2 µg/kg in the bottom sample representing the year 1975 to a maximum of 31 µg/kg at a sediment depth of 37.5 cm (~1984) and then decreased to 3 µg/kg in the top-level sample from year 1986 onwards.The concentrations of the polychlorinated biphenyls (PCB) according to Ballschmiter and Zell (1980) were at a high level of more than 600 µg/kg in the bottom layer, dropped down to a level of 128 µg/kg in 1986 and then decreased constantly to 24 µg/kg in the top layer. The levels of PAHs had also a maximum of 11 mg/kg in the sample from year ~1984, decreased to 2 mg/kg in the sample at 18.5 cm (year: ~1995) and then increased again to a value of 5 mg/kg. Maximum concentrations of those compounds occurred during the 1970s in the Rhine catchment (Japenga and Salomons 1993, Van Eck and De Rooij 1993). The water body and sediments of Rhine were highly polluted in the 1960s to 1980s. In the following years different international agreements for the protection of the Rhine (e.g., Berne Agreement on the International Commission for the Protection of the Rhine from 1963 and Convention for the protection of the Rhine against chemical pollution from 1976; ICBR 2012) were passed.

Furthermore, HCB and PCBs are listed as persistent organic pollutants and are worldwide banned by the Stockholm convention since 2001 (UNEP 2001). PCBs were restricted in open and closed systems from 1985 in the European Community (European Council 1985). Decreasing concentrations of regulated industrial chemicals show the success of the

restriction efforts (Heim 2005). PAHs are released by energy production as well as industrial and natural processes and thus the decline was not as clear as that of the PCBs and HCB.

2.3.3.2 Sediment core S1 from Güdingen (Saar)

In Figure 2-5 are shown the results of target screening analysis of sediment core S1. The contents of PCBs increased in depth from not detectable in the uppermost sample to a level of 193 µg/kg in the deepest sample (year: ~1986). The concentrations of the PAHs also declined from 12 mg/kg in the bottom sample to 2 mg/kg in the top sample. HCB and chlorinated compounds others than PCBs were not found in this sediment core. The concentrations of PAHs and PCBs in lower sediment layers were comparable to those levels found in SPM samples from 1990 upstream of the sampling site (Breitung and Bergmann 1993).

2.3.4 PAHs in suspended particulate matter

PAHs were found in ranges from 2 mg/kg to 9.5 mg/kg in the Saar samples and from 0.6 to 9.7 mg/kg in the Saar and the Rhine samples (Figure 2-6A). PAH concentrations in the Saar samples from sites S1 and S2 were significantly higher than in those of the Upper and the Middle Rhine samples (sites R1–R3; $p<0.05$; Kruskal-Wallis ANOVA with Dunn's posttest), but they were not differently from the Lower Rhine sample R4. The elevated PAH contents in the Saar samples could be assigned to former and recent coal mining activities and burning of coal for power generation in the Saarland coal mining and steel production district (Pies et al. 2007, RAG 2012).

The concentrations of PAHs in the SPM samples from site R4 were significantly different ($p<0.05$; Kruskal-Wallis ANOVA with Dunn's posttest) from the other sampling sites of the Rhine (R1–R3), but similarly to those of the Saar samples. The higher contents of PAHs at site R4 might be explainable by an important contribution of PAHs contaminated SPM from the Ruhr, the Emscher and the Lippe. These rivers drain the Ruhr coal mining and steel district and flow into the Rhine between 50 and 85 kilometers upstream from site R4. Heim et al. (2006, 2003) found total PAHs concentrations of 13 mg/kg and more than 1 mg/kg in top sediment layers of the Lippe (near Wesel, Germany) and the Rhine (Duisburg harbor), respectively.

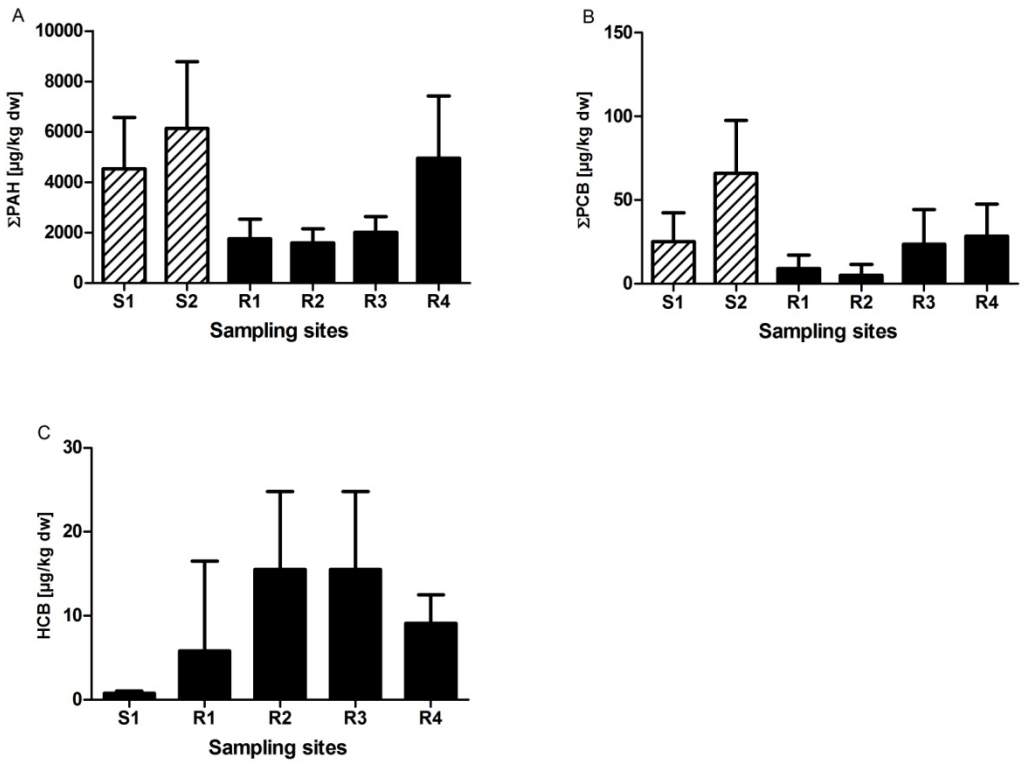


Figure 2-6 Concentrations of (A) PAHs, (B) PCBs and (C) HCB in suspended particulate matter samples of year 2005; Saar (pin striped), S1: Güdingen, S2: Rehlingen; Rhine (solid black), R1: Weil, R2: Iffezheim, R3: Koblenz, R4: Bimmen; the data are given in mean concentrations and standard deviations in µg/kg dry weight (dw)

2.3.5 Source appointment of PAHs

Comparison of ratios of different PAHs is a frequently used tool for the source appointment of PAHs (Budzinski et al. 1997, Lake et al. 1979, Lipiatou and Saliot 1991, Yunker et al. 2002). The definition of PAH ratios is based on the interpretation of different stabilities of PAHs on different types of combustion materials in order to relate a certain PAH signature to petrogenic or combustion sources (Yunker et al. 1996).

For the differentiation between combustion (i.e. coal, petroleum, wood, grass) and petroleum sources the parent PAHs of molecular mass 178 (phenanthrene, Phen; anthracene, Ant), 202 (fluoranthene, Flu; pyrene, Pyr), 228 (benzo[a]anthracene, BaA; chrysene, Chry) and 278 (benzo[ghi]perylene, Bghi, indeno[1,2,3-cd]perylene, Ind) were used in this study (Table 2-4; Yunker et al. 2002). A comprehensive overview and interpretation of PAHs' ratios is presented in Yunker et al. (2002).

The values of the ratio Flu/(Flu + Pyr) were in the range of 0.2–0.8, those of the ratio Ant/(Ant + Phen) in the range of 0.09–0.7, those of the ratio BaA/(BaA + Chry) in the range of 0.1–0.8, and those of the ratio Ind/(Ind + Bghi) in the range of 0.38–0.52 (Figure 2-7 A-C).

Most of the PAHs in the investigated samples originated from combustion sources, mainly from coal, wood or grass combustion (location R4, R3, R2, particularly R1, S1, S2), but in some cases also from combustion of petroleum (R1, R2, R3, S1, S2). There was no clear evidence if PAHs in some samples from Saar (S1, S2) originated from petroleum or not. Some samples with Flu/(Flu+Pyr) ratios below 0.4 (Figure 2-7 A-C) may induce petroleum influence, but the other PAH ratios point to combustion sources.

Ratios of substituted PAHs such as the ratio methylphenanthrene to phenanthrene and *n*-alkanes ratios could give more evidence to petroleum sources (Pies 2009), but substituted PAHs and *n*-alkanes were not quantified in this study.

Table 2-4 Ratios of different parent PAHs and threshold values for source appointment of PAHs; Ant: anthracene, Phen: phenanthrene, Fluo: fluoranthene, Pyr: pyrene, BaA: benzo[a]anthracene, Chry: chrysene, Ind: indeno[1,2,3-cd]perylene, Bghi: benzo[ghi]perylene (Yunker et al. 2002)

Ratio	**Threshold values for the interpretation of sources**	
$\frac{Ant}{(Ant + Phen)}$	<0.1	petroleum
	>0.1	combustion
$\frac{Flu}{(Flu + Pyr)}$	<0.4	petroleum
	0.4 – 0.5	petroleum combustion
	>0.5	grass/wood/coal combustion
$\frac{BaA}{(BaA + Chry)}$	<0.2	petroleum
	0.2 – 0.35	mixed sources
	>0.35	combustion
$\frac{Ind}{(Ind + Bghi)}$	<0.2	petroleum
	0.2 – 0.5	petroleum combustion
	>0.5	grass/wood/coal combustion

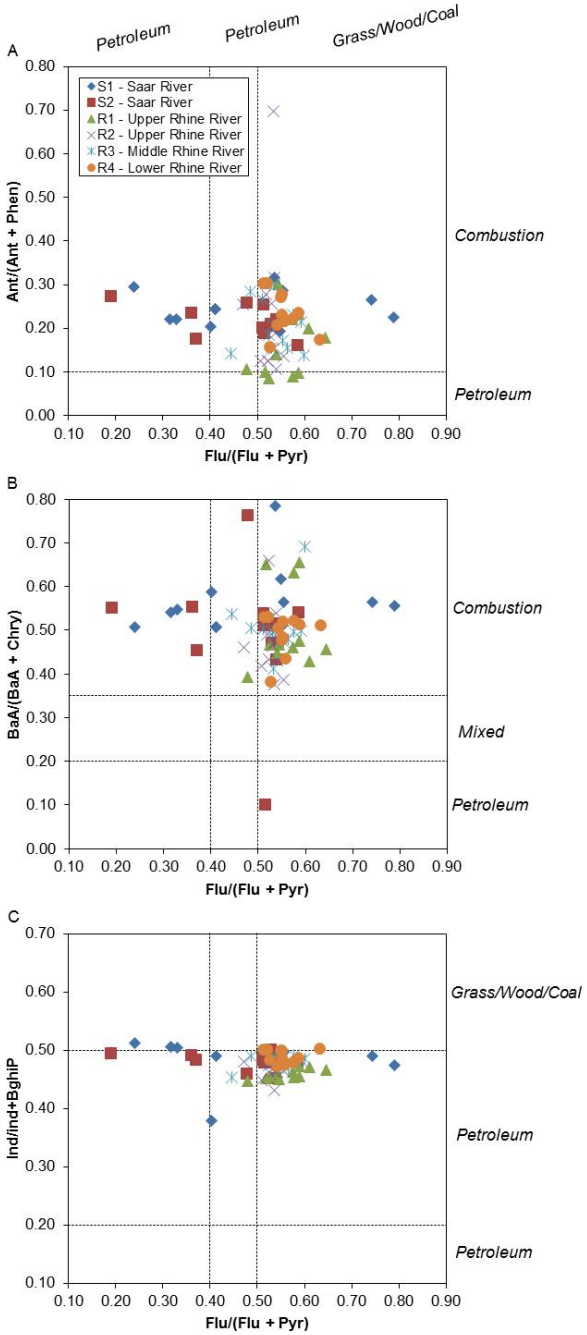


Figure 2-7 PAH scatter plots for the ratios of (A) Ant/(Ant + Phen) vs. Flu/(Flu + Pyr), (B) BaA/(BaA + Chry) vs. Flu/(Flu + Pyr) and (C) Ind/(Ind + Bghi) vs. Flu/(Flu + Pyr) in suspended particulate matter samples of year 2005 according to Yunker et al. (2002)

2.3.6 PCBs in suspended particulate matter

The total PCB levels in the Saar samples reached values between 11 µg/kg and 114 µg/kg and in the Rhine samples of below detection limits to 83 µg/kg (Figure 2-6B). The PCB concentrations found in the Saar sample S1 and the samples from Rhine (R1 to R4) were in the ranges of other recently investigated sediment sites (Hilscherova et al. 2010). The maximum concentrations in one sample from site R4 and the most samples of site S2 were above the concentrations analyzed by Hilscherova et al. (2010). However, superfund sites are known such as the Hudson River (USA) with sediment concentrations of up to 1.5 mg/kg of total PCBs (Bopp et al. 1998).

Comparing the levels found in the Saar and the Rhine, the sites R1 and R2 were significantly different from the other sites (Kruskal-Wallis ANOVA, $p<0.05$). Constraining to the sole rivers, the sites R1 and R2 were significantly different from the site R3 and R4 in the Rhine ($p<0.05$, Kruskal-Wallis ANOVA, $p<0.05$) and S1 was significantly different from S2 in the Saar (Wilcoxon matched-pairs signed rank test, two-tailed, $p=0.001$). One point source of PCBs that was recently identified is the former mine of Reden located at the little creek Blies that flows into the Saar some kilometers upstream of sampling site S1 (MUEV 2011). In the Blies were found 48 µg/kg ΣPCBs in a SPM sample taken in 2005 (IKSMS 2005b). However, there may be a lot of point and diffuse sources of PCBs in the Saarland area such that the total concentrations in the SPM from site S2 are twofold of those found at site S1. The PCB contamination of the Saar samples might be related to mining activities in the Saar coal mining and steel production district due to usage of PCBs as hydraulic fluids in mining machines and dielectric fluids in capacitors and transformers as well as in other applications.

The significantly increased concentrations at site R3 and R4 could be assigned to diffuse sources of PCBs from the industrialized zones along the Rhine (Heise and Förstner 2006, IKSR 2009). Known production locations of PCBs and maybe point sources were Ludwigshafen and Leverkusen (Heise et al. 2004, cf. Burgos et al. 2008). Point sources in the Ruhr coal mining and steel productions district may exist, but without significant effects on the PCB levels at site R4. The Rhine tributaries Neckar, Main and Moselle were also identified as major contributors to the increased PCB loads in the Middle and Lower Rhine (Pohlert et al. 2011b). The Rhine sediment management plan (IKSR 2009) indicates two main areas of risk – Eggersheim barrage (Main River) and the Duisburg barrage (Ruhr River) – which could serve as secondary sources of PCBs due to sediment remobilization.

2.3.7 HCB in suspended particulate matter

HCB reached concentrations of 0.5–1.1 µg/kg in samples from site S1 in the Saar and of 0.7–61 µg/kg in the Rhine samples (Figure 2-6C). HCB was not detected at site S2. The concentrations in the Saar are very low and may be related to diffuse sources in the catchment area. The HCB concentrations found in samples of the Rhine were highly variable as shown by the high standard deviations at sites R1, R2 and R3, but not in R4. Furthermore, the HCB levels at site R1 were significantly different from R2 and R3 (p<0.05, Kruskal-Wallis ANOVA). This variability was also confirmed by newer data of these sites with HCB levels of 5.6 ± 6.2 µg/kg (R1), 20.2 ± 4.5 µg/kg (R2), 17.9 ± 11.2 µg/kg (R3) and 15.6 ± 0.9 µg/kg (R4) shown as mean values of yearly composite SPM samples 2006–2009 (data source: UBA 2012). Pohlert et al. (2011b) reported generally decreasing HCB loads in the Rhine.

The discharge of untreated wastewater from a chemical plant (Dynamit Nobel) in Rheinfelden (Germany, Rhine km 148.4) was identified as the main source of HCB in the Rhine (Fiedler et al. 1995). In this plant, HCB was synthesized as precursor for the production of pentachlorophenol sodium (PCP-Na) until 1986 (Fiedler et al. 1996a). Dynamit Nobel was one of the main producers of PCP-Na in the world and the only one in former West Germany (Fiedler et al. 1996a). Since 1986, chlorosilanes have been produced by the Hüls AG (today Evonic Industries AG), but HCB was as an unintentional byproduct due to hydrochlorination of silicon at high temperatures (Heise and Förstner 2006). The discharge of HCB contaminated wastewater was discontinued in 1993 (Fleig et al. 2006, Heise and Förstner 2006).

It is unclear whether there are recent sources of HCB or not and why the concentrations show such variability (Fleig et al. 2006). Further point or diffuse sources of HCB in the Rhine catchment could be the tire production in Karlsruhe or the former chemical plant in Mulhouse (France; Rhodia 2007). Inputs from the Lippe, which drains parts of the Ruhr district, are also likely (Pohlert et al. 2011b). The HCB loads from the main source in Rheinfelden were distributed longitudinal over the stretch of the Upper Rhine barrages between Basel and Karlsruhe such that the sediments in this area could serve as secondary sources of HCB (IKSR 2009, Pohlert et al. 2011b). Remobilization from those sediment repositories is maybe the cause of the high variability in HCB concentrations in samples from locations R1, R2 and R3.

2.3.8 Spatiotemporal assessment of suspended particulate matter

The regional and seasonal dynamics of occurrence and distribution of target compounds in the Rhine were investigated using the self-organizing map technique (SOM). Since remobilization of contaminated sediments could influence the quality of SPM (e.g., contents of pollutants and total organic carbon as well as grain size distribution), the discharge at gauging stations related to the sampling sites was considered to address possible relationships. Quarterly aggregated data by calculation of the average values of each factor was used in order to reduce the number of samples. The individual factors were contents of PAHs, PCBs, HCB and TOC as well as the grain sizes (K630: fraction <630 µm; K200: fraction <200 µm; K63: fraction <63 µm; K20: fraction <20 µm), turbidity (FNU)[12] and the discharge (Q)[13].

2.3.8.1 Self-organizing maps

Figure 2-8 shows the SOM planes with a 5 x 4 matrix and a related U-matrix grouped by the factors. The U-matrix in the upper left of the figure shows the overall nodes of all single SOM assigned to the factors and additional intermediate nodes. In the U-matrix is also depicted a so-called hit diagram (magenta hexagons) that are related to map areas with high loadings of samples as visible in the last plane «Labels». The comparison of the SOM patterns gives evidences for qualitative relationships between the factors (Jin et al. 2011).

The SOMs have elevated areas in red to yellow colors, intermediate regions with light green to light blue colors and lower districts with blue colors. For example, the grain size fraction <20 µm (K20) has an elevated area in the bottom of the SOM and the PCB plane is vice versa. In contract, the factors discharge (Q) and turbidity (FNU) have similar patterns with an elevated ridge in the top and upper right corner. PAH has also a higher region in the upper right corner and thus it might be in concordance with the factors FNU and Q. HCB has two elevations in the upper left corner and in the lower right corner.

[12] FNU data for year 2005 was kindly provided by Amt für Umwelt und Energie Basel-Stadt (R1, Switzerland), LUBW (R2, Karlsruhe, Germany) and BfG (R3). SPM contents data for location R4 was kindly provided by the Regional Environmental Authority North Rhine-Westphalia (Düsseldorf, Germany). It was converted to FNU values using the empirical model $SPM = 0.695 \times FNU + 15.2$ (Pfannkuche and Schmidt 2003).

[13] Discharge data for year 2005 was kindly provided by the Federal Waterways Administration (WSA) Freiburg/Breisgau (Germany; locations R1 and R2), WSA Duisburg-Rhein (Germany; location R4) and Federal Institute for Hydrology Koblenz (BfG, Germany, location R3).

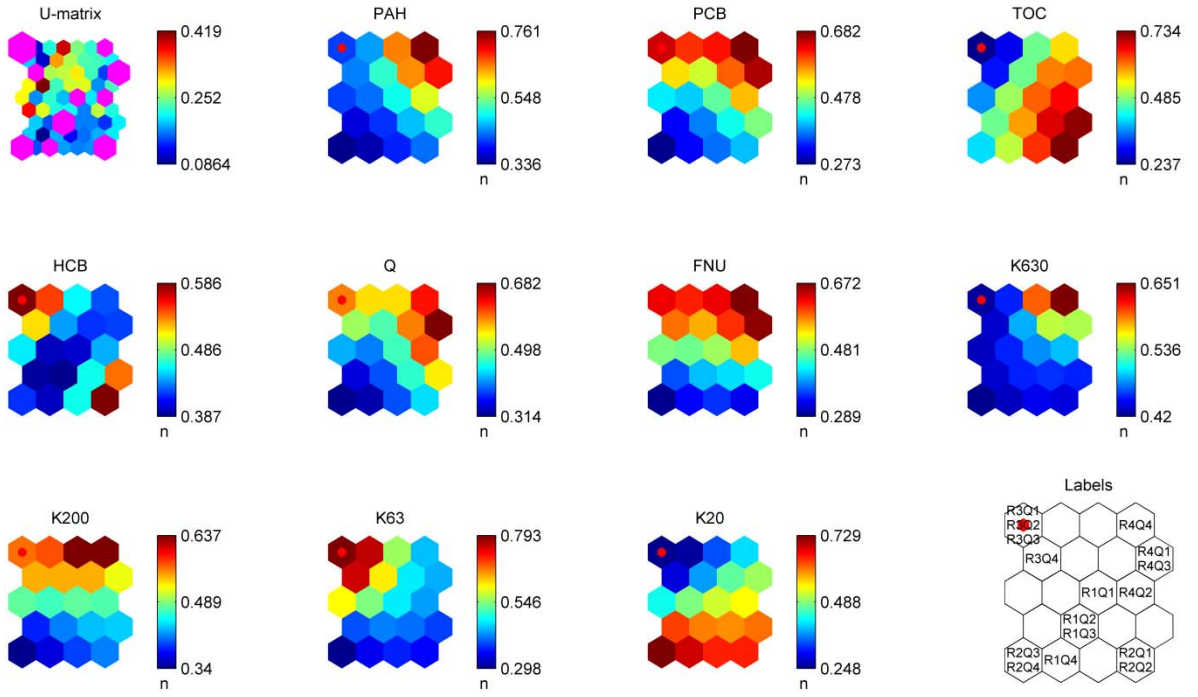


Figure 2-8 *SOM planes grouped by the measured factors in the quarterly aggregated SPM samples of the Rhine; the U-matrix is an aggregation of every plain shown with intermediate regions and a hit-diagram (magenta hexagons) indicating nodes with high factor loadings; the last plane depicts the loading of each factor to a curtain node (see text for abbreviations)*

2.3.8.2 Classification of patterns in self-organizing maps

In terms of optimization the number of clusters for *k*-means clustering, the Davies-Bouldin validity index (DBI) was calculated (Figure 2-9). The minimum DBI was five (A) that was chosen as input value for *k*-means analysis. The five clusters are visualized in SOM in Figure 2-9C. The samples of site R4 were assigned to Cluster 1. The samples R1Q1, R1Q2 and R1Q3 as well as R2Q1 and R2Q2 were loaded to Cluster 2. The Cluster 3 includes the samples R1Q4, R2Q3 and R2Q4. Cluster 4 has no loadings. Cluster 5 contains all samples of sampling site R3. The samples from site R3 and R4 are associated with individual clusters (Cluster 1 and Cluster 5). The samples of site R1 and R2 are assigned to two mixed clusters (Cluster 2 and Cluster 3). Some nodes include only one sample: R1Q1, R1Q4 and R4Q4. R1Q4 is in neighborhood to R2Q3 and R3Q4, which are classified together to Cluster 3.

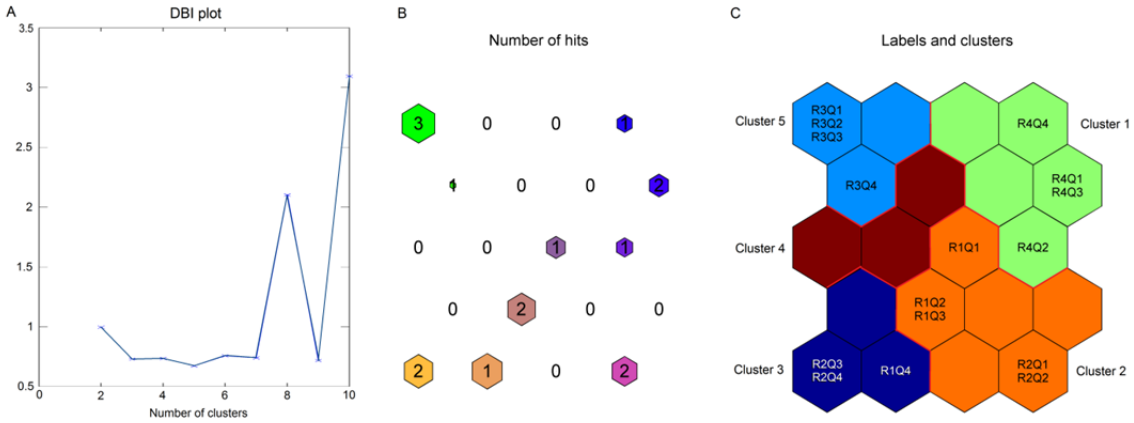


Figure 2-9 Plots of the Davies-Bouldin index (DBI; A) of SOMs plotted in Figure 2-8; numbers of hits assigned to each node (B); labels of quarterly aggregated samples for all sampling sites of the Rhine loaded to each node and classification map of five clusters (C)

2.3.8.3 Interpretation of the self-organizing maps

The samples of the Upper Rhine region with exception of sample R1Q1 were rather influenced by the contents of fine grain sizes below 20 µm (K20) than by other factors. In comparison, the samples of sites R3 and R4 had coarser grain sizes due to high elevations of the factors K63, K200 and K630 in the Cluster 1 and Cluster 5. The turbidity (FNU) and the grain size fraction below 200 µm (K200) were in good concordance because of similar SOM patterns with an elevation in the Middle and Lower Rhine region and a depression in the Upper Rhine region. The factor TOC revealed no obvious evidences aside from some interrelations between the sites R1, R2 and R4 – maybe by chance. Discharge (Q) showed only a clear interaction with PCBs but not with other factors and sites. The elevated area of the factor PAHs was assigned to the Cluster 1 containing the samples from site R1. A similar picture draws the factor PCB that was loaded to Cluster 1 and Cluster 5 representing sites R4 and R3, respectively. Thus, the factors PCB and PAH had a clearly regional, but no seasonal variability due to missing or only low differences between the node loadings. With respect to HCB, the elevations was associated with the upper left node that is related to the samples R3Q1, R3Q2 and R3Q3 as well as to the lower right node, which is loaded with the samples R2Q1 and R2Q2. In conclusion, the SPM contamination patterns in the Rhine are related rather to regional influences than by seasonal effects. Discharge might have an influence to PCB contamination but not with other factors, maybe caused by remobilization of PCB containing sediments during flood events.

2.3.9 Identification of suspect and nontarget compounds

Different groups of anthropogenic markers were investigated by detailed suspect screening using GC-MS. The list of suspect was compiled during the project «Development of a Standard Operation Procedure Sediments and Suspended Particulate Matter» (Schulze et al. 2005b) – based on previous nontarget screening studies (e.g., Ricking et al. 2003a, Schwarzbauer 1997).

The analysis was applied to all SPM and sediment core samples to unravel the spatial and temporal distribution as well as changes in contents of lipophilic organic compounds in the investigated samples. If there were evident peaks of not listed compounds present in the chromatograms of different samples, a nontarget identification approach by using library and literature search as well as *in silico* fragmentation prediction for confirmation was used.

In the Rhine, a time series of up to 12 SPM samples collected in 2005 at locations R1–R4 and a dated sediment core taken in 2003 at location R3 were investigated. Table 2-5 summarizes the compounds found in the samples. In the Saar, a time series of up to 12 SPM samples obtained at locations S1 and S2 collected in 2005 and a dated sediment core taken at location S1 in 2003 were analyzed. Table 2-6 lists the compounds found in the Saar samples. A detailed overview of compounds identified in each sample could be found in Table S2–3 to Table S2–9 (pp. XI et seq. in Appendix).

Table 2-5 Organic contaminants sediment and SPM samples of the Rhine

Technical additives and precursors
Dimethyl phthalate
Di-iso-butyl phthalate
Di-n-butyl phthalate
Bis(2-ethylhexyl) phthalate
Tri-n-butylphosphate
Tris(2-ethylhexyl)phosphate
Tris(chloropropyl)phosphate
N-phenylnaphthylamine
Methyldiphenylmethane
Methylbisdiphenylmethane
Dioctyldiphenylamine

Surfactants
C10 – C13 Linear alkyl benzenes

Personal care products
Galaxolide
Tonalide
α-tocopherol
α-tocopherol acetate

Halogenated compounds
Polychlorinated biphenyls
1,3,5-trichlorobenzene
1,2,4-trichlorobenzene
1,2,3-trichlorobenzene
1,2,3,5-/1,2,4,5-tetrachlorobenzene
1,2,3,4-tetrachlorobenzene
Pentachlorobenzene
Hexachlorobenzene
Trichloroaniline
Polychlorinated biphenyls

Sulfones
a,a-dinaphthylsulfon
a,b-dinaphthylsulfon
b,b-dinaphthylsulfon

Polycyclic aromatic compounds (PAC)
Naphthalene*
C_1-Naphthalenes
Biphenyl
Acenaphthylene*
Acenaphthene*
Fluorene*
C_1-Fluorenes
C_2-Fluorenes
1-Phenylnaphthalene
2-Phenylnaphthalene
9-Vinylanthracene
Phenanthrene*
Anthracene*
C_1-Phenanthrenes/anthracenes
C_2-Phenanthrenes/anthracenes
C_3-Phenanthrenes/anthracenes
4H-Cyclopenta[def]phenanthrene
Fluoranthene*
Pyrene*
Benzo[a]fluorene
C_1-fluoranthenes/pyrenes
C_2-fluoranthenes/pyrenes
Ethylfluoranthenes/-pyrenes
o-terphenyl
m-terphenyl
p-terphenyl
Benzo[c]phenanthrene
Cyclopenta[cd]pyrene
Benzo[a]anthracene*
Triphenylene
Chrysene*
1,1'-binaphthyl
1,2´-binaphthyl
2,2´-binaphthyl
Benzo[b,j]fluoranthene*
Benzo[k]fluoranthene*
Benzo[e]pyrene
Benzo[a]pyrene*
Perylene
C_1-benzo[e]pyrenes/perylenes
Indeno[1,2,3-cd]pyrene*
Benzo[ghi]perylene*
Dibenzo[a,h]anthracene*
Benzo[b]chrysene
Dibenzo[def,mno]chrysene
Coronene

S-heterocycles
Dibenzothiophene
Benz(b)naphtho[2,1-d]thiophene
Benz(b)naphtho[1,2-d]thiophene
Benz(b)naphtho[2,3-d]thiophene

O-heterocycles
Dibenzofuran
Benz(b)naphtho[2,1-d]furan
Benz(b)naphtho[1,2-d]furan
Benz(b)naphtho[2,3-d]furan

N-heterocycles
9H-Carbazole
C_1-carbazoles
C_2-carbazoles
Benzocarbazole

Aromatic ketones
9H-fluroene-one
Methylfluorene-one
9,10-anthraquinone

C_1: methyl substitutes; C_2: dimethyl substitutes; C_3: trimethyl substitutes; *: EPA-PAHs

Table 2-6 Organic contaminants sediment and SPM samples of the Saar

Technical / industrial compounds
Dimethyl phthalat
Di-iso-butylphthalat
Di-n-butylphthalat
Bis(2-ethylhexyl)phthalat
Tri-n-butylphosphate
Tris(2-ethylhexyl)phosphate
Tris(chloropropyl)phosphate
N-Phenylnaphthylamine
Methyldiphenylmethane
Methylbisdiphenylmethane
Dioctyldiphenylamine
Ugilec 141

Surfactants
C10 – C14 Lineare alkyl benzenes

Personal care products and biocides
Galaxolide
Tonalide
α-tocopherol
α-tocopherol acetate
Triclosan

Halogenated compounds
Hexachlorobenzene
Polychlorinated Biphenyls
Trichloroaniline

Polycyclic aromatic compounds (PAC)
Naphthalene*
C_1-Naphthalenes
C_2-Naphthalenes
C_3-Naphthalenes
Biphenyl
Acenaphthylene*
Acenaphthene*
Fluorene*
C_1-Fluorenes
C_2-Fluorenes
1-Phenylnaphthalene
2-Phenylnaphthalene
9-Vinylanthracene
Phenanthrene*
Anthracene*
C_1-Phenanthrenes/anthracenes
C_2-Phenanthrenes/anthracenes
C_3-Phenanthrenes/anthracenes
4H-Cyclopenta[def]phenanthrene
Fluoranthene*
Pyrene*
Benzo[a]fluorene
C_1-Fluoranthene/pyrene
C_2-Fluoranthene/pyrene
Ethyl-Fluoranthene/pyrene
o-terphenyl
m-terphenyl
p-terphenyl
Benzo[c]phenanthrene
Cyclopenta[cd]pyrene
Benzo[a]anthracene*
Triphenylene
Chrysene*
1,1'-Binaphthyle
1,2´-Binaphthyle
2,2´-Binaphthyle
Benzo[b,j]fluoranthene*
Benzo[k]fluoranthene*
Benz(e)pyrene
Benz(a)pyrene*
Perylene
C_1-Benzo[e]pyrenes/-perylenes
Indeno[1,2,3-cd]pyrene*
Benzo[ghi]perylene*
Dibenzo[a,h]anthracene*
Benzo[b]chrysene
Dibenzo[def,mno]chrysene
Coronene

S-PAC
Dibenzothiophene
C_1-dibenzothiophene
Benzo[b]naphtho[2,1-d]thiophene
Benzo[b]naphtho[1,2-d]thiophene
Benzo[b]naphtho[2,3-d]thiophene

O-PAC
Dibenzofurane
C_1-dibenzofurane
C_2-dimethyldibenzofurane
Benzo[b]naphtho[2,1-d]furane
Benzo[b]naphtho[1,2-d]furane
Benzo[b]naphtho[2,3-d]furane

N-PAC
9H-Carbazole
C_1-carbazole
C_2-carbazole
Benzocarbazole

Aromatic ketones
9H-fluroene-one
Methylfluorene-one
9,10-anthraquinone

C_1: methyl substitutes; C_2: dimethyl substitutes;/ C_3: trimethyl substitutes

2.3.9.1 Anthropogenic and natural markers

The most abundant group of substances present in all investigated samples was the large class of polycyclic aromatic compounds (PAC). The sources of PACs are primarily petrogenic (i.e. crude oil, coals and refined products) and pyrogenic (i.e. incomplete combustion of organic matter; Neilson 1998). However, the analysis of preindustrial sediment cores showed that parent PACs and their alkylated homologues could be formed from biologic precursors (i.e. diterpenoids, triterpenoids, steroids and hopanoids; Neilson and Hynning 1998, Wakeham et al. 1980). Perylene 1 is an example for such a compound (Figure 2-10). In SPM and recent sediment samples, it is often detected in low concentrations, but in low contaminated or preindustrial sediments, it is the most abundant PAC (Hallare et al. 2005, Wakeham et al. 1980). Silliman et al. (1998) showed that perylene may be formed from nonspecific aquatic and terrestrial organic material and thus it is an indicator for the deposition rather than of the sources of organic precursors.

Parent polycyclic aromatic compounds

The marker PACs (i.e. the 16 EPA-PAHs; not shown) were determined in all investigated samples. Additionally, the following PACs were tentatively identified in the sediment and SPM samples (Figure 2-10; structures 1–12): perylene 1, 4H-cylopenta[def]phenanthrene 2, benzo[a]fluorene/benzo[c]fluorene 3, benzo[c]fluoranthene 4, biphenyl 5, cyclopenta[cd]-pyrene 6, triphenylene 7, benzo[e]pyrene 8, benzo[b]chrysene 9, dibenzo[def,mno]chrysene 10, picene 11, and coronene 12.

Perylene 1 is a frequently occurring polycyclic aromatic hydrocarbon and nested with benzo[a]pyrene and benzo[e]pyrene 7. The role of perylene 1 in sediments was discussed above. Furthermore, it was confirmed as an indirect mutagen in the Ames bioassay (strain TA98; Brack et al. 2005b).

Biphenyl 5 (Figure 2-10) was used as a food preservative (E230) and as the precursor for the production of PCBs 47 (Figure 2-18). Furthermore, biphenyl is applied as a component in heat transfer agents (Jackson et al. 1975, Klein et al. 1995).

Triphenylene 6 frequently appears with the marker PAHs benzo[a]anthracene and chrysene. The other compounds shown (i.e. biphenyl 5, 4H-cyclopenta[def]phenanthrene 2, benzo[a]fluoranthene/benzo[c]fluoranthene 3, cyclopenta[cd]pyrene 4, benzo[b]chrysene 8, dibenzo[def,mno]chrysene 9, picene 10, and coronene 11) are typically occurring PACs as well (Biselli et al. 2005, Brack et al. 2003, Ricking et al. 2003a, Schwarzbauer 1997).

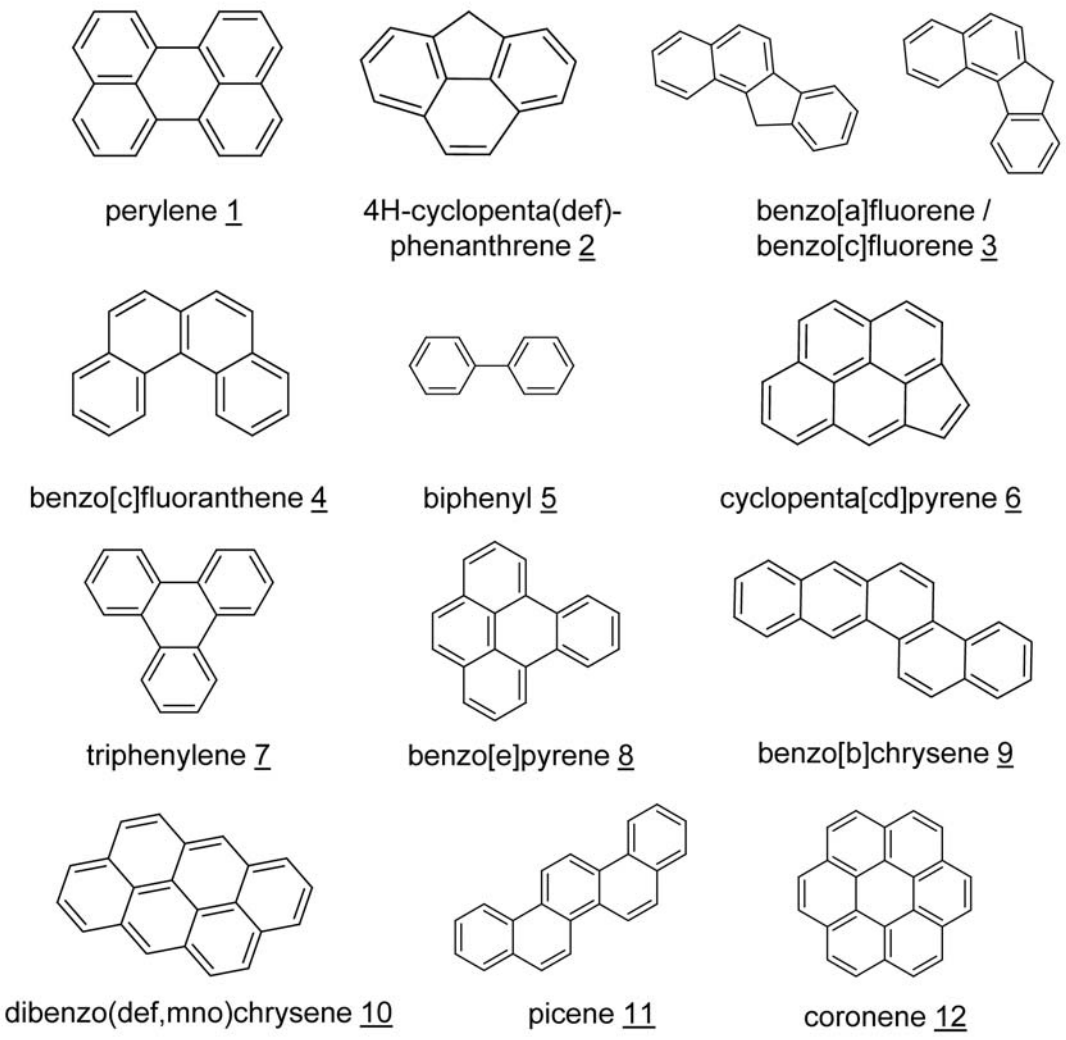


Figure 2-10 Structures of parent polycyclic aromatic compounds

Alkylated polycyclic aromatic compounds

Beyond the parent PACs, the group of mono- (C1), di- (C2) and tri-alkylated (C3) PACs (Figure 2-11; structures 13–30) were abundant in most of the samples. Since the alkylated PACs analogues could have a relevant contribution to sediment pore waters toxicity and are often found in high concentrations (Hawthorne et al. 2007), the identification of those compounds is very important. Alkylated PACs originate either from petrogenic (e.g., crude oils, petroleum, coal tar, and diagenesis) or from pyrogenic sources (Yunker et al. 1996).

The C1-naphthalenes 13, C1-C3-phenantrenes/anthracenes 16–19 and C1-C2-chrysenes 26, 27 are indicators of unweathered crude oil or light petroleum products input (Boehm et al. 1998, Savinov et al. 2000, Steinhauer and Boehm 1992). However, the C1-naphthalenes should be regarded with attention due to facilitated weathering of those compounds (Sporstøl et al. 1983).

Although alkylated fluorenes 14, 15 were analyzed in different sediment samples, only few studies appointed them to petrogenic sources, because they are components of coal tars, bituminous sands and crude oils (Paschke et al. 1992, Zeigler and Robbat 2012).

C1-/C2-fluoranthenes 20, 21 and C1-/C2-pyrenens 22, 23 were detected in heavily contaminated sediments (Kaisarevic et al. 2009) and in high concentrations in an urban lake in South Korea (Kim et al. 2008).

C1-benzo[a]anthracenes 25 and C1-chrysenes 26 were identified as major dioxin-like compounds in sediment of the creak Spittelwasser near Bitterfeld (Germany; Brack and Schirmer 2003). Furthermore, Machala et al. (2008) showed that they are aryl hydrocarbon (AhR) antagonists. C1-chrysenes were also found in sediments of the Danube near Novi Sad (Serbia), which had significantly dioxin-like potentials (Kaisarevic et al. 2009).

As methyl substituted analogues of PAHs with m/z 252 were detected the C1-benzo[a]pyrenes 28, C1-benzo[e]pyrenes 29 and C1-perylenes 30 that were previously identified by other studies (Brack et al. 2005b, Kaisarevic et al. 2009, Schwarzbauer 1997). According to Brack et al. (2005b), they may contribute to the mutagenic potential of contaminated sediments.

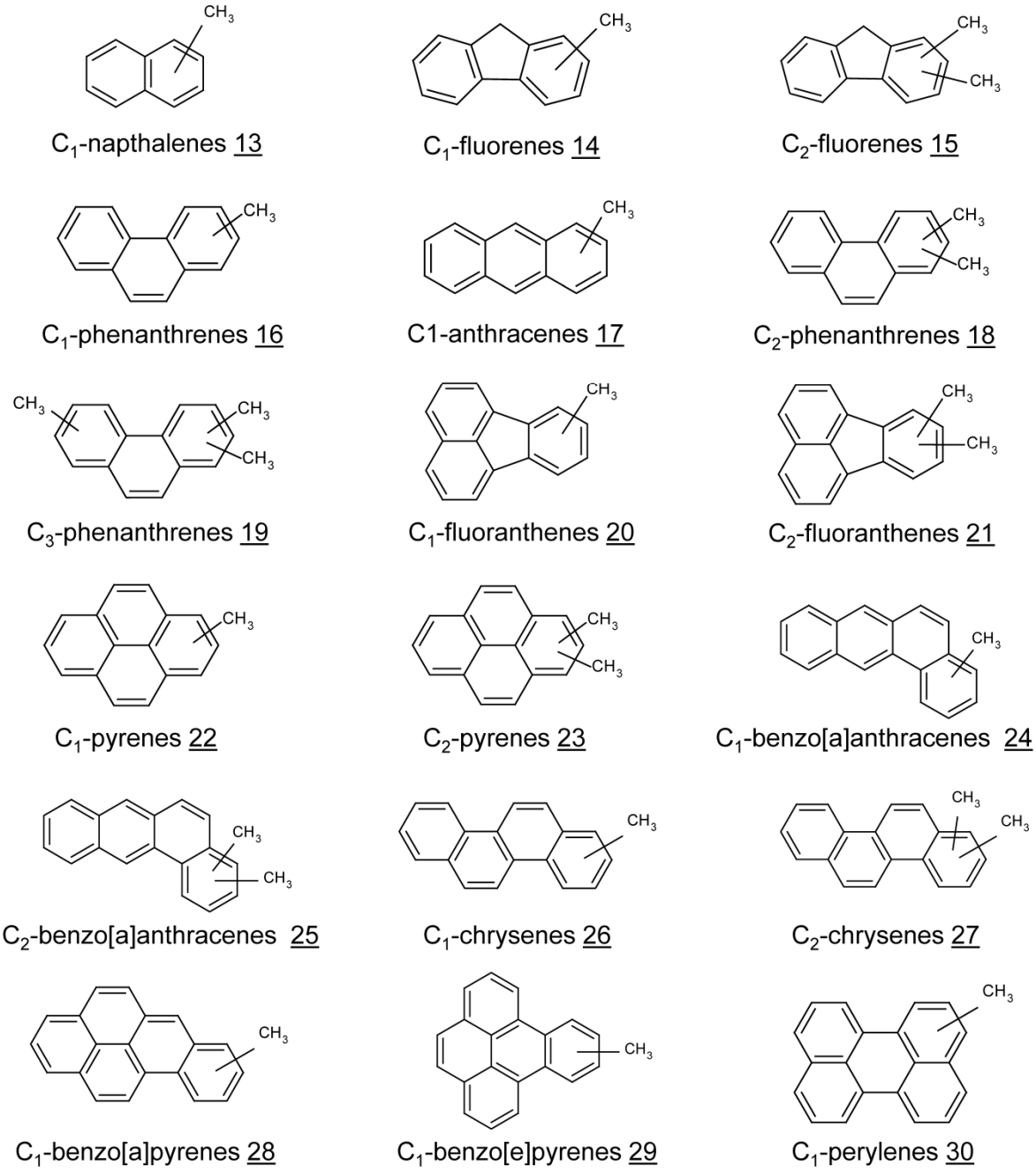


Figure 2-11 Structures of alkylated polycyclic aromatic compounds

Heterocyclic compounds

The third group of environmentally relevant polycyclic aromatic compounds included the heterocycles (Figure 2-12, structures 31–42). In their aromatic skeletons, one or more C atoms are substituted with oxygen, nitrogen or sulfur. Main sources of N-heterocycles are the pyrolysis of N-containing material (Harvey 1998). Sources of O- and S-heterocycles are for example coal tars, soot, shale oils, crude oils, tobacco smoke, automobile exhaust and emissions from burning of fossil fuels and wood (Harvey 1998, Jacob et al. 1991, Nishioka et al. 1986, Willey et al. 1981). Usually, N-, S- and O-heterocycles have benzo- and naphtho-condensed structures of the thiophene, furan or carbazole type, respectively, supplemented by alkyl-substituted homologues (Heinzel 2006). Heterocycles and their C1- and C2-substitutents were found in many of the investigated samples including the sediment core samples. The presence of those compounds in river sediments is widely known, for example in those of the Elbe and the Rhine river systems (Heim et al. 2004, Heinzel 2006, Schwarzbauer 1997). Lübcke-von Varel et al. (2011) detected mutagenic effects in the Ames fluctuation assay and AhR-agonistic responses in the DR-CALUX assay in heterocycles-containing sub-fractions of sediment extracts from the Elbe system in a comprehensive effect-directed analysis (EDA) study. AhR-agonistic effects were confirmed in an EDA study of crude oils and refined oil products (Vrabie et al. 2012).

Aromatic ketones

Aromatic ketones were also present in the sediment core samples (Figure 2-12, structures 42–44) as well as in the majority of SPM samples of the Saar, but only particularly in the SPM samples of the Rhine. Oxy-PACs are emitted from the same sources as the parent PAHS, but they are also formed by environmental processes such as chemical oxidation, photooxidation or biotransformation (Lundstedt et al. 2007, Ramdahl 1983). Thus, the occurrence of aromatic ketones is in coincidence with higher abundances of EPA-PAHs (Figure 2-6). The effectiveness of oxy-PACS in several *in vitro* bioassays with different endpoints such as acute toxicity, oxidative stress, endocrine disruption and genotoxicity gives evidence for an environmental impact thereof (Lundstedt et al. 2007).

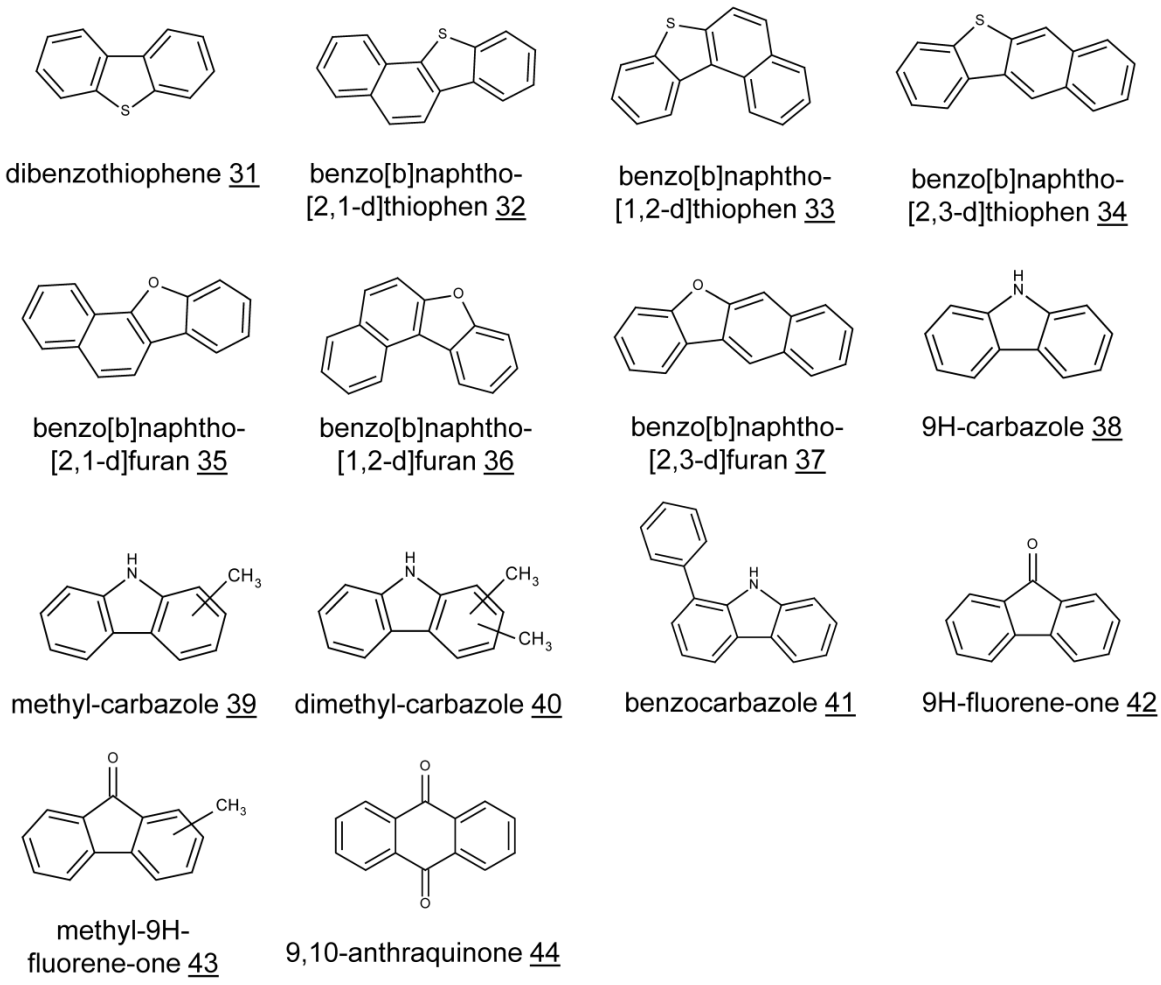
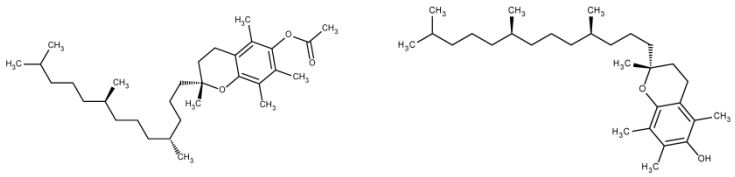


Figure 2-12 Structures of heterocyclic aromatic compounds and aromatic ketones

2.3.9.2 *Markers from personal care products and biocides*

Since the last decade, environmental organic chemistry focuses on the occurrence of organic compounds such as pharmaceuticals, illicit drugs, biocides and personal care products in wastewater, surface water and groundwater (Buchberger 2011, Daughton and Ternes 1999, Drewes et al. 2003, Kümmerer 2011, Lapworth et al. 2012, Petrovic et al. 2003, Schirmer et al. 2007). In majority, they have polar and hydrophilic properties, but some of those are nonpolar or hydrophilic and thus may partition to solid phases (Langford et al. 2011).

A fairly less known marker for municipal wastewater discharge to surface water is α-tocopherol acetate 45 (α-TA; Figure 2-13). It is a derivative of natural α-tocopherol 46 (α-TP; «vitamin E»; Figure 2-13) that is an essential substance for human and animal life due to its antioxidant, anti-proliferative and anti-inflammatory properties (Doods 2003, Eganhouse and Kaplan 1985, Takada et al. 1997).



α-tocopherol acetate 45 α-tocopherol 46

Figure 2-13 Structures of α-tocopherol and α-tocopherol acetate

Alpha-TA is more stable than α-tocopherol regarding oxidation and thus favourable for long-term storage (Eganhouse and Kaplan 1985). Alpha-TP and α-TA are industrially produced for nutrition supplement and medication as well as an anti-oxidant in cosmetics and technical applications (Eganhouse and Kaplan 1985, Meyer 2001). Alpha-TA was reported in a few studies as an anthropogenic marker in sediments (Coakley and Poulton 1991, Eganhouse and Kaplan 1985) and never in SPM. However, the major part of α-TP and α-TA in wastewater should adsorb to sewage sludge during wastewater treatment due to their strong lipophilicity with log K_{ow} of 12.2 and 12.3 (calculated with EPI Suite™; US-EPA 2008), respectively. The estimation of the biodegradability in a sewage treatment plant model using EPI Suite™ supports this thesis. Alpha-TP showed a removal capacity of 94.0% (93.2% total sludge adsorption + 0.8% biodegradation) and α-TA of 94.0% (93.6% total sludge adsorption

+ 0.4% biodegradation). In sediments, both compounds are rather persistent due to half-lives of more than four years according to a fugacity level III model (US-EPA 2008).

Figure 2-14 shows the ion chromatograms of α-TP and α-TA. The identity of the peaks were confirmed using library search with match values of 952 for α-TP and 828 for α-TA using the Wiley 9 and NIST08 mass spectra libraries (NIST/EPA/NIH 2008, Wiley 2009), AMDIS (NIST 2005) and NIST MS Search 2.0 (NIST 2008).

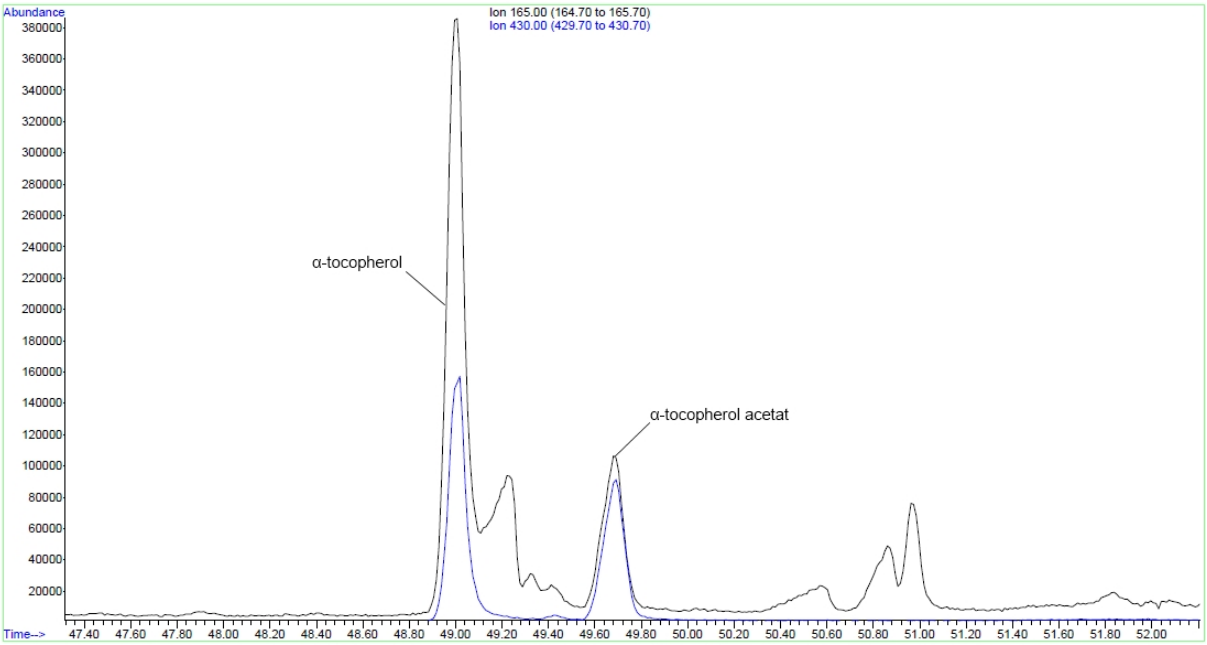


Figure 2-14 Ion chromatogram of sample S1 04/05-1 F6 in scan mode with extracted ions with m/z 165 and m/z 430 showing peaks of α-tocopherol and α-tocopherol acetate

Figure 2-15 depicts the mass spectrum of α-TP extracted with AMDIS (NIST 2005) and fragment annotations generated with MetFrag (Wolf et al. 2010). The mass spectrum is characterized by a base ion peak at m/z 165 (M-265; [C10H12O2]), a high molecular ion peak at m/z 430 as well as a fragment at m/z 205 (M-225; [C13H17O2]).

Figure 2-16 shows the mass spectrum of α-TA extracted with AMDIS and fragment annotations generated with MetFrag. The mass spectrum is defined by a base ion peak at m/z 165 (M-307; [C10H13O2]), a molecular ion peak at m/z 472 as well as a big fragment peak m/z 430 (M-42; [C28H46O3]). The main characteristics are similarly to those in a mass spectrum of α-TA published by Eganhouse and Kaplan (1985).

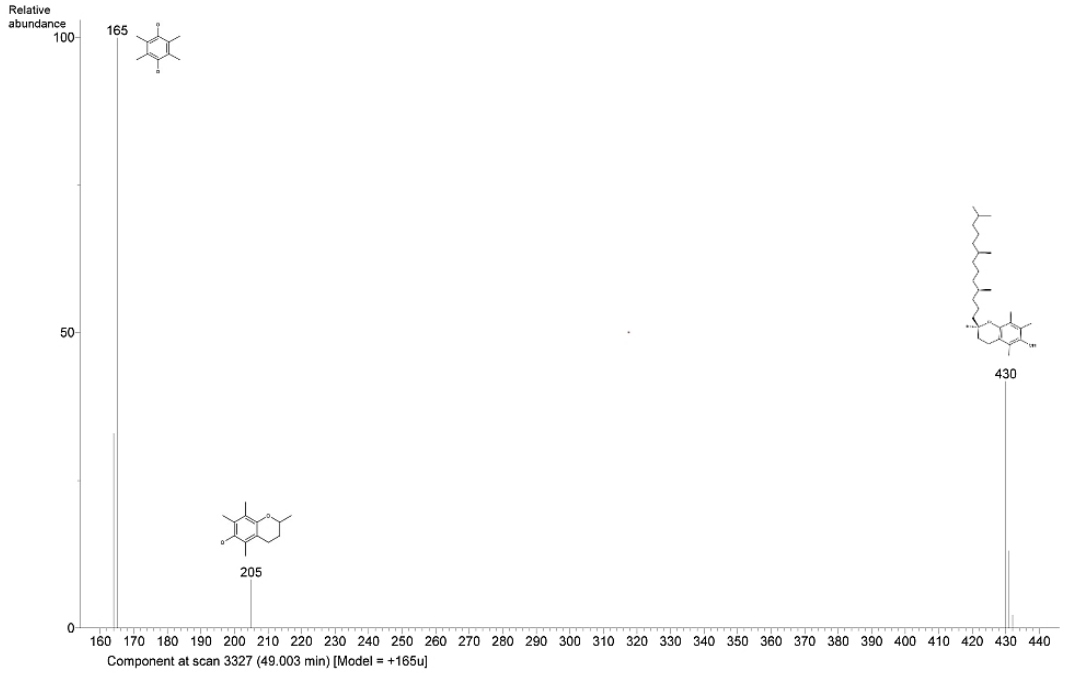


Figure 2-15 Mass spectrum of α-tocopherol and fragment annotation using MetFrag (scan 3327 at 49.00 min in sample S1 04/05-1 F6)

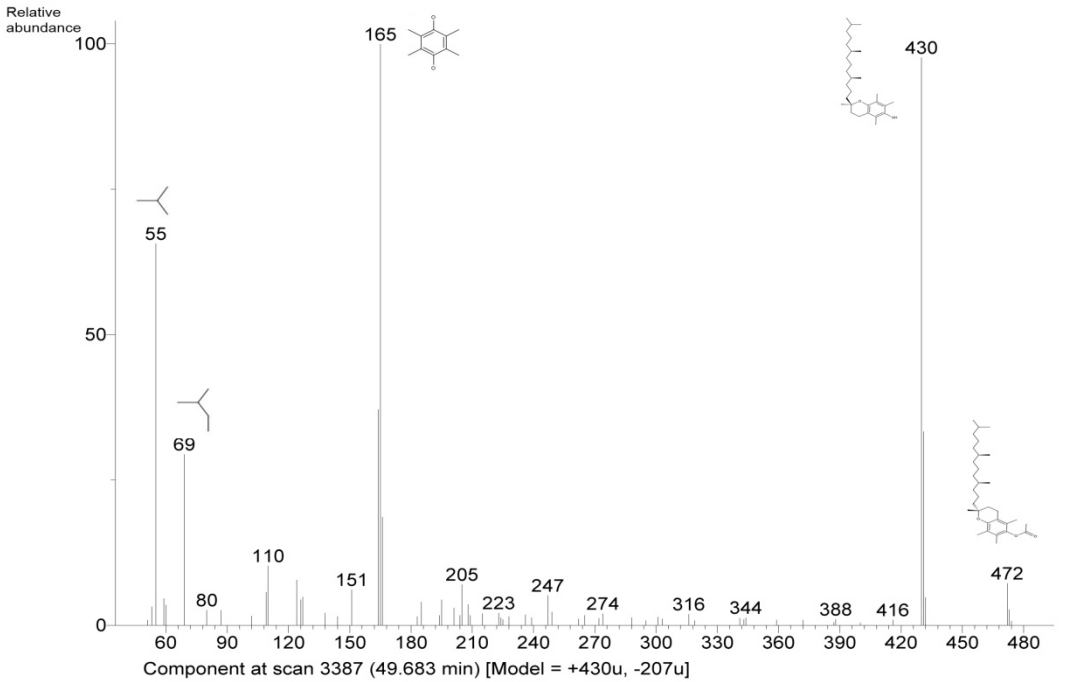


Figure 2-16 Mass spectrum of α-tocopherol acetate and fragment annotation using MetFrag (scan 3338 at 49.68 min in sample S1 04/05-1 F6)

Alpha-TP and α-TA were present in the majority of SPM samples from locations S1, S2, and R1. At sites R2, R2 and R4, they were found in only few samples, particularly due to chromatograms with a low resolution and thus insufficient quality. Furthermore, α-TP was abundant in all sediment core samples from site S1 and R4 because of its natural sources. Interestingly, α-TA was even not found in the top samples of the sediment cores maybe due to degradation of the comparing to a less abundant α-TP. This observation is supported by different nontarget screening studies reporting presence of α-TP and its degradation products in sediment samples but no α-TA (Ricking et al. 2003a, Schwarzbauer 1997). This is in contrast to the findings of Eganhouse and Kaplan (1985) as well as Coakley and Poulton (1991), who stated the traceability of α-TA in sediments. Alpha-TA was reported to be present in surface water samples (Meyer 2001, Schwarzbauer and Ricking 2010).

The polycyclic musk compounds (PCM) galaxolide 47 and tonalide 48 (Figure 2-17) are frequently found substances in aquatic environments (Heberer et al. 1999, Ricking et al. 2003b, Rimkus 1999, Schwarzbauer 1997). They are used as cheap fragrances in consumer products such as soaps, softeners, laundry detergents and perfumes (Rimkus 1999). Due to their high lipophilicity, they accumulate in sediments and sewage sludge as well as in biota and thus they are considered as emerging pollutants (Richardson and Ternes 2011).

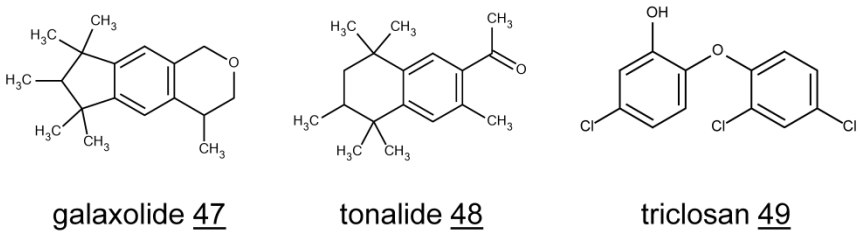


Figure 2-17 Structures of galaxolide, tonalide and triclosan

Whereas, PCM are investigated in several chemical monitoring studies, there are only few studies considering the ecotoxicological potentials thereof. Strong inhibition effects of galaxolide and tonalide were discovered on the early life stages of the copepod species *Acardia tonsa* (Wollenberger et al. 2003) and *Nitocra spinipes* (Breitholtz et al. 2003), but only weak effects on full life stages of *N. spinipes* (Breitholtz et al. 2003). Simmons et al. (2010) investigated the interaction of galaxolide with the human and trout estrogen receptor-α with the result that galaxolide did not act as an estrogen agonist, but as a strong estrogen antagonist and thus inhibited estrogenic activity.

Thus, there are concerns regarding adverse effects of PCM. Furthermore, it was shown that galaxolide, celestolide, tetralide and traseolide are able to inhibit the multixenobiotic resistance defense system in aquatic organisms by affecting the P-glycoprotein (P-gp; Smital et al. 2006, Smital et al. 2004). The P-gp is the best-known transmembrane protein of the adenosine triphosphate binding cassette super family, which are responsible for the active and ATP consuming transport of drugs and xenobiotics out of biological cells (Smital et al. 2004). Galaxolide and tonalide were found in the sediment core S1 and in the top samples of sediment core R1. They were abundant in the SPM samples of location S2, but not at the sites S1 as well as in the Rhine samples R1–R4, except sample R4 08/05-1. Methyl-galaxolide – the main transformation product of galaxolide (Bester 2005) – was not detected in any of the samples.

Triclosan 49 (Figure 2-17) s an antimicrobial biocide that is used in personal care products such as soaps, toothpaste and deodorants as well as in items such as sportswear, plastic cutting boards and furniture and released by municipal wastewater treatment plants (WWTP) in the environment (von der Ohe et al. 2012). Triclosan was identified as much more effective as for example prioritized EPA-PAHs in the green algae inhibition test with *Scenedesmus vacuolatus* using an partitioning-based dosing approach due to an enhanced bioaccessibility because of its polar nature (Bandow 2011). Triclosan was found in sediment samples at location S2 (Rehlingen) from year 2002 (Schulze et al. 2005b). Despite its intrinsic toxicity as well as worldwide usage and presence in rivers, triclosan was not considered as priority substance in many countries ignoring the fact that it is one of the major causes of adverse effects to several aquatic species (von der Ohe et al. 2012).

2.3.9.3 Industrial and technical markers

In Figure 2-18 (structures 50–57), Figure 2-19 (structures 58–67), Figure 2-20 (structures 68–76) and Figure 2-23 (structures 77–79) are depicted structures of compounds of industrial and technical origin. The polychlorinated biphenyls 50 are a group of chlorinated anthropogenic compounds with in total 209 congeners (Ballschmiter and Zell 1980) that do not occur in nature. The main applications of PCBs were cooling agents and dielectric fluids in capacitors and transformers as well as hydraulic fluids, plasticizers, flame-retardants, lubricants and paper finishers (Müller and Korte 1973). PCBs are toxic, persistent and bioaccumulative and thus they were banned in the 1980s by the European Community (European Council 1985) and worldwide by the Stockholm Convention in 2001 (UNEP 2001).

Due to their persistence, they are ubiquitously distributed in the atmosphere, biosphere, hydrosphere, and pedosphere.

The second group of halogenated compounds in the samples included the chlorinated benzenes compounds trichlorobenzenes 51–53, tetrachlorobenzene 54, 55, pentachlorobenzene 56 and hexachlorobenzene 57. HCB was abundant in the majority of the samples, but tri- and, tetrachlorobenzenes as well as pentachlorobenzene were only present in the sediment core R3 below 20 cm sediment depth (Table S2–3 in Appendix).

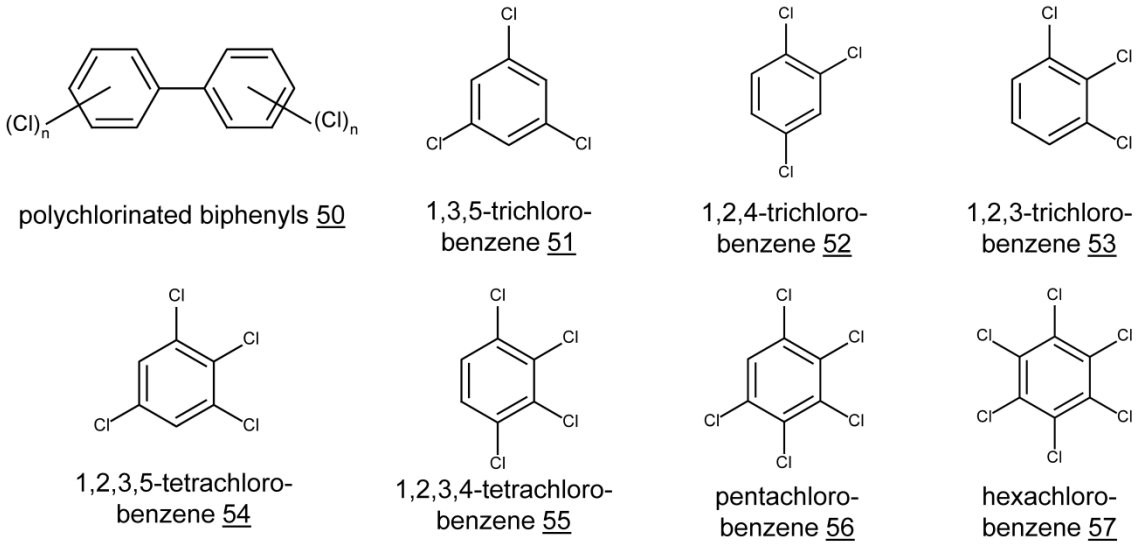


Figure 2-18 Structures of polychlorinated biphenyls and chlorinated benzenes

The 2,4,6-trichloroaniline 56 (Figure 2-19) conformer is used as an intermediate compound for the production of azodyes, chlorinated benzene derivatives, and fungicides (Botalova 2010, Scholz and Palauschek 1988). Trichloroanilines are known as acutely toxic for water organisms (Chen et al. 2007, Dom et al. 2010). It was only found in the sediment core from site R3 in layers below 10 cm sediment depth. However, recent release from an industrial WWTP effluent in the Rhine area was reported (Botalova 2010, Botalova et al. 2011).

All three isomers of binaphthyl sulfon 57–59 (Figure 2-19) were present in the sediment core from site R3 in layers below 10 cm sediment depth. These compounds were firstly described in environmental samples by Sheldon and Hites (1978) and also identified in sediment samples from the Elbe catchment (Schwarzbauer 1997) as well as in effluents of tanneries (Sheldon and Hites 1978, Thruston and McGuire 1981). Sheldon and Hites (1978) suggested that binaphthyl sulfons are by-products of sulfonated polymers production. Lofrano et al.

(2008) identified tentatively binaphthyl sulfons in a retanning resin. Thus, tannery manufactory and industry may be the main source of those substances. However, there is only scarce information regarding their environmental occurrence and toxicity available.

The 9-vinylanthracene 62 (Figure 2-19) is used for the production of photosensitive polymers (Biswas and Uryu 1986, Reucroft 1975) and semiconductors (Ando et al. 2005, Meng et al. 2005). Another source may be the possible usage as a fuel additive (Krull et al. 2004) and as a precursor in production of vinylaromatic polymers and rubbers (Mamak and Peri 2011, Sarraf and Jenkins 1998). Simmon and Baden (1980) reported 9-vinylanthracene as directly mutagenic in the Ames assay with TA98, TA100 and TA1535 strains. It was abundant in all samples and thus natural sources for 9-vinylanthracene may be considered. Although, there are only few evidences for natural sources such as coal and shale oils (Sadeghi et al. 1994).

Phenylnaphthalenes 63, 64 (Figure 2-19) were reported to be present in sediment extracts that were analyzed using bioassay-directed approaches. Brack et al. (1999) identified 2-phenylnaphthalene in an algal toxic subfraction of a sediment sample from the Spittelwasser (Saxony-Anhalt, Germany) and it was shown as genotoxic in the Ames assay (West et al. 1988).

Terphenyls 65–67 (Figure 2-19) are possible precursors for dyes (Jones and Reeve 1980, Pauluth and Tarumi 2004) as well as disinfectants or tannins (Hueffer et al. 2009). Dyes based on terphenyls and biphenyls, play an important role for the production of liquid crystal fluids (Jones and Reeve 1980, Pauluth and Tarumi 2004). Terphenyls were formerly used for the production of polychlorinated terphenyls (PCT). PCTs were used similarly to PCBs since 1929 until the 1970s – with a comparable impact to the environment (Jensen and Jørgensen 1983, Wester et al. 1996).

However, compounds 62–67 occur naturally as well due to their appearance in ancient sediments, crude oils and other fossil material (Alexander et al. 1986, Marynowski et al. 2001, Schwarzbauer 1997, Steinheimer et al. 1981).

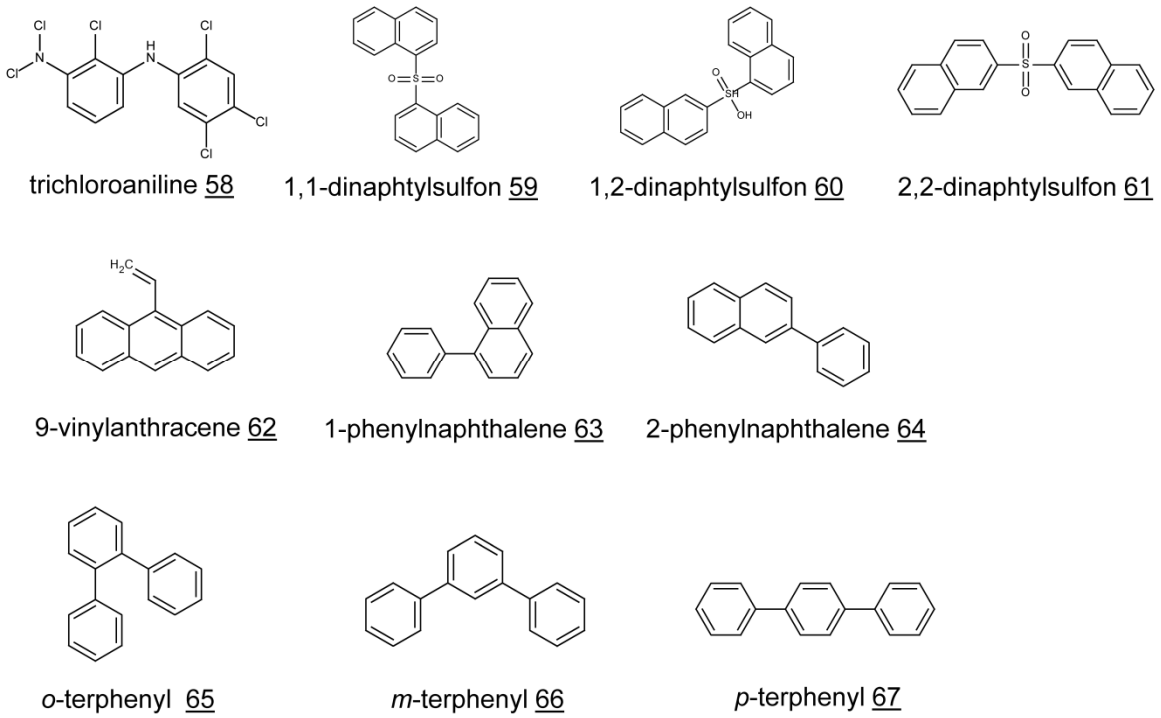


Figure 2-19 Structures of industrial and technical chemicals

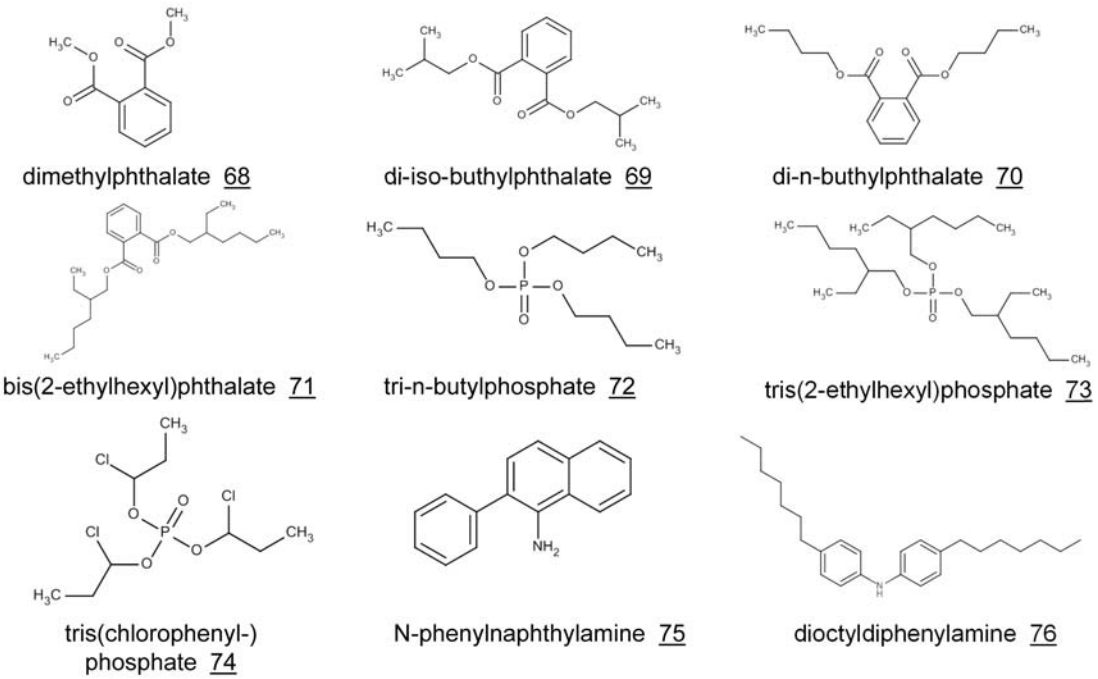


Figure 2-20 Structures of phthalates, phosphoric acid esters and amines

Phthalate esters 68–71 (Figure 2-20) are technical chemicals widely used as plasticizers in polyvinyl chloride and other polymers such as polyvinyl acetates, cellulosics, and polyurethanes (Stales et al. 1997). They are ubiquitously distributed and can be found in many environmental compartments (Furtmann 1993). The second group of plasticizers found in the sediments and SPM of the Saar and the Rhine are the tributyl-, triphenyl- and trioctyl-phosphoric acid esters 72–74. Furthermore, these compounds are used as flame-retardants and they are reported to be present in wastewater (Rodil et al. 2005), surface water (Andresen et al. 2004) and sediments (Schwarzbauer 1997).

N-phenylnaphthylamine (PNA) 75 (Figure 2-20) is used as an antioxidant in the rubber production and in lubricants. In this study, PNA was found in the sediment core samples from Güdingen (whole core) and Koblenz (layers below 33 cm sediment depth representing years 1975–1986) but neither in the SPM samples of the Saar nor in those of the Rhine. Previously, PNA was identified in sediments of the Spittelwasser Creek near Bitterfeld (Saxony-Anhalt, Germany) as a major site-specific toxicant in the *Daphnia magna* immobilization test (Brack et al. 1999).

A further amine, the diphenylamine derivative dioctyldiphenylamine (DODPA) 76 (Figure 2-20) was identified as nontarget compound in all SPM samples except in those from site S1. It is a high-temperature antioxidant additive in lubricants and has been previously determined in sediments of the Lippe (Kronimus et al. 2004) and of the Mediterranean Sea (Mille et al. 2007). A newer study reported the presence of DODPA in sand samples from Mediterranean beaches (Galgani et al. 2011). This study reports the occurrence of DODPA in SPM for the first time. Derivatives of diphenylamine are the most commonly used antioxidant additives in lubricants and rubbers (Baderna et al. 2012, Bernabei et al. 2000, Dalene and Skarping 1985, Thompson et al. 2006) and other applications such as production of dyes, plastics, elastomers, pharmaceuticals, pesticides and explosives (Christodoulatos et al. 1997, Drzyzga 2003). Although, they are produced and applied worldwide, only scarce information is available regarding their environmental occurrence and fate (Baderna et al. 2012, Thompson et al. 2006). Furthermore, there are concerns of adverse effects to environmental health due to known toxicity of aromatic amines (Drzyzga 2003) and only fair biodegradation in marine sediments (Thompson et al. 2006).

In Figure 2-21 shows the ion chromatogram of DODPA, it co-elutes with benzo[k]fluoranthene.

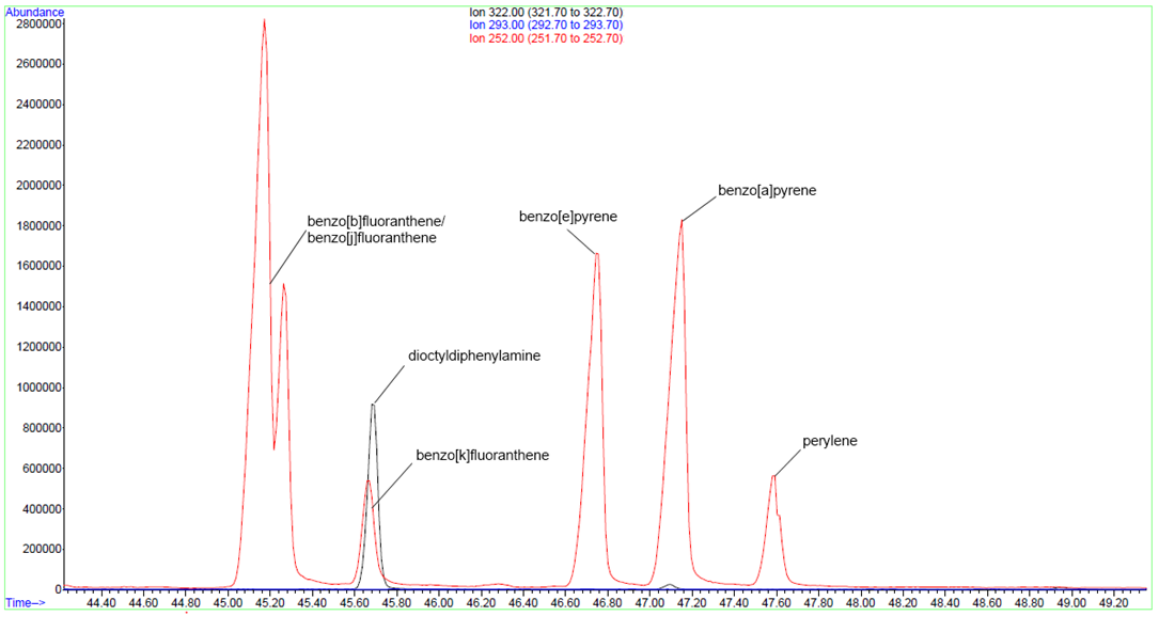


Figure 2-21 Ion chromatogram of sample R2-09/5-1 F4 with extracted m/z 322, 293 (Dioctyldiphenylamine) and m/z 252 (PAHs); dioctyldiphenylamine co-eluates with benzo[k]fluoranthene

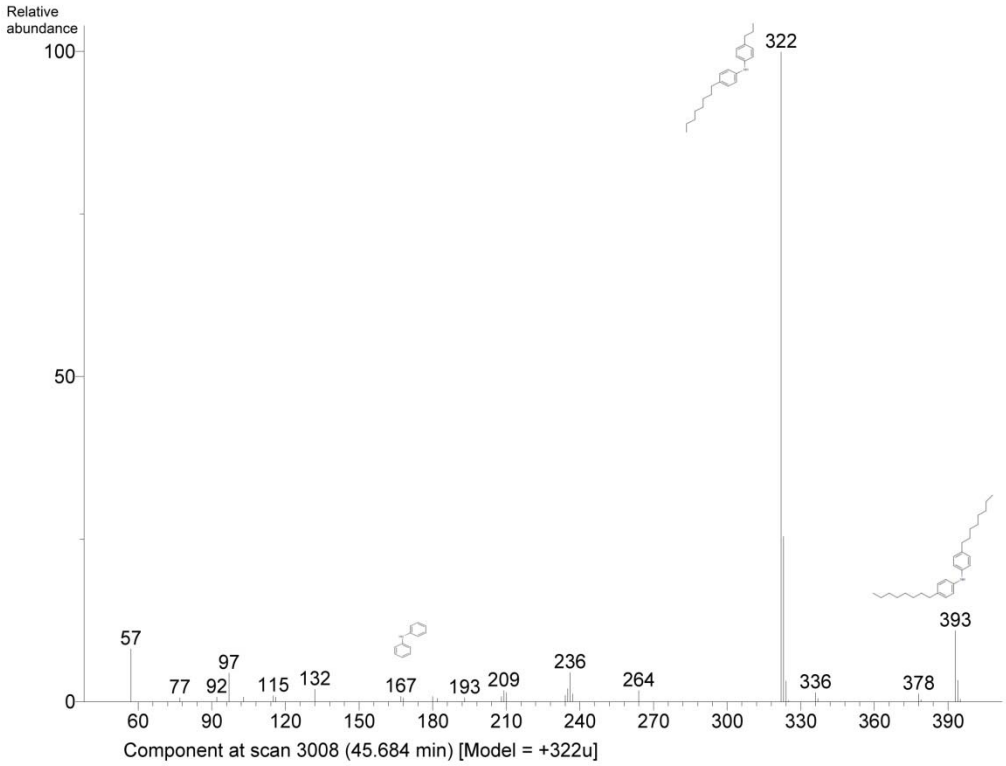
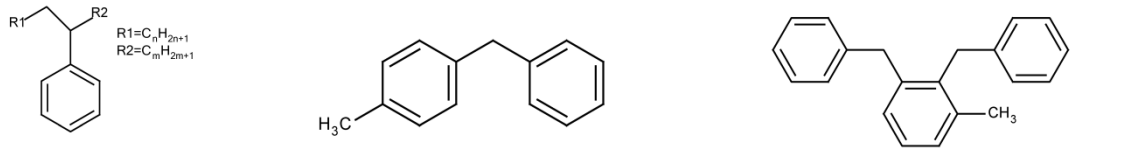


Figure 2-22 Mass spectrum of dioctyldiphenylamine and annotation using MetFrag (scan 3008 at 45.68 min in sample S2 09/05-1 F4)

The mass spectrum of DODPA is depicted in Figure 2-22. The fragment annotation with MetFrag predicted three fragments with a base peak at m/z 322 (M-71; [C23H32N]), the molecular ion at m/z 393 [C28H43N] as well as smaller peaks at m/z 323 (M-70; [C23H33N] and at m/z 167 (M-226; [C12H9N]). The match value in NIST MS search was 855 referring to NIST08 library. The fragments were confirmed by findings of Moldavan et al. (2000) and Thomson et al. (2006).



C_{10}-C_{14} linear alkyl benzenes 77 | methyldiphenylmethane 78 | methylbisdiphenylmethane 79

Figure 2-23 Structures of linear alkyl benzenes and diphenyl methane derivates

Commercial available linear alkyl benzenes 77 (LAB; Figure 2-23) are a homologues group of 26 compounds with alkyl chains in the range of C_{10}–C_{14} (Eganhouse et al. 1988, Fernández et al. 2002). LABs are produced in high volumes and a precursor for the fabrication of linear alkyl benzene sulfonates (LAS; Binetti et al. 2000) that may contain 1–3% unsulfonated LABs residues (Gledhill et al. 1991). A small volume of 5% is used in paper fabrication, as detergents or in industrial fluids (Gledhill et al. 1991). Due to their lipophilic nature with log K_{ow} between 7.5 and 9.1 (Binetti et al. 2000), they are expected to accumulate in sediments and biota (Binetti et al. 2000, Fernández et al. 2002). Thus, they are mainly removed by wastewater treatment plants (Gledhill et al. 1991).

Eganhouse et al. (1988) considered them as markers for untreated domestic wastewater or sewage sludge discharge. However, they were found in concentrations up to 20 µg/kg in sediments downstream of a WWTP (UK Department of the Environment 1994, c.f. Binetti et al. 2000) and thus they may occur if a WWTP has malfunctions or treatment is insufficient. A comprehensive risk assessment of LABs demonstrated that a risk to the aquatic environment is negligibly if effective wastewater treatment plants are available (Binetti et al. 2000).

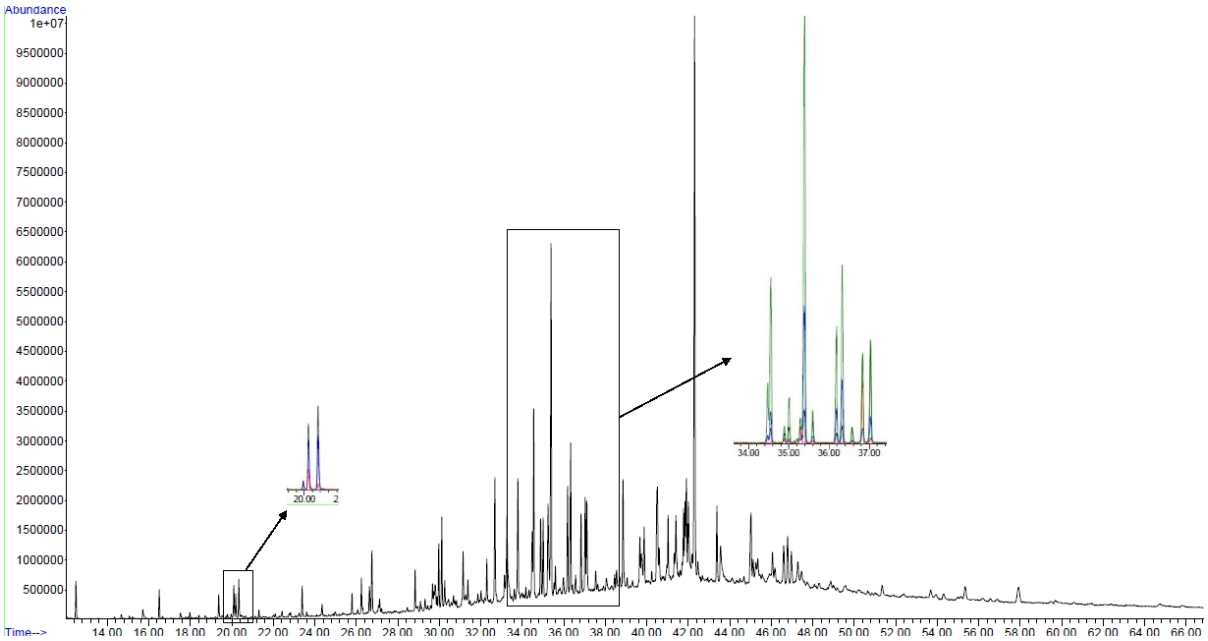


Figure 2-24 Total ion chromatogram of sample R1 11/05-1 F4 in scan mode; the inserts show the characteristic patterns of the methyldiphenylmethane (MDPM; left insert) and the methylbisdiphenylmethane (MBDPM; right insert) isomers (extracted ions with m/z: 272,182,167,104)

In fraction 4 of some samples, characteristic peak patterns of compounds with large molecular ion peaks at m/z 182 and at m/z 272 as well as base ion peaks at m/z 167 and at m/z 181, respectively, were observed (Figure 2-24). Library search resulted in two compounds with a diphenyl methane moiety: methyldiphenylmethane (MDPM; CAS-RN 713-36-0) and methylbisdiphenylmethane (MBDPM; CAS-RN 29589-57-9). Figure 2-23 shows the structures of MDPM 78 and MBDPM 79 (Figure 2-23).

In Figure 2-25 are depicted the mass spectrum and MetFrag fragment annotations of MDPM with a base ion peak at m/z 167 (M-15; [C13H11]) and a big molecular ion peak at m/z 182 as well as smaller fragments at m/z 104 (M-78; [C8H8]), at m/z 89 (M-93; [C7H5]) and at m/z 77 (M-105; [C6H5]). The match value in NIST MS search was 892 referring to the Wiley 9 library entry and MetFrag explained five of the most abundant peaks in the mass spectrum. Furthermore, the main characteristics of mass spectrum of MBDPM are similarly to those published by Trolio et al. (1996). Thus, three independent references confirm the identity of MDPM.

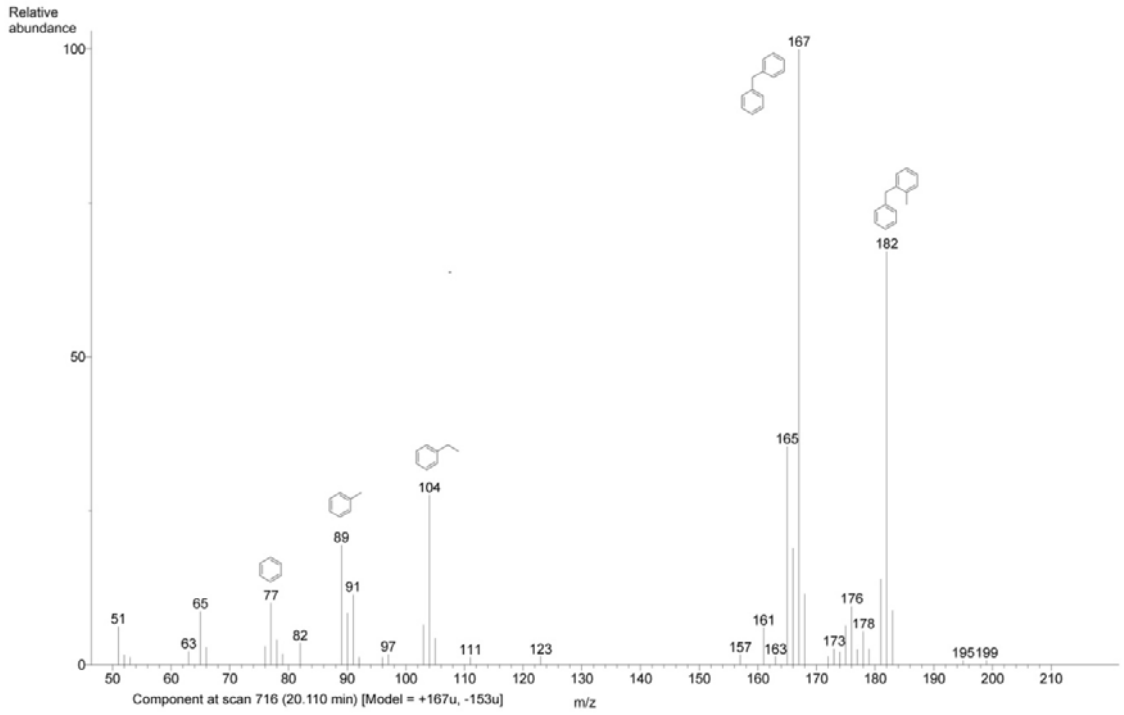


Figure 2-25 Mass spectrum of methyldiphenylmethane (MDPM) and fragment annotation using MetFrag (scan 716 at 20.11 min in sample R1 11/05-1 F4)

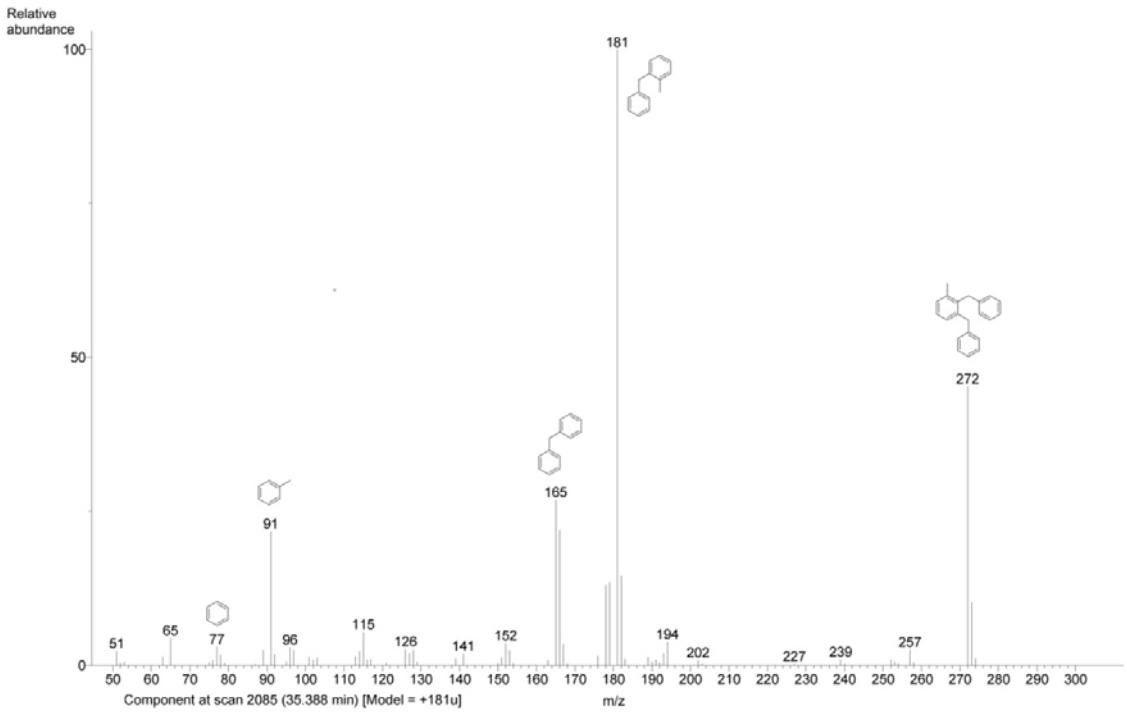


Figure 2-26 Mass spectrum of methylbisdiphenylmethane (MBDPM) as well as fragment annotation using MetFrag and manual interpretation (scan 2085 at 35.39 min in sample R1 11/05-1 F4)

Figure 2-26 shows the mass spectrum of MBDPM and MetFrag fragment annotations of MBDPM with a base ion peak at m/z 181 (M-91; [C14H13]) and a big molecular ion peak at m/z 272 as well as smaller fragments at m/z 165 (M-107; [C13H9]), at m/z 91 (M-181; [C7H7H]) and at m/z 77 (M-195; [C6H5H]). The match value in NIST MS search was 819 referring to Wiley 9 library entry and MetFrag explained four of the most abundant peaks in the mass spectrum. However, MetFrag did not predict the peak at m/z 165. Hence, manual interpretation and calculation was necessary. The comparison with the spectrum of MDPM in Figure 2-25 shows similarities and thus three evidences confirm the identity of MBDPM as well.

MDPM and MBDPM are used as substituents for PCBs in transformers and capacitors as well as in heat transfer applications (Martin and van Kessel 2006, Miranda et al. 2000). MBDPM is distributed under the commercial names Marlotherm® S or Neo-Sk Oil 1400. One study reported crude oil as a possible source thereof (Belkina et al. 2008). To my knowledge, benzyltoluenes were not systematically investigated as SPM contaminants in a monitoring study before. Thus, this investigation reported these compounds for the first time as pollutants in SPM. According to the European inventory of existing commercial chemical substances (European Commission 2012), they are registered as low product volume compounds. Even though, they were detected several times in the Rhine catchment and thus they should be considered as emerging contaminants in future monitoring activities.

2.4 Conclusions

The first comprehensive suspect and nontarget screening of sediments and suspended particulate matter of the Rhine and the Saar revealed a broad range of nonregulated compounds with adverse effect potentials to aquatic ecosystems. The role of the anthropogenic compound α-tocopherol acetate (α-TA) as a marker for municipal wastewater discharge in SPM and sediments was discussed and confirmed due to its presence in most SPM samples. The *in silico* fragmentation program MetFrag was used to confirm the tentative identification of α-TA, dioctyldiphenylamine, methyldiphenylmethane and methylbisdiphenylmehthane as only rarely reported anthropogenic marker compounds in sediments and SPM. This is one of the first applications of MetFrag for the confirmation of electron ionization mass spectra of environmental samples. The combination of multitarget analysis with univariate and multvariate statistical methods such as analysis of variance and

self-organizing maps helped to draw a vital picture of the regional distribution and possible sources of nonpolar organic pollutants in the Rhine and the Saar catchments.

2.5 Acknowledgements

I am grateful to Dr. Christa Schröter-Kermani from Federal Environmental Agency (UBA, Berlin, Germany) Dr. Martin Krauss from Helmholtz Centre for Environmental Research (UFZ, Leipzig, Germany) for review and helpful comments on the manuscript. I am particularly grateful to the student's assistants and technicians from Department of Geosciences (Freie Universität Berlin) that helped me with the field and laboratory work. The dating of the sediment cores and interpretation of radiometric data was realized by the Department of Environmental Physics (Prof. Dr. Augusto Mangini and Dr. Clemens Woda, Heidelberg Academy of Science, Heidelberg, Germany) funded by a third-party sub-contract. This study was funded by the German Federal Environmental Agency (FKZ 301 02 013, FKZ 301 02 018). A free academic license of Marvin Sketch and Calculation Plugins (Chemaxon Ltd., Budapest, Hungary) was used to draw chemical structures and calculate chemical properties.

Chapter B

On the extractability and effect potentials of organic compounds in river sediments

3 Comparison of different exhaustive and one biomimetic extraction techniques for chemical and biological analysis of polycyclic aromatic compounds in river sediments [14] [15]

3.1 Introduction

A river basin management that accounts for the probability of contaminant emission and their potential impact on the environment is an inherent part of the European Water Framework Directive (WFD, European Community 2000). Thus, risk-based sediment management strategies considering these issues have been recommended (Apitz 2006, Chapman and Hollert 2006, Förstner 2002, Quevauviller 2006) to estimate negative effects of dredging (Babut et al. 2006, den Besten et al. 2003) and floods (Baborowski et al. 2005, Hollert et al. 2000, Wölz et al. 2010b). In this approach, river sediments are recognized as secondary sources of contaminants, and suspended particulate matter (SPM) act as a carrier of these contaminants (Power and Chapman 1992, Schulze et al. 2007a).

The consideration of bioaccessible and bioavailable fractions of contaminants in sediments and riparian soils is a key part of the assessment of their likely risks to the aquatic environment (Brack et al. 2009b, Brack and Burgess 2011). However, no comprehensive concept for analysis exists (Brack et al. 2009b, Ehlers and Loibner 2006, Reichenberg et al. 2006). Although sediment contact tests are tools to characterize best the bioavailability in toxicity testing (Kosmehl et al. 2006, Kraaij et al. 2002), they do not allow to identify toxicant and receptor targets. In effect-directed analysis (EDA) or toxicity identification evaluation procedures, the bioavailability is considered as a main challenge to avoid bias in the prioritization of toxic fractions and compounds towards compounds which are poorly or not bioavailable (Brack et al. 2009b, Brack and Burgess 2011). New partition-based dosing techniques for the direct usage in biotests were recently published (Bandow 2011, Bandow et al. 2009, Bougeard et al. 2011, Brack et al. 2009b, Fai et al. 2009, Schwab et al. 2009, Smith et al. 2010, Smith et al. 2009).

[14] The original publication is available at www.springerlink.com: **Schulze, T.**; Seiler, T.-B.; Streck, G.; Braunbeck, T.; Hollert, H.: Comparison of different exhaustive and one biomimetic extraction techniques for chemical and biological analysis of polycyclic aromatic compounds in river sediments; Journal of Soils and Sediments 12, 1419-1434 (10.1007/s11368-012-0574-1)

[15] A previous manuscript of this section is part of the PhD thesis of Dr. Thomas-Benjamin Seiler (2010): Total or biomimetic extracts or direct contact exposure? Comparative research towards a realistic ecotoxicological characterisation of sediments; PhD thesis, Ruperto Carola University of Heidelberg, Heidelberg, 340 pp

The definition of bioavailability and bioaccessibility is a topic of controversial discussion (Brack et al. 2009b, Reichenberg et al. 2006, Semple et al. 2004). Bioavailability is a complex phenomenon of several processes including environmental availability or bioaccessibility (i.e. physico-chemical interactions of the contaminants with water and sediment), environmental bioavailability (i.e. uptake or partitioning of freely dissolved molecules into the organism according to the chemical activity), and toxicological bioavailability (i.e. toxicokinetics in the organism including internal transport, metabolism and excretion resulting in a toxicant concentration at the site of the target receptor; Brack et al. 2009b, DIN EN ISO 17402, Seiler et al. 2008). Bioaccessibility is defined as the fraction of a contaminant that is readily desorbable from the sediment particles encompassing the actual bioavailable fraction along with that what is potentially bioavailable (Semple et al. 2004). However, bioaccessibility is operationally defined and related to extraction procedures mimicking the rapid desorption of loosely bound compounds (Brack et al. 2009b, Reichenberg et al. 2006). Residual compounds, which are not readily available or extractable are either absorbed inside the sediment matrix with (very) slow long-term desorption or chemically bound to the particles (bound residues; Puglisi et al. 2007b) that are hardly accessible for the majority of organisms under natural conditions (Ehlers and Luthy 2003b, Mayer and Reichenberg 2006, Northcott and Jones 2000, Semple et al. 2004).

The extractability of organic compounds highly depends on the individual sample matrix and the extraction technique providing different levels of contaminant fractions in order of the bioaccessible and residual fraction as well as the bound residues (Ehlers and Loibner 2006). Various physico-chemical extraction methods were developed for the assessment and prediction of the bioaccessible and the determination of the residual fractions, respectively. Methods mimicking bioaccessibility are extraction with absorber resins such as Tenax®-TA (Cornelissen et al. 1997, Kraaij et al. 2002, Leppänen and Kukkonen 2006, MacRae and Hall 1998, Schwab et al. 2009, Schwab and Brack 2007, Ten Hulscher et al. 2003) or XAD (Cornelissen et al. 1997, Lei et al. 2004, Lu et al. 2006) as well as extraction with surfactants (Brown 2007, Cuypers et al. 2002, Guha et al. 1998) or hydroxypropyl-β-cyclodextrin (HBCD; Allan et al. 2006, Bergknut et al. 2007, Cuypers et al. 2002, Sabaté et al. 2005). Methods using weak solvents for extraction such as butanol (Kelsey et al. 1997, Swindell and Reid 2006, Tang et al. 1999) or mixtures of methanol and water (Chung and Alexander 1998, Kelsey et al. 1997) may overestimate the bioavailable fraction depending on the sediments' or soils' properties (Kelsey et al. 1997). Commonly used vigorous methods for the extraction

of the residual fractions are for example, pressurized liquid extraction (PLE), membrane dialysis extraction (MDE), microwave-assisted extraction (MAE), Soxhlet extraction (SOX) and ultrasonic-assisted extraction (USE; e.g., Bandh et al. 2000, Bossio et al. 2008, Dean and Xiong 2000, Gevao et al. 2000, Heemken et al. 1997, Kronimus and Schwarzbauer 2007, Northcott and Jones 2000, Richnow et al. 1997, Saim et al. 1997, Schwarzbauer et al. 2003b, Song et al. 2002, Sporring et al. 2005). Extraction of the bound residue or nonextractable fraction requires degradation procedures such as alkaline and acidic hydrolysis, boron tribromide treatment or ruthenium tetraoxide oxidation in order to cleave ether and ester bonds as well as to degrade aromatic structures and activated carbon-carbon bonds formed by compound and organic matrix interactions (Eschenbach et al. 1994).

The exhaustive extraction of PAHs from soils, sediments or suspended particulate matter is commonly performed using vigorous extraction techniques such as MAE with acetone, a mixture of *n*-hexane/acetone (1:1,v/v) or toluene (Dean and Xiong 2000, Saim et al. 1997), PLE with acetone, mixtures of acetone/dichloromethane (1:1,v/v), *n*-hexane/acetone (1:1,v/v) or dichloromethane (DCM; Bandh et al. 2000, Dean and Xiong 2000, Heemken et al. 1997), SOX with acetone (Hollert et al. 2000, Saim et al. 1997), MDE with *n*-hexane (Seiler et al. 2006) or USE with *n*-hexane/acetone, or isopropyl alcohol (Banjoo and Nelson 2005, Bossio et al. 2008). For the determination of the bioaccessible fraction of PAHs, TENAX-TA® extraction in sediment-water slurries over 6 h or 24 h (Cornelissen et al. 2001, Leppänen and Kukkonen 2006, Schwab and Brack 2007, Ten Hulscher et al. 2003), 50 mM HBCD extraction over 20 h (Cuypers et al. 2002, Doick et al. 2005a, Reid et al. 2000), or passive sampling methods such as semipermeable membrane devices, polyoxymethylene, or polydimethylsiloxane (Jonker and Koelmans 2001, Leppänen and Kukkonen 2006, Mayer et al. 2000) are used.

HBCD is a water-soluble torus-shaped cyclic oligosaccharide with a hydrophilic shell and a hydrophobic center (cavity) of 7.5-8.3 Å (Shieh and Hedges 1996) that has the ability to form 1:1 (target compound : HBCD molecule) or 1:2 inclusion complexes with hydrophobic compounds (Shieh and Hedges 1996, Wang and Brusseau 1993). In consequence, the water solubility of such compounds is elevated; because (1) water tension at the water-sediment interface is decreased (Wang and Brusseau 1993) and (2) the HBCD complexes with substances show a hydrophobic affinity (Mayer et al. 2005). For the extraction of the bioaccessible fraction of sediment or soil bound hydrophobic organic compounds HBCD is

used (Allan et al. 2006, Cuypers et al. 2002, Doick et al. 2005b, Papadopoulos et al. 2007, Reid et al. 2000, Sabaté et al. 2005, Stokes et al. 2005, Swindell and Reid 2006, van der Heijden and Jonker 2009). Van der Heijden and Jonker (2009) have shown a good, but not significant relationship between HBCD extraction and PAHs uptake in the aquatic worm *Lumbriculus variegatus*, but remarked that overestimation of PAHs may occur due to co-extraction of dissolved organic matter bound PAHs. Furthermore, it was suggested that higher condensed PAHs such as benzo[a]pyrene may not desorb very well from sediments due to low water solubility (Cuypers et al. 2002) and that large molecules and cavity sizes do not fit (Brack and Burgess 2011).

The presence and quality of black carbon in river sediments has an influence on the sorption of planar hydrophobic organic compounds such as PAHs and thus affects bioaccessibility of these substances (Johnsen and Karlson 2007, Oen et al. 2006, Rhodes et al. 2008). Hence, the consideration of black carbon in risk assessment of sediments is highly recommended in the literature (Cornelissen et al. 2005, Koelmans et al. 2009, Oen et al. 2006).

The purpose of the present study was to examine contaminated river sediments for extractability and toxicity with procedures representing nondepletive and vigorous extraction (Soxhlet extraction (SOX), membrane dialysis extraction (MDE), hydroxypropyl-β-cyclodextrin extraction (HBCD), ultrasonic extraction (USE), hydrolysis) using two biotests (the neutral red test for cytotoxicity and the 7-ethoxyresorufin-O-deethylase induction assay (EROD)) induction assay) with the permanent fish cell line RTL-W1) in order to improve the understanding and interpretation of lab-based sediment assessment. A schematic overview on the extraction and analytical steps is shown in Figure 3-1. The role of black carbon present in the sediments will also be discussed with respect to risk assessment. A confirmation of the effects was performed using different approaches namely biological toxicity equivalents (Bio-TEQ) and chemical toxicity equivalents (Chem-TEQ) values as well as the index of confirmation quality according to Grote et al. (2005).

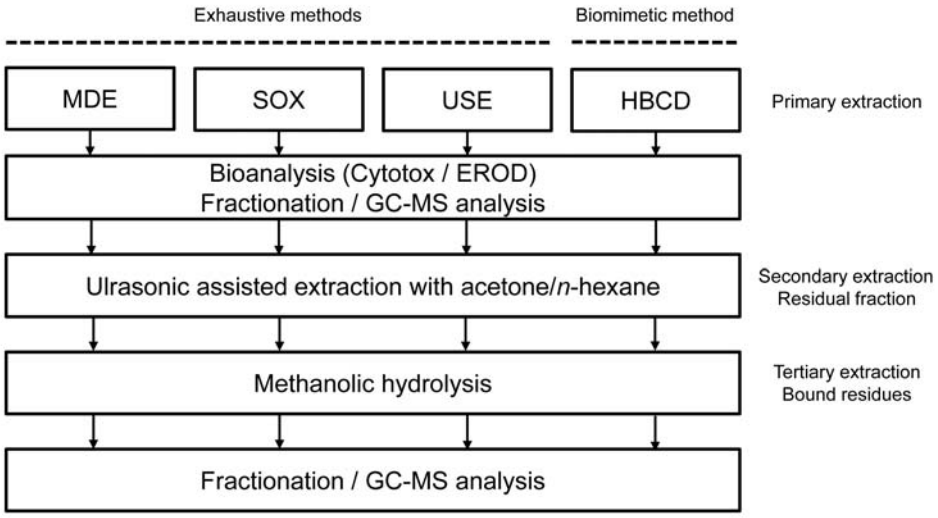


Figure 3-1 Scheme of the study design with the different extraction and analysis steps; MDE: membrane dialysis extraction with n-hexane, SOX: Soxhlet extraction with acetone, USE: ultrasonic assisted extraction with acetone, HBCD: extraction with 2-hydroxypropyl)-β-cyclodextrin, Cytotox: neutral red retention assay, EROD: 7-ethoxyresorufin-O-deethylase induction assay, GC-MS: gas chromatography – mass spectrometry

3.2 Materials and methods

3.2.1 Chemicals

The solvents used were Picograde® purchased from LGC Promochem (Wesel, Germany) if not noted otherwise. All certified reference standard solutions for chemical analysis were obtained from Dr. Ehrenstorfer (Augsburg, Germany) or LGC Promochem (Wesel, Germany). Other chemicals were supplied by Sigma-Aldrich (Deisenhofen, Germany) or Merck (Darmstadt, Germany). All gases (helium 5.0, nitrogen 5.0) used were delivered by Messer (Griesheim, Germany).

3.2.2 Study area and sampling

The sediment samples were collected at two locations at the Saar (Germany) using a stainless steel Van Veen grab sampler (Hydrobios, Kiel, Germany). Site S1 (49°-22'-18''-N, 7°-02'-16''-E; Saar km 90.1) is located at the east harbor (Saarbrücken, Germany), whereas site S2 (49°-36'-95'-N, 6°-70'-00''-E; Saar km 54.7) is located upstream to Rehlingen Barrage

(Schulze et al. 2007). The Saar is characterized as a heavily modified water body according to (European Community 2000).

3.2.3 Sample storage and preparation

The samples were homogenized manually for 5 min by means of a polypropylene spatula in a stainless steel tub and transferred to stainless steel containers. Afterwards, they were shock-frozen using dry ice and stored at -18 °C. The frozen samples were freeze-dried (Christ, Osterode Germany), sieved through a 2 mm stainless steel test sieve with a hole-plate (mesh: 2 mm; Retsch GmbH, Haan, Germany) and stored in amber bottles at -30 °C in the dark until analysis.

The unfrozen wet samples for analysis of grain size distribution were stored at 4 °C and processed within a week to account for possible alteration. For analysis of grain size distribution, a portion of the unfrozen and homogenized samples were stored in polyethylene bottles for a maximum of 1 week analysis of grain size distribution and stored at 4 °C. to account for possible alteration.

3.2.4 Grain size distribution, total organic carbon and black carbon

Standard procedures were used for analysis of grain size distribution according to ISO 11277 (meshes: 2 mm, 630 µm, 200 µm, 63 µm, 20 µm). Differing from ISO 11277, the sediments were not treated with acid or hydrogen peroxide to remove carbonate or organic matter. Loss on ignition (LOI) was determined at 550 °C using a muffle furnace (Heraeus, Hanau, Germany) according to DIN 19684-3. The contents of total organic carbon (TOC) were analyzed by means of a C-Mat 500 (Stroehlein Instruments, Juwe GmbH, Viersen, Germany) according to ISO 10694. Briefly, contents of total carbon (TC) were determined by incineration of the sediments at 1000 °C in a constant flow of oxygen and infrared spectrometric analysis of carbon dioxide. Inorganic carbon (TIC) was analyzed by decomposition of the carbonates using phosphoric acid (42%) at a temperature of 70 °C and infrared determination of carbon dioxide. The TOC was calculated as TOC=TC-TIC. Pure calcium carbonate (Merck, Darmstadt, Germany) was used for calibration.

Incineration of sediments at 375 °C for 24 h in a muffle furnace in an oxygen-rich atmosphere and subsequent analysis of organic carbon contents is a common method for estimation of

black carbon contents (Cornelissen et al. 2004, Gustafsson et al. 1997, Sundelin et al. 2004). TOC contents in the incineration residue were defined operationally as the BC. Aliquots of 0.5 g of freeze-dried sediments were treated accordingly.

3.2.5 Extraction methods

3.2.5.1 Soxhlet extraction

Soxhlet extraction (SOX) was carried out with 20 g portions of freeze-dried sediment, weighed into 100 ml extraction thimbles (Schleicher and Schuell, Dassel, Germany) and extracted for 8 h at 12 cycles per hour using 200 ml acetone (Hallare et al. 2005) to obtain the acetone-extractable fraction (Hallare et al. 2005, Ulrich et al. 2002).

3.2.5.2 Membrane dialysis extraction

Membrane dialysis extraction (MDE) was applied according to Seiler et al. (2006) with slight changes modifications: as detailed below. Portions of dry sediment (2.5 g) were filled into pre-extracted (48h, *n*-hexane, p.a. grade, Merck) low-density polyethylene (LDPE) dialysis membranes (Jencons, Leighton Buzzard, UK). Sediments were evenly distributed and spread through the interior of the membrane, and any air was expelled by means of a bent glass rod, prior to introduction of the membrane into a 250 ml brown glass jar containing 200 ml *n*-hexane (p.a.; Merck). Membrane ends were secured and sealed with the surface grinded lid to give a seal-to-seal length of 75 cm (equivalent to a diffusive surface area of 375 cm2). Dialysis was then allowed to proceed for 48 h at room temperature.

3.2.5.3 Ultrasonic extraction

Ultrasonic extraction (USE) was performed with 10 g portions of freeze-dried sediment under different conditions: (1) Sediments were one-time extracted ultrasonically for 15 min with 40 ml acetone (Babić et al. 1998) after vortex mixing for 2 min and horizontally shaken for 1 h at room temperature and 100 rpm. (2) Sediments were double double-extracted ultrasonically at 35 kHz (Sonorex Super RK 514, Bandelin, Berlin, Germany) for 15 min with 40 ml of 1:1 mixture of *n*-hexane/acetone (1:1; Banjoo and Nelson 2005) after vortex mixing for 2 min and shaken for 1 h at room temperature and 100 rpm using an orbital shaker (IKA, Stauffen, Germany). (2) Sediments were extracted once by means of ultrasonication for 15 min with 40

ml acetone after vortex mixing for 2 min (IKA, Stauffen, Germany) and horizontally shaken for 1 h at room temperature and 100 rpm).

3.2.5.4 Extraction with 2-hydroxypropyl-β-cyclodextrin

Extraction with 2-hydroxypropyl-β-cyclodextrin (HBCD) was carried out according to Reid et al. (2000). Briefly, 5 g of freeze-dried sediments were extracted in centrifugation jars with polytetrafluorethylene (PTFE)-coated screw caps with 100 ml of 50 mM HBCD (Sigma-Aldrich) in purified water (SERALPUR Pro 90 CN, Seral, Gelman Sciences Inc., Ann Arbour, U.S.A.) by means of orbital shaking for 20 h at 20 ± 2 °C and 100 rpm (IKA, Stauffen, Germany). The supernatant was removed by centrifugation at 2000 × G and subsequently liquid-liquid extracted 3 x 5 minutes with 20 ml dichloromethane (DCM) at pH 2 (acidified with 1 M hydrochloric acid, Suprapur®, Merck; Schwarzbauer et al. 2003a), thus dissipating the HBCD complexes to glucose at pHs <3 (Saenger 1980, Schwarz-Barać 2003).

3.2.5.5 Extraction and hydrolysis of sediment residues

Sediment residues derived from SOX, MDE, HBCD and USE method 1 were extracted twice by ultrasonic extraction with *n*-hexane/acetone as describe above (USE method 2). Sediment residues of this extraction step were hydrolyzed using 2 M KOH (p.a. grade, Merck) in methanol (Eschenbach et al. 1994) for 1 h at 70 °C in centrifugation jars with PTFE-coated screw caps. The supernatant was removed, passed through a glass microfiber filter (GF/C, Whatman, Brentfort, UK) and liquid-liquid-extracted as described above. Following extraction, the organic extracts were dried with Na_2SO_4 (organic analysis grade, Merck), reduced in volume by means of rotary evaporation and then concentrated close to dryness under a gentle N_2-stream. Finally, the solvent was changed to 1 ml of *n*-hexane. Vortex mixing for 2 min was used to dispense sediments and solvents in centrifugation jars.

3.2.6 Extracts preparation

Extracts from primary extraction step using SOX, MDE, HBCD and USE approaches were reduced in volume using rotary evaporation and split into equal aliquots for mass spectrometric and ecotoxicological analysis. The aliquots were evaporated close to dryness under a gentle stream of N_2 and dissolved in 0.5 ml *n*-hexane for chemical or in 0.5 ml

dimethylsulfoxide (DMSO; Merck, Darmstadt, Germany) for ecotoxicological analysis resulting in final concentrations of 5 g/ml (HBCD extracts) or 10 g/ml (all other extracts).

3.2.7 Silica gel fractionation

Organic extracts were separated into six fractions by column chromatography (2 g silica gel 60, Merck, Darmstadt, Germany; Ricking and Terytze 1999). Mixtures of n-pentane, dichloromethane and methanol were used as eluents (Bundt et al. 1991, Heim et al. 2005). Extracts from hydrolysis were separated into two fractions using dichloromethane (fraction 1) and methanol (fraction 2) as eluents (Schwarzbauer et al. 2003a). The fractions were reduced to 200 µl under a gentle stream of N_2.

3.2.8 Gas chromatography – mass spectrometry

Gas chromatographic – mass spectrometric (GC-MS) analysis was carried out on a Hewlett-Packard HP 5890 II GC coupled to a HP MSD 5971 A (Agilent, Palo Alto, USA), equipped with a 60 m x 0.25 mm i.d. x 0.25 µm film DB-XLB fused capillary silica column (Agilent J&W, Folsom, USA). Chromatographic conditions were as follows: 300 °C injector temperature, 2-µl splitless injection at an oven temperature of 80 °C, then programmed at 4 °C/min to 310 °C (25 min isotherm). Carrier gas velocity (Helium 5.0) was 25 cm/s.

The mass spectrometer was operated in electron ionization mode (EI+, 70 eV) with a source temperature of 180 °C. External five-point-calibration was used for quantification of the 16 EPA-PAHs and additionally benzo[e]pyrene and perylene in single ion monitoring mode (SIM). The latter two compounds were quantified using the response ratio to benzo[a]pyrene. Results were corrected for errors due to injection and matrix effects using an internal standard solution containing 4.0 ng/µl acenapthene-D_{10}, phenanthrene-D_{12}, chrysene-D_{12} and perylene-D_{12}, which were that was added to the samples prior to analysis with recoveries from 63% to 101%. The instrumental limit of detection (LOD) was in the range of 0.03–1.2 ng/g dry weight (dw), and the limit of quantification (LOQ) was in the range of 0.3–4 ng/g dw. The LOD was defined as three times the signal-to-noise ratio (S/N) and the LOQ as ten times the S/N of the analyte peak, respectively.

Selected samples were analyzed in scan mode with 50–550 amu and a scan time of 1.5 scans/s under the identical chromatographic conditions. Since sediments of the sampling

locations carry only very low amounts of polychlorinated biphenyls and no organochlorine compounds such as HCH, HCB or DDX (DDT, DDD, DDE; Schulze et al. 2005b), these compounds were not included in this study.

3.2.9 Toxicity testing

3.2.9.1 Neutral Red retention assay

Acute cytotoxic effects were determined using the neutral red retention assay (Babich and Borenfreund 1992) with slight modifications as described by Seiler et al. (2006). Cells from CYP1A-expressing cell line RTL-W1 (Bols et al. 1999, Lee et al. 1993) were exposed to serial dilutions of sediment extracts along seven wells in six replicates of a 96-well microtitre plate (TPP, Trasadingen, Switzerland) at a final concentration range of 1.56–100 mg/ml. As a positive control, 3,5-dichlorophenol at a concentration of 40 mg/l was used. After incubation at 20 °C for 48 h, cells were incubated with neutral red (2-methyl-3-amino-7-dimethylamino-phenanzine) for 3 h, and neutral red retention was determined after solution at 540 nm with a reference wavelength of 690 nm using a GENios plate reader (Tecan, Crailsheim, Germany).

3.2.9.2 EROD induction assay

The dioxin-like activity of sediment extracts was assayed using the 7-ethoxyresorufin-O-deethylas induction assay (EROD; Behrens et al. 1998). RTL-W1 cells were seeded into 96-well microtitre plates and exposed to sediment extracts in 8 dilution steps with 6 replicates. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was serially diluted on two separate rows of each plate as a positive control. Following incubation at 20 °C for 72 h, EROD induction was terminated by disrupting the cells at -80 °C. Subsequently, 100 µl of the substrate 7-ethoxyresorufin were added to each well, before deethylation was initiated for 10 min with nicotinamide adenine dinucleotide phosphate in phosphate buffer. The reaction was stopped by adding 100 µl of fluorescamine in acetonitrile. The production of resorufin as a metabolite of the substrate was recorded fluorometrically at 544 nm (excitation) and 590 nm (emission) using a GENios plate reader. Whole protein was also determined fluorometrically using the fluorescamine method (excitation 360 nm, emission 465 nm; Hollert et al. 2002, Kennedy and Jones 1994). Fluorescent units were converted to mass of resorufin and protein with the aid via calibration curves. The variability of the positive control TCDD in our test system was ± 35% (EROD EC_{25}; n = 59; Keiter et al. 2008).

3.2.10 Data analysis

3.2.10.1 Concentration-response relationships

Concentration-response relationships for neutral red retention and EROD induction were plotted with GraphPad Prism® 5 (GraphPad 2007) using a sigmoid log-logistic Hill-function or a second order polynomial function.

3.2.10.2 Bio-TEQ values

Bioassay-derived TCDD equivalents (Bio-TEQs) were calculated through by relating the biological EROD activities of the samples to the positive control TCDD using the fixed effect level quantification method (Brack et al. 2000, Wölz et al. 2008). Mean TCDD-EC_{25} and standard deviation (SD) values were determined using a sigmoid log-logistic Hill-function with GraphPad Prism® 5. Bio-TEQs with concentrations in pg TCDD per gram of sample equivalent (SEQ) were computed given as pg per gram (Equation 3–1):

$$Bio - TEQ\left[\frac{pgTCDD}{gSEQ}\right] = \frac{TCDD{-}EC_{25}[pgTCDD/ml]}{sample{-}EC_{25}[gSEQ/ml]} \qquad (3\text{–}1)$$

3.2.10.3 Chem-TEQ values

In order to explain Bio-TEQs, chemically derived TEQ values (Chem-TEQs) were calculated using relative potency factors (REP; Bols et al. 1999; Equation 3–2):

$$Chem - TEQ\left[\frac{pgTCDD}{gSEQ}\right] = \sum_i (c_i \times TEF_i) \qquad (3\text{–}2)$$

Where for a given chemical *i*, c_i is the measured concentration in the sample and TEF_i is the toxic equivalency factor for each compound relative to TCDD. For calculation TEFs according to Clemons et al. (1997) derived for RTL-W1 cells were used.

3.2.10.4 Effects confirmation of EROD induction

Confirmation of the EROD induction was performed based on artificial mixtures prepared in order of the identified compounds in the samples and their concentrations using the index of confirmation quality (ICQ) approach (Grote et al. 2005). The ICQ is a quantitative measure of

the similarity or dissimilarity between the concentration-response relationships of the original sample and the identified compound(s) or an artificial mixture thereof. It is defined as the ratio of the effects concentration of the original fraction $EC_x^{original}$ and the artificial mixture EC_x^{mix} at a specific effect level x and can be calculated over a range of different effect levels (Equation 3–3):

$$ICQ = \frac{EC_x^{original}}{EC_x^{mix}} \quad (3–3)$$

It was applied here for effect levels from 15% to 90%. Analysis of the ICQ data was performed using a sigmoid log-logistic Hill-model or a second order polynomial model with GraphPad Prism® 5 and EC_x calculation using Microsoft Excel®. It has been proven as suitable tool for effects confirmation in different effect-directed analysis related studies (Bandow 2011, Grote et al. 2005, Schulze et al. 2010, Schwab et al. 2009).

3.2.10.5 Statistics

Significance testing was performed using one-way analysis of variance (ANOVA) followed by Tukey's posttest or, in cases Bartlett's test showed significant differences of variances, the nonparametric Kruskal-Wallis ANOVA followed by Dunn's posttest to elucidate significant differences between the extraction methods using GraphPad Prism® 5. The significance level was α=0.05 and *p*-values <0.05 were considered as significant. Vapor pressure values were computed using EPISUITE™ 4.1 (US-EPA 2008).

3.3 Results and discussion

3.3.1 Grain size distribution

The grain size distribution revealed a high content of particles below 63 µm for both sediments (Table 3-1). However, the sediment S2 contained a larger fraction of finder-grained sediments than sediment S1. Overall, in comparison with sediment data from other German rivers, S1 could be classified as a coarse and S2 as a medium-grained sediment (Schulze et al. 2007a). Due to possible alterations during sieving procedures, the sediments were only sieved below 2 mm to normalize for the further experiments.

Table 3-1 Sample codes sand physico-chemical characterization of the sediment samples (LOI: loss on ignition; TOC: total organic carbon; BC: black carbon). LOI, TOC and BC data are given as means with standard deviations

Sample code	Carbon Contents (%)			Grain size (%)			
	LOI	TOC	BC	<20 µm	20-63 µm	63-200 µm	200-2000 µm
S1	7.4 ± 1.4	3.0 ± 0.8	0.4 ± 0.1	22.4	19.4	43.4	14.8
S2	10.3 ± 0.1	4.5 ± 0.1	0.9 ± 0.1	44.1	25.6	28.5	1.8

3.3.2 Extractability of PAHs

The primary extraction step was performed with different extraction approaches mimicking bioaccessibility (HBCD; Cuypers et al. 2002, Fai et al. 2009, Reid et al. 2004, Rhodes et al. 2010, van der Heijden and Jonker 2009), vigorous extraction (MDE, SOX; Seiler et al. 2006, Seiler et al. 2008) and additionally with ultrasonic assisted extraction (USE; Banjoo and Nelson 2005).

The sum concentrations of PAHs ranged between 256 ng/g dw for HBCD and 6948.5 ng/g dw for SOX extraction for S1 and between 242.8 ng/g dw for HBCD and 6741.9 for MDE extraction for S2 for the primary extraction step. With both sediment samples, the HBCD extraction resulted in the lowest amounts after the primary extraction step with clear differences compared to USE, MDE and SOX (Figure 3-2) and on average 3.4% of the whole PAH fraction in the sediments. The USE extraction yielded significantly higher amounts than HBCD (p<0.05, Kruskal-Wallis ANOVA with Dunn's posttest), but was less vigorous than

MDE and SOX (Figure 3-2a). MDE and SOX, which that were considered to be the depletive extraction methods (Hawthorne et al. 2000, Seiler et al. 2006), gave elicited the highest concentrations for the first extraction step (Figure 3-2a).

The second extraction step was carried out with residual sediments from extraction step 1 to estimate the completeness of the first extraction step. As expected, considerable amounts of PAHs (8322.3 ng/g dw (S1) and 5414 ng/g dw (S2) were extracted in the second extraction step of HBCD extraction (Figure 3-2a). The residues in sediment S1 after MDE extraction were bigger than that of S2 due to possibly incomplete extraction. The extraction using SOX might have been vigorous in the primary extraction step (Figure 3-2a). However, in total, there appeared to be a no significant loss of PAHs during SOX extraction and subsequent procedures (Figure 3-2a; $p>0.05$, Kruskal-Wallis ANOVA with Dunn's posttest). The loss of PAHs was not related to volatilization of compounds, because the PAH fractions grouped by numbers of aromatic rings were comparable for both sediments and extraction methods (Figure 3-2a).

Subsequently, the third extraction step including hydrolysis was applied to determine the bound residues from macromolecular sediment organic matter due to cleavage of ester bonds by chemical degradation (Eschenbach et al. 1994, Hawthorne et al. 2000, Schwarzbauer et al. 2003a). This step resulted in significant two- to ten-fold higher residues in HBCD extraction compared to USE, MDE and SOX extraction (Figure 3-2a). Residues after USE, MDE and SOX extraction were comparable. For the sum PAH concentrations from all extraction steps, no significant divergences between the total amounts of PAHs extracted were found (Figure 3-2a).

Results for MDE and SOX extraction indicated similar extractability or extraction power of the primary extraction step. USE showed an extraction power between the HBCD approach and the MDE as well as SOX and MDE method. HBCD gained a low quantity of 3.4% of the whole PAH fraction in the first extraction step – operationally defined as the bioaccessible fraction. These latter results were comparable to the findings of Reichenberg et al. (2010) who found an average of 4% of PAH accessible in a gas plant soil. It has to be considered that sediments were shock-frozen and freeze-dried, which may have modified physical properties of the sediments and thus desorption kinetics.

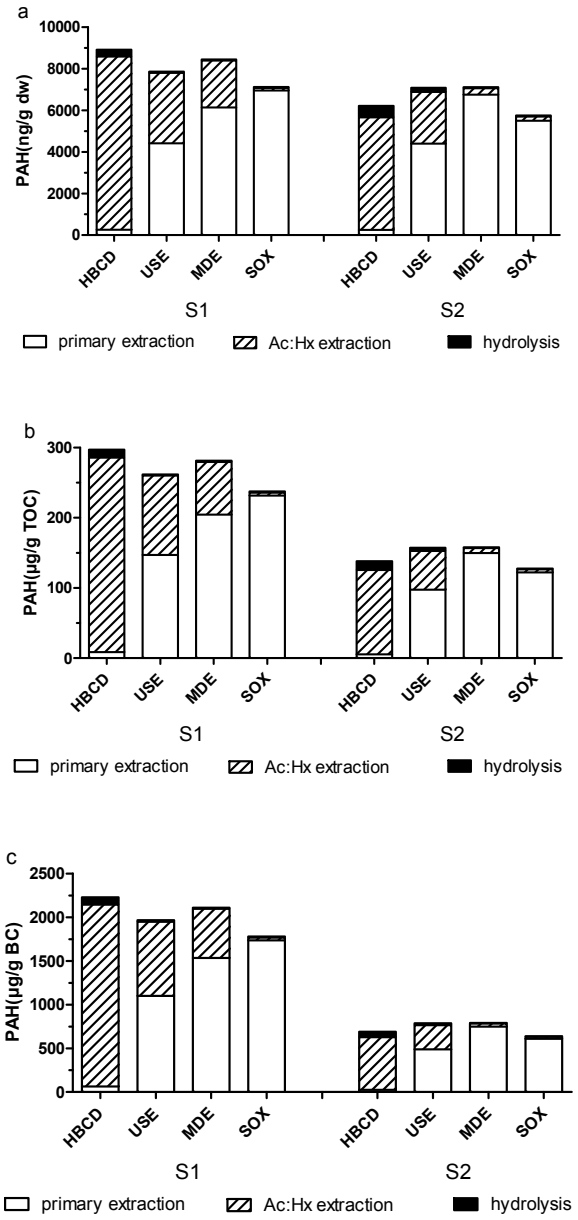


Figure 3-2 Sum of PAH concentrations in extracts for sediment samples S1 and S2 from the primary extraction step (open), the second extraction step with n-hexane/acetone (diagonal pinstriped) and the hydrolysis step (solid); HBCD: 2-hydroxypropyl)-β-cyclodextrin; USE: ultrasonic extraction; MDE: membrane dialysis extraction; SOX: Soxhlet extraction); (A) concentrations given in ng/g dw (dw: dry weight), (B) concentration normalized to the content of total organic carbon (TOC), (C) concentrations normalized to the content of black carbon (BC).

In consequence, chemical composition and toxicity may be altered (Seiler et al. 2008, Zielke et al. 2011). Furthermore, HBCD could show effects at least as extracts obtained with MDE or Soxhlet in the zebrafish (*Danio rerio*) embryo test (Zielke et al. 2011). The authors suggested short aging periods of the investigated spiked sediments to be responsible for the unexpected results. This could have lead to a high level of accessibility and extractability of the test substances and, thus, equalized the differences in extraction power of the SOX, MDE and HBCD procedures. The findings are therefore not in contrast to observations of several studies that have classified HBCD as a biomimetic method (e.g., Allan et al. 2006, Bergknut et al. 2007, Cuypers et al. 2002, Reid et al. 2000, van der Heijden and Jonker 2009).

In these studies, HBCD extraction was compared with the bioavailability of PAHs in soils and sediments to bacteria communities (Allan et al. 2006, Cuypers et al. 2002, Reid et al. 2000), aquatic worms (*L. variegatus*, Bergknut et al. 2007, Gomez-Eyles et al. 2010, van der Heijden and Jonker 2009, and earth worms (*Eisenia fetida*). In the first two cases, good correlations were found between the fraction of PAHs accumulated in *L. variegatus* or mineralized by bacteria and those extracted by HBCD. In the latter case, only poor relationships were found due to different exposition pathways. In the sediment-water slurry used in bacteria tests and HBCD extraction, partitioning between sediment and water influences the partitioning of PAHs to bacteria, aquatic worms or HBCD, whereas earth worms are exposed to soils directly or by dietary uptake (Bergknut et al. 2007).

3.3.3 Compound specific extractability of PAH

Figure 3-3 illustrates PAH fractions grouped by the numbers of aromatic rings. With the first extraction step, similar fractions were extracted through USE, SOX and MDE (Figure 3-3a, and Figure 3-3b). The relative compositions of the HBCD extracts were different from those yielded with the other extraction methods confirmed by principal component analysis (Figure S3-1 in Appendix). Especially, the fractions of five- and six-ring PAHs in HBCD extracts were found in slightly higher relative abundances compared to lower condensed PAH fractions. HBCD is a water-soluble torus-shaped cyclic oligosaccharide with a hydrophilic shell and a hydrophobic center (cavity) that has the ability to form inclusion complexes with hydrophobic compounds (Wang and Brusseau 1993). In consequence, the water solubility of such compounds is elevated, because (1) water tension at the surface water-sediment is

decreased (Wang and Brusseau 1993) and (2) the HBCD complexes with substances show a hydrophobic affinity (Mayer et al. 2005).

However, the sole change of solubility may not result in an enhanced extraction of PAHs using HBCD than those that were bioavailable for bacterial biodegradation due to a lower affinity of high molecular to HBCD (Cuypers et al. 2002). Therefore, the relative higher extractability of five- and six-ring PAHs in this study could be explained by their only readily sequestration in the investigated surface sediments due to a recent input of airborne PAHs (Brion and Pelletier 2005). The secondary extraction step with a mixture of acetone and *n*-hexane revealed similar patterns for HBCD and USE (Figure 3-3c, and Figure 3-3d).

The patterns of MDE extraction in both sediments were comparable. In contrast, the patterns of the SOX extracts were different. In S1 the fractions of two- and three-ring PAHs dominated, and in S2 the two- and three-ring PAHs and of four-ring PAHs were quite similar, but the five-ring fractions was small and the six-ring fraction was absent. This could be explained by the loss of these fractions during sample preparation of S2 using the SOX extraction.

The hydrolysis step yielded high portions of more than 80% of two- and three-ring PAHs after MDE extraction and similar patterns of all PAH fractions for USE and SOX extraction (Figure 3-3e, and Figure 3-3f). The patterns of S1 and S2 were different in HBCD extraction: In S1 the fraction of two- and three-ring PAHs dominated with more than 75%, but in S2 the two- and three-ring PAH accounted for less than 40% due to loss of the more volatile small molecular PAHs during sample preparation. The comparison of the patterns of the summarized values of all extraction steps for each fraction, extraction method and samples showed only minor differences (Figure 3-3g, and Figure 3-3h) despite the putative loss of compounds during analytical procedures.

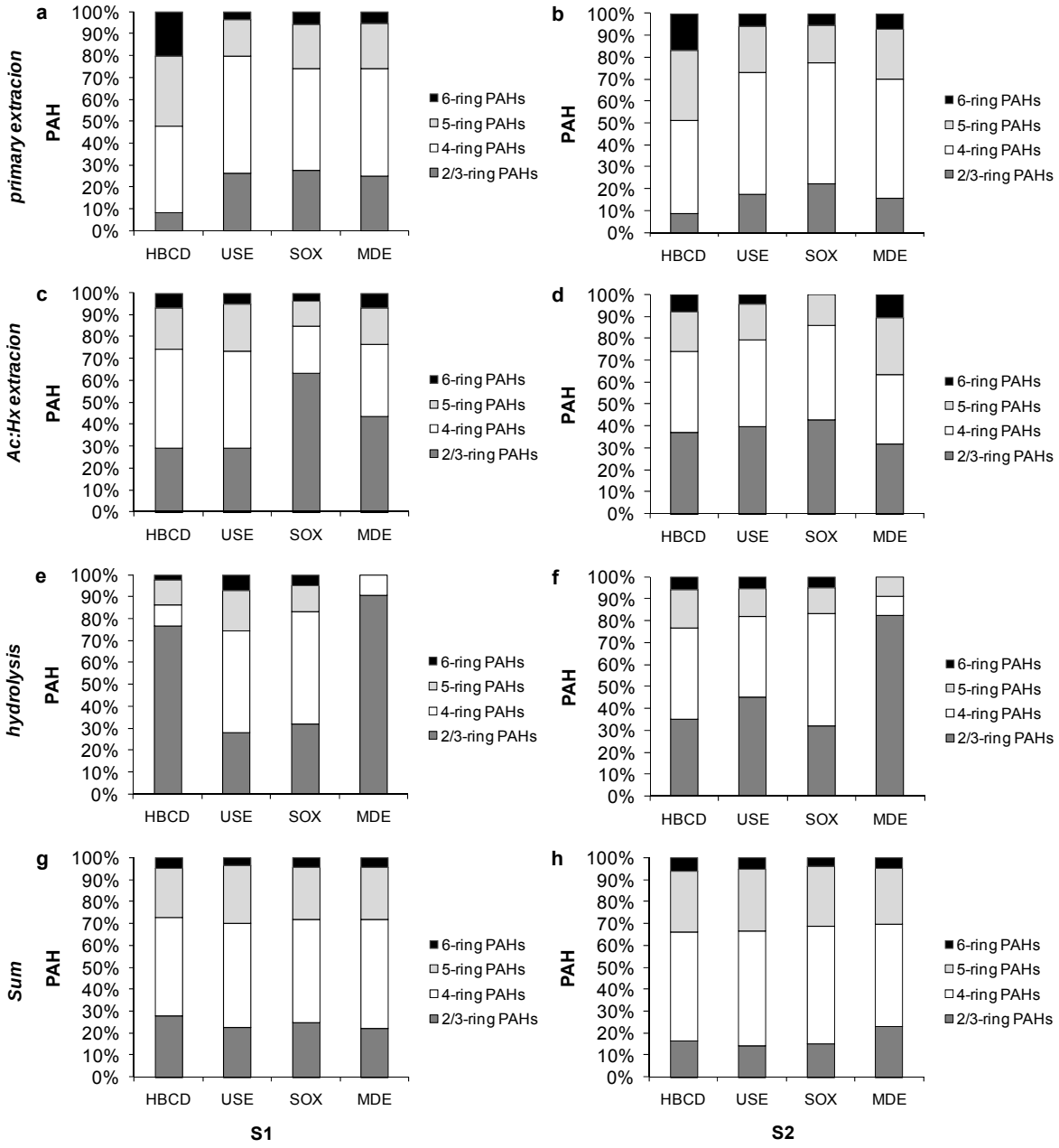


Figure 3-3 Comparison of PAHs fractions grouped by numbers of aromatic rings and samples extracted by (A) primary extraction with either HBCD, USE, Soxhlet or MDE (a,b), (B) secondary extraction step using a mixture of n-hexane/acetone (1:1;v:v) in ultrasonic extraction (c,d) and (C) hydrolysis (e,f). Data are given as means and mean absolute deviations (n=2) of sediments S1 and S2 and are normalized to the sum of PAHs extracted with each extraction method and step).

3.3.4 Role of organic and black carbon on the extractability

The sediments S1 and S2 had total organic carbon contents (TOC) of 3% and 4.5%, respectively (Table 3-1). These results were in accordance with the study by Pies et al. (2007), who found TOC levels between 3.2 and 8.7% in floodplain soils of the Saar. Compared to other sediments from German rivers, these were low to medium-high TOC values (Schulze et al. 2007a). The BC levels of 0.4% and 0.9% were very low in comparison to other sediments (Cornelissen et al. 2005). The BC:TOC ratio was 13% and 20% for S1 and S2, respectively.

Comparing the results of both sediments related to PAH-to-solid phase ratios, there are only small differences appearing with an S1:S2 ratio of 1.2 ± 0.2 (Figure 3-2a). With respect to PAH-to-TOC and PAH-to-BC ratios (Figure 3-2b, Figure 3-2c) the higher contents of TOC and BC resulted in lower concentrations of PAHs for S2 than for S1 with a S1:S2 ratio of 1.9 ± 0.3 (PAH-to-TOC) and 2.8 ± 0.4 (PAH-to-BC).

Hence, amounts of PAHs extracted from S2 were 1.5-fold and 2.3-fold lower than in sample S1. The consideration of total organic carbon (TOC) and BC takes into account possible influences of the quality of organic carbon in the sample on the assessment of the PAH amounts extracted and any putative ecotoxicological risks. The higher the contents of TOC and BC in the sample are the lower is the bioaccessibility of PAHs (Oen et al. 2006, Rhodes et al. 2008).

3.3.5 Cytotoxic potency and EROD induction of the primary extracts

For SOX S1 and MDE S1, cytotoxicity gave similar results, but significant differences between those samples and USE S1 (p<0.05, ANOVA with Tukey's posttest; Figure 3-4a). SOX S2 was significantly divergent from MDE S2 and USE S2 (p<0.05, ANOVA with Tukey's posttest). The comparison of cytoxicity among the extraction methods gave only a significant dissimilarity between SOX and USE extracts (p<0.05, Kruskal-Wallis ANOVA with Dunn's posttest). No or only negligibly cytotoxic responses were recorded for HBCD extracts (data not shown). The EROD data showed more differences (Figure 3-4a). In both sediments, the HBCD extracts gave significantly lower effects compared to the other extraction methods (p<0.05, ANOVA with Tukey's posttest).

For S1, MDE compared to SOX and USE were similar, but SOX and USE had significantly divergent effects (p<0.05, ANOVA with Tukey's posttest). For S2, EROD induction was similar in USE compared to MDE, but SOX and MDE or USE showed significantly different effects (p<0.05, ANOVA with Tukey's posttest). Comparison of EROD induction among the extraction methods showed only significant differences between HBCD and all other extraction methods (p<0.05, ANOVA with Tukey's posttest).

Differences in cytotoxicity effect potentials cannot be explained with the compound-specific composition of the extracts, since at least SOX and MDE extracts were highly comparable in terms of both PAHs concentrations and composition of the PAHs fractions (Figure 3-3a, Figure 3-4a.The USE extracts showed a similar composition of PAHs, but yielded lower compound concentrations. Hence, other differences between SOX and MDE extracts might be responsible for the existing, but insignificant dissimilarities in effect potentials. Sulfur and sediment organic matter (SOM) are regularly extracted alongside contaminants by vigorous extraction (Salizzato et al. 1998, Schwarzenbach et al. 2003).

Whereas sulfur can occur in both extract types, the appearance of SOM is limited to SOX extracts. The retardation effect of LDPE membranes used in MDE keeps organic macromolecules inside the membrane during dialysis (Seiler et al. 2006). Diffusion of large compounds is tailed-off due to molecular interactions with the polymer chains and decreased availability of free solvent cavities in the membrane (Schulze et al. 2012a). Furthermore, sulfur is known to have a cytotoxic potential (Ricking et al. 2004, Svenson et al. 1996, Svenson et al. 1998).

In the EROD assay, this effect is avoided by applying test concentrations below a cytotoxic impact. On the other hand, SOM comprises countless chemically functional groups (Steinberg et al. 2000, Steinberg et al. 2003), with a strong potential to interact with cellular mechanisms, such as the monooxogenase detoxification system (Steinberg et al. 2006). Hence, SOM contents could be a possible cause for the elevated reactions of the cells upon exposure to SOX extracts.

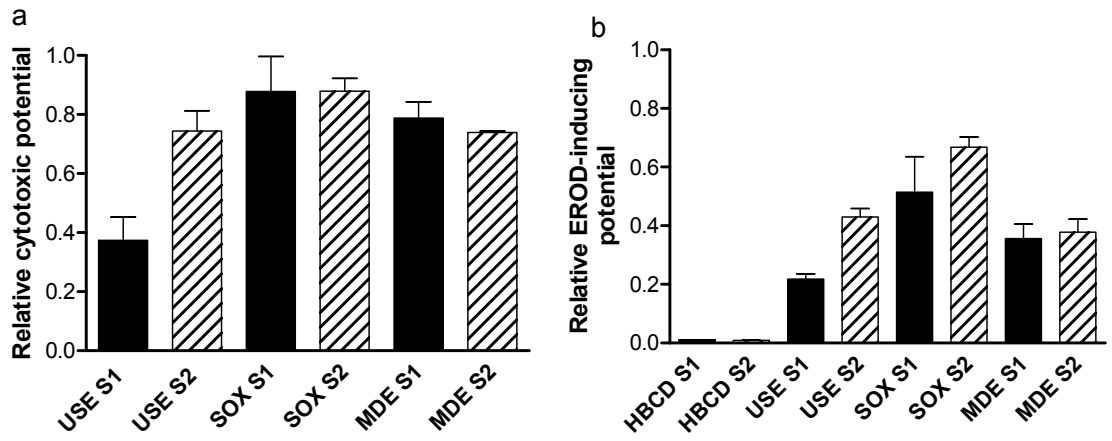


Figure 3-4 Relative effect potentials for the primary extraction methods HBCD, USE, SOX and MDE in neutral red assay for cytotoxicity (a; n=3) and EROD induction assay regarding for dioxin-like activity (b; n=2). Data are given as means with mean absolute deviations.

3.3.6 Effect-confirmation using toxicity equivalents and artificial mixtures

3.3.6.1 Effect-confirmation based on toxicity equivalents

In environmental samples with multiple contaminants present, effect confirmation of responsible compounds is often a challenge (Altenburger et al. 2004, Andersson et al. 2009, Grote et al. 2005, White 2002). In order to identify similarities and differences between the extraction methods and between chemical and biological analyses, the Bio-TEQs of the original extracts and of artificial mixtures of the quantified PAHs as well as the Chem-TEQs of the PAHs were calculated based on the EC_{25}-values in the EROD assay (Figure 3-5a, Figure 3-5b; Table S3–1 in Appendix). For both sediments, SOX extracts caused the strongest effects in RTL-W1. The effects of USE and MDE extracts were in the same range for both sediments. The HBCD extracts caused the lowest effects in both sediments as well. Few of the analyzed PAHs are known to induce EROD activity (Behrens et al. 2001, Bols et al. 1999, Bosveld et al. 2002, Hollert et al. 2002). However, in any cases, the Chem-TEQs were not able to explain the effects in the bioassays. The confirmation of the effects using Chem-TEQs was between 2% for MDE S1 and 16% for HBCD S2 (Table S3–1 in Appendix). The testing of artificial mixtures representing the concentrations of the determined compounds in the original extracts yielded different results (Figure 3-5b). EROD induction results of the MDE and USE extracts were explained very well by the artificial mixtures at percentages between 68% and 116% at the EC_{25}-level, but not at higher effect levels. The

effects of the HBCD extracts were overestimated four- to eight-fold and those of the SOX extracts were underestimated two-and-a-half-fold by the artificial mixtures (Table S3–1 in Appendix). In the case of the HBCD extracts, the latter results could be explained in case of the HBCD extracts by analytical uncertainties due to measurement near the analytical determination limits and due to specific modes of action (MOA) of single compounds dominating the effectiveness at low effect levels (Grote et al. 2005).

3.3.6.2 Effect-confirmation based on comparison of whole concentration-response curves

The index of confirmation quality (ICQ; Figure 3-5c) was calculated for USE, SOX and MDE extracts. An ICQ of one equals indicates 100% overlap and thus perfect confirmation of the observed effects, while smaller or greater ICQs indicate that the identified compound or the artificial mixture of identified compounds do not fully explain the observed toxicity. Unidentified compounds in the original sample that are not present in the artificial mixture as well as matrix components may explain both lower solubility and lower bioavailability in the original sample (Schulze et al. 2010, Schwab et al. 2009). At higher concentrations, unspecific narcotic MOAs are expected to represent concentration-addition or mixture-toxicity effects (Grote et al. 2005). The ICQ curves of USE S1 (curve A, Figure 3-5c), MDE S1 (curve C) and MDE S2 (curve F) had ICQ values greater than one in the majority of effects levels indicating that effects of the MDE extracts were overestimated up to two-fold by the artificial mixture at higher effect levels. However, the effects were explained quite well comparing to SOX S1 (curve B) and USE S2 (curve D). Both ICQ curves revealed ICQ values less than one at any effect level and did not explain the effects. Interestingly, the effects of SOX S2 (curve E) resulted in low ICQ values at lower effects levels, but if values are approximately one at higher levels showing a strong mixture toxicity MOA at these levels. In conclusion, the effects of the extracts were not explained well by the artificial mixtures in the most cases and at most effect levels. However, the ICQ approach is a much better method to compare and confirm effects than the Bio-TEQ and Chem-TEQ could address, because the Bio-TEQ shows only the effects at the 25% level and the Chem-TEQ is strongly reliant to the availability of REPs of possible EROD-inducing compounds. Nevertheless, the interpretation and evaluation of the ICQ values regarding explanation or confirmation of effects is still not well-established (Bandow et al. 2009b, Schulze et al. 2010, Schwab et al. 2009).

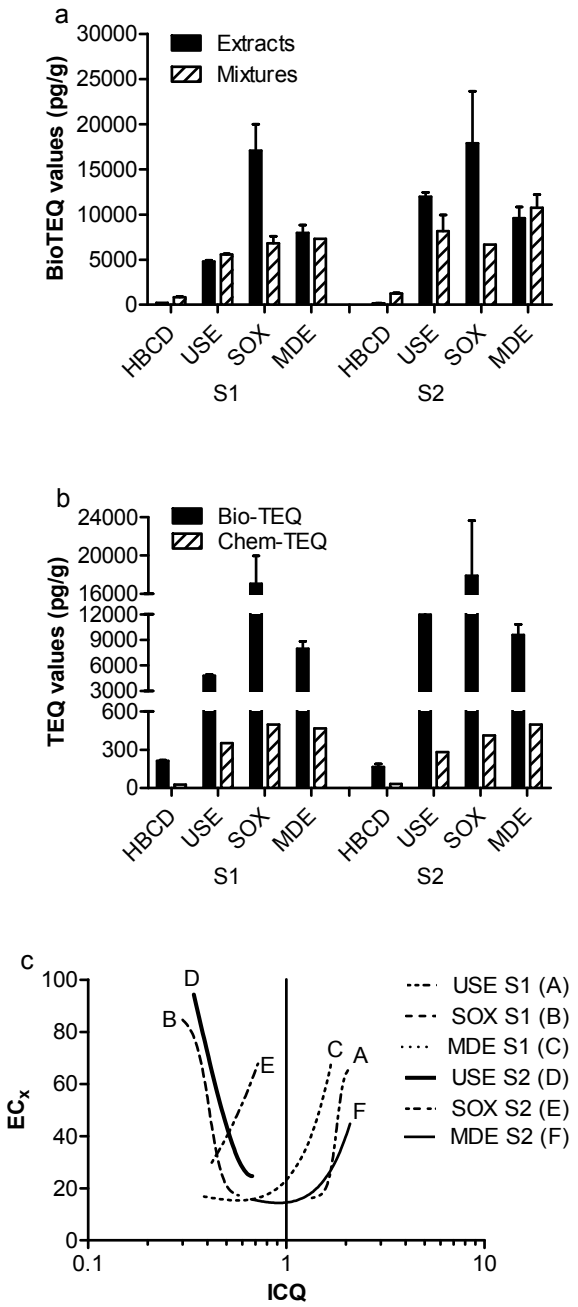


Figure 3-5 a) Bio-TEQ values of primary extracts and artificial mixtures in pq/g; (b) Bio-TEQ vs. Chem-TEQ values of primary extracts in pg/g; (c) Index of confirmation quality (ICQ) of the primary extracts from both sampling locations (the vertical line at 1 represents the original sample, i.e. the toxicity that has to be explained). Data are given as means with mean absolute deviations

3.4 Implications for the risk assessment of sediments

This study documents that comprehensive and holistic investigation strategies for the lab-based risk assessment of sediments have to be recommended. Such holistic investigation strategies should always consider different lines of evidence and a confirmation step to arrive at the highest possible standard of realism and security in sediment risk assessment. Therefore, the following recommendations should be recognized:

- Different exhaustive extraction methods should be included and compared to gain unbiased information about the whole contaminant spectrum due to possible losses or alterations of effects-causing compounds during extraction (Seiler et al. 2008).
- A flexible battery of biotests *in vitro* and *in vivo* biotests including whole sediment tests should be conducted to account for different exposition pathways and effect levels (Ahlf et al. 2002, Chapman and Hollert 2006, Höss et al. 2010, Tuikka et al. 2011).
- Biomimetic extraction and dosing approaches should be considered to gain the bioaccessible fractions of contaminants from sediments and to allow an unbiased and realistic determination of effects-causing contaminants that may be available *in situ* (Bandow et al. 2009, Brack et al. 2009b, Smith et al. 2010).
- Confirmation of identified chemicals and determined concentrations by testing of artificial mixtures is necessary to provide evidences on the correctness of the found cause-effect relationships and to avoid prioritization of unimportant compounds (Bandow et al. 2009, Brack et al. 2009b, Grote et al. 2005, Schulze et al. 2010).
- The residual chemical fractions and bound residues should be determined to account for a whole contaminant mass balance. The latter supports the delimitation and decision on the complete extraction of a distinct fraction that is reflected by each extraction step.

3.5 Conclusions

The extractability and potential toxicity of PAHs' contaminated river sediments was investigated to gain information about the extraction power of four different extraction approaches. Results for membrane dialysis extraction (MDE) and Soxhlet extraction (SOX) indicated similar extractability or extraction power of PAHs. Cytotoxicity tests confirmed these results, whereas the EROD-induction was lower in the MDE extracts. This was possibly due to retardation effect of the polyethylene membrane such that organic macromolecules were not extracted and might not have provoked toxic effects. USE showed an extraction power between the HBCD approach and the MDE as well as SOX and MDE method. Thus, MDE and SOX are vigorous techniques recommended for the exhaustive extraction of PAHs from sediments and to investigate the black carbon associated fraction of PAHs. However, SOX may not lead to correct PAHs patterns due to possible volatilization of PAHs with high vapor pressures. Furthermore, MDE and SOX are appropriate methods for the investigation of the worst case hazardous or toxicological potentials of particulate bound PAHs. Research regarding the influence of the LDPE membrane and the experimental conditions (e.g., solvents, temperature, and extraction time) on recoveries of compounds with different physical-chemical properties in MDE is recommended. This should include the establishment of quantitative-structure relationships or other model for the prediction of diffusion of substances with varying properties to define a chemical domain for which this method is appropriate. HBCD was confirmed as a method providing a certain, putatively bioaccessible fraction for the analysis of its toxicological effect potential. However, more mechanistic research is required regarding desorption and uptake kinetics of particularly bound compounds with different physico-chemical properties (e.g., nonpolar/polar compounds, nonionic/ionic compounds, small/large molecules) in the solid phase-water-HBCD system to unravel the possible discrimination of compounds with specific properties. Furthermore, comparisons with other partitioning-based nondepletive extraction methods such as Tenax®-TA and in situ solid-phase microextraction in combination with a multi-compounds analysis and different in vitro and in vivo biotest as well as bioaccumulation experiments appear necessary to gain more and unbiased information on the bio-mimicking mechanisms of these approaches for broad range of organic compounds with different physical-chemical properties. The ICQ approach was confirmed as a powerful concept to compare and confirm effects rather than Bio-TEQ and Chem-TEQ. Further research is needed towards a better

interpretation and evaluation of the ICQ values regarding explanation and confirmation of effects.

3.6 Acknowledgments

This paper is dedicated to Prof. Dr. Asaf Pekdeger who passed before his time in May 2011. This project was partly funded by the German Federal Environmental Agency (UBA; under FKZ 301 02 013 and FKZ 301 02 018). We feel particularly grateful to our student assistants and laboratory technicians. For technical and logistical support, we are very grateful to the German Federal Waterways and Shipping Administration and the Department of Biogeography of the University of Trier. The authors would like to express their thanks to Drs. Niels C. Bols and Lucy Lee (University of Waterloo, Canada) for providing RTL-W1 cells. The authors are grateful to three anonymous referees whose valuable comments helped to improve the paper. We are also thankful to Dr. Christa Schröter-Kermani from UBA for reviewing an earlier version of the manuscript.

4 Excursus: On the comparability of procedures for sediment extraction in environmental assessment [16] [17]

4.1 Introduction

The assessment of sediment contamination is a common task in applied ecotoxicology (e.g., Heise 2009, Karlsson et al. 2008, Wenning and Ingersoll 2002, Wölz et al. 2009a). It is a pre-requisite for decisions on the treatment of dredged materials (den Besten et al. 2003) and part of the evaluation of water quality of lakes, rivers and streams under the EU Water Framework Directive (WFD; Brils 2004). Furthermore, sediment pollution plays a key role in the estimation of possible adverse effects during floods, which can massively disturb legacy sediment layers (Förstner et al. 2004, Hilscherova et al. 2007, Salomons 2005, Wölz et al. 2009b, Wölz et al. 2008), turning these into secondary sources of contamination, with suspended particulate matter as the carrier (Power and Chapman 1992, Schulze et al. 2007a). Because of climate change, such events are considered to increase in number and severity within the coming centuries (Wilby et al. 2006).

Comprehensive assessment of sediment contamination should always be based on several parallel lines of evidence, including biological effects, chemical analyses and investigations into community structure (Chapman and Hollert 2006). Whereas chemical analyses are applicable for the identification of contaminants, only bioanalysis can deliver information about possible adverse effects of sediments on the screening or risk prioritization level.

In vitro biotests provide high efficiency in ecotoxicological high-throughput investigations, and increase reliability due to standardized methods under permanently controlled conditions. A considerable number of test systems are available for various different lethal and sublethal endpoints, providing acute toxicity data as well as results on mechanism-specific effectiveness (e.g., Hilscherova et al. 2000, Hollert et al. 2000, Hollert et al. 2003b, Keiter et

[16] This section is a synopsis of two manuscripts that are suggested for submission to Science of the Total Environment: Seiler, T.-B.; Streck, G.; **Schulze, T.**; Schwab, K.; Brack, W.; Braunbeck, T.; Hollert, H.: On the comparability of procedures for sediment extraction in environmental assessment. Part A: Bioanalytical investigations / Streck, G.; **Schulze, T.**; Seiler, T.-B.; Schwab, K.; Brack, W.; Braunbeck, T.; Hollert, H.: On the comparability of procedures for sediment extraction in environmental assessment. Part B: Chemical analytical investigations

[17] The underlying manuscripts are part of the PhD thesis of Dr. Thomas-Benjamin Seiler (2010): Total or biomimetic extracts or direct contact exposure? Comparative research towards a realistic ecotoxicological characterisation of sediments; PhD thesis, Ruperto Carola University of Heidelberg; Heidelberg; 340 pp

al. 2008, Kosmehl et al. 2004). A comprehensive literature review could be found in Seiler et al. (2006).

Toxicity testing and assessment of putatively contaminated sediments can be accomplished using various sample types derived from the original sampling site. Possible exposure scenarios are pore water, aqueous elutriates, native sediment samples, and extracts (Ahlf et al. 2002, Harkey et al. 1994). A broad range of procedures is available for the preparation of sediment extracts (Dean and Xiong 2000, Hollert et al. 2009, Puglisi et al. 2007a).

Extractability of contaminants, however, highly depends on the individual sample matrix and the specific technique, providing different levels of extraction power (Ehlers and Loibner 2006). Correspondingly, several rather mild extraction procedures like hydroxypropyl-β-cyclodextrin extraction or Tenax®-TA extraction, which focus on the rapidly desorbing contaminant fractions, are considered to mimic bioaccessibility of – at least – hydrophobic compounds in subsequent biotests (Cornelissen et al. 2001, Eisentraeger et al. 2004, Reid et al. 2000, Schwab et al. 2009). However, recent studies in effect-directed analysis have shown a shift in effectiveness from hydrophobic to more polar fractions if partitioning based dosing methods were used in biotesting instead of dosing with dimethylsulfoxide (DMSO; Bandow et al. 2009, Brack et al. 2009b). Together with whole sample investigations using sediment contact assays (Höss et al. 2010), these data can provide an enhancement of screening and risk prioritization approaches.

In contrast, vigorous methods such as pressurized liquid extraction (PLE; trademarked as Accelerated Solvent Extraction (ASE®) by Dionex, Sunnyvale, CA, USA), microwave assisted extraction, membrane dialysis extraction (MDE) and the Soxhlet technique aim at the preparation of total extracts (Bandh et al. 2000, Dean and Xiong 2000, Seiler et al. 2008), including also very slowly desorbing contaminants, which would be only hardly accessible for the majority of organisms under natural conditions (Ehlers and Luthy 2003a, Ehlers and Loibner 2006, Mayer and Reichenberg 2006, Reichenberg et al. 2006, Semple et al. 2004).

Extracts resulting from each individual procedure therefore represent distinct contaminant fractions with corresponding bioavailability, and each extract type reveals a particular hazardous potential of the original sample (de Maagd 2000, Deboer 1988, Hynning 1996).

In the present study, extracts from eight extraction procedures (five exhaustive techniques versus three mild ones mimicking bioaccessibility; Figure 4-1) were compared with respect to their extraction power using biotesting for cytotoxicity, dioxin-like activity and embryo toxicity and chemical analysis for polycyclic aromatic hydrocarbons (PAHs) and organochlorine compounds. The data assessment was performed with respect to extraction power, repeatability and applicability by means of effect concentration values and determined target compounds values compared among the used techniques.

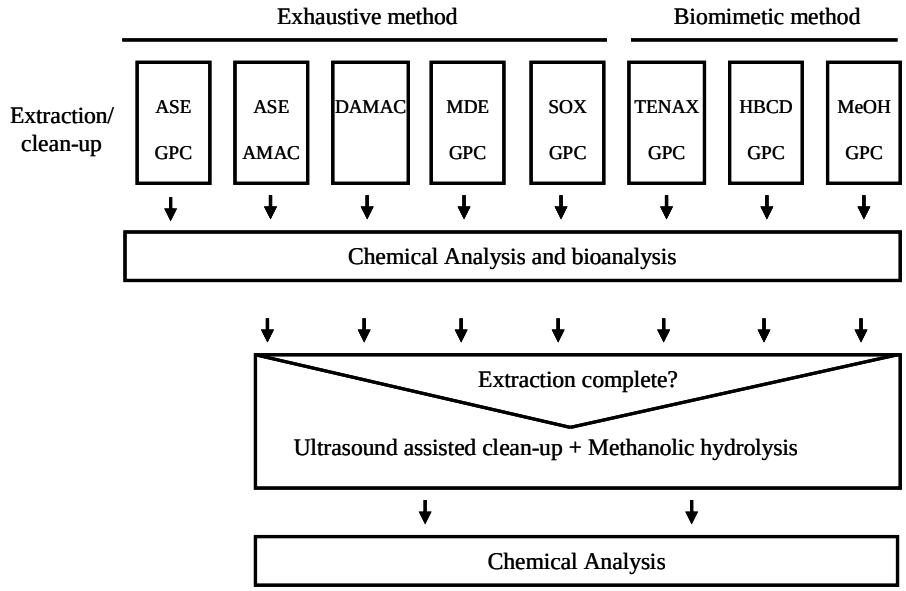


Figure 4-1 Scheme of the study design with the different extraction and analysis steps

4.2 Materials and methods

4.2.1 Study area, sampling and sample preparation

Near-surface sediments from the Elbe catchment close to the towns of Bílina (Bílina Creek) and Přelouč (Elbe River), Czech Republic, were sampled in 2005 using an Ekman-Birge dredge and stored cooled at 4 °C for transport. In laboratory, subsamples were centrifuged for pore water removal, shock-frozen at -30 °C and freeze-dried (Lycvac GT2, Heraeus Leybold, Cologne, Germany). Subsequently, dried sediments were sieved using a mesh <63 µm and stored at 4 °C until extraction. Two subsamples of each sediment were then extracted in parallel with the compared extraction techniques described below, giving four extracts from each approach. The sampling sites were characterized within the integrated EU

project MODELKEY (Brack et al. 2005a, Hein et al. 2010), and the sediments were considered suitable for a comparison of extraction techniques.

4.2.2 Chemicals and solvents

Certified standard solutions containing the 16 EPA-PAH was purchased from Dr. Ehrenstorfer (Augsburg, Germany) and chlorinated compounds (PCBs, DDX, CBs and HCHs) as well as benzo[a]pyrene-d_{12} were obtained from Promochem (Wesel, Germany). Solvents were purchased from Merck (Darmstadt, Germany) or Promochem (Wesel, Germany) and of Suprasolv®, Lichrosolv® or Picograde® grade unless otherwise noted.

4.2.3 Extraction and clean-up

4.2.3.1 Extract treatment and storage

Following the respective extraction or clean-up procedure, extracts were reduced to a volume of approximately 2 ml using rotary evaporation and then close to dryness under a gentle nitrogen stream. Residues were re-dissolved in 2 ml *n*-hexane and split into two equal aliquots for biological and chemical analysis. For bioassay, extracts were reduced again and re-dissolved in dimethylsulfoxid (DMSO; p.a. Merck, Darmstadt, Germany). All samples were stored at -20 °C until further use.

4.2.3.2 Soxhlet extraction

Soxhlet extraction (SOX) was based on the method applied by Hollert et al. (2000). An appropriate number of Soxhlet extraction thimbles (Schleicher & Schuell, Dassel, Germany) were pre-extracted using *n*-hexane. Portions of 10 g of dried sediment were weighed into the thimbles and extraction with acetone was carried out at 8 to 10 cycles per h over 8 h. Acetone has been recommended for the extraction of PAH by several authors in order to break up aggregates and a higher accessibility to more hydrophilic domains (Berset et al. 1999, Hartmann 1996) and it was used in previous studies (Hallare et al. 2005, Ulrich 2002, Ulrich et al. 2002)

4.2.3.3 Membrane dialysis extraction

Membrane dialysis extraction (MDE) was carried out according to Seiler et al. (2006), with slight changes as detailed below. In brief, 2.5 g of dried sediment were inserted into 80 cm of pre-extracted LDPE membrane tubes (Jencons, Leighton Buzzard, UK). The sediment was evenly distributed by means of a bent glass rod prior to introduction of the membrane into a 250 ml brown glass jar containing 200 ml *n*-hexane (p.a.; Merck). Membrane ends were secured and sealed with the surface grinded lid. Dialysis was allowed to proceed for 48 h at room temperature.

4.2.3.4 Methanol/Water extraction

Methanol/water extraction (MEOH) was performed according Kelsey et al. (1997). Portions of 10 g dried sediment sample were weighed into individual centrifuge jars with a 1:1 mixture of 50 ml methanol and 50 ml purified water (SERALPUR Pro 90 CN, Seral, Gelman Sciences Inc., Ann Arbour, U.S.A.). The jars were sealed with polytetrafluoroethylene (PTFE)-coated screw caps, vortexed for 2 min and orbitally shaken at 100 rpm for 2 h at 20 °C (IKA, Stauffen, Germany). The supernatant was separated by centrifugation at 2000 × G , filtered over GF/C microfiber filters (Whatman, Brentford, UK) and re-extracted by means of 3 x 5 minutes liquid-liquid extraction with each 20 ml dichloromethane at ph 2 (acidified with 1 M hydrochloric acid, Suprapur®, Merck; Schwarzbauer et al. 2003b).

4.2.3.5 Hydroxypropyl-β-cyclodextrin extraction

Hydroxypropyl-β-cyclodextrin extraction (HBCD) followed the protocol by Reid et al. (2000). Portions of 10 g of dried sediment samples were weighed into separate centrifuge jars with PTFE-coated screw caps, and 100 ml of a 50 mM HBCD solution in purified water (SERALPUR Pro 90 CN) were added. The jars were vortexed for 2 min and orbitatally shaken at 100 rpm for 2 h at 20 °C (IKA). The supernatant was separated by centrifugation at 2000 × G , filtered over GF/C microfiber filters (Whatman) and re-extracted by means of liquid-liquid extraction with dichloromethane as described above.

4.2.3.6 Tenax®-TA extraction

Tenax®-TA extraction (TENAX) was carried out as described by Schwab and Brack (2007). Briefly, Tenax®-TA beads obtained from Alltech International (mesh 60-80, Deerfield, IL, USA) were cleaned by pressurized liquid extraction with solvents of different polarity. After drying the beads in a nitrogen stream for 2 h at 60, 110, and 200 °C using a tailor-made glass tube, fresh sediment (equivalent of 125 g dry weight), 180 g of clean Tenax®-TA beads and approximately 3 l of deionised water were vigorously stirred for 24 h at 20 °C. Following separation of the sediment suspension from the Tenax®-TA, loaded beads were washed until the water phase was clear, and then extracted using 2.5 l of acetone followed by 2.5 l of *n*-hexane. The solvent phase was reduced in volume close to dryness and residues re-dissolved in dichloromethane. Finally, particles were removed using a combination of glass microfiber filters and PTFE frits. Particle-free extracts were purified by gel permeation chromatography (GPC).

4.2.3.7 Accelerated solvent extraction

Accelerated solvent extraction (ASE) of dried sediments were subjected to a 2-step extraction procedure with an ASE® 200 device (Dionex, Sunnyvale, CA). The first step applied 3 static cycles of 10 min with *n*-hexane/dichloromethane 50:50 (v/v) at 80 °C, 10 MPa and 60% flush volume. The second step consisted of 3 static cycles using toluene at 140 °C as solvent, while other parameters remained the same. Immediately after extraction, resulting extracts were purified using either GPC or accelerated membrane assisted clean-up (AMAC).

4.2.3.8 Gel permeation chromatography

Prior to the gel permeation chromatography (GPC) clean-up procedure, samples were evaporated close to dryness under a gentle nitrogen stream and re-dissolved in dichloromethane (Merck, Darmstadt, Germany). Extracts were filtrated using a glass cartridge containing a combination of GF/C glass microfiber filters (Whatman) and PTFE frits to remove solid particles. Clean-up was performed applying an automated gel permeation chromatography system (AccuPrep MPS™, Antec GmbH, Sindelsdorf, Germany). The chromatography column (3.5 x 38 cm) was filled with BioBeads S-X3 (200–400 mesh, J2 Scientific, MO, USA), and dichloromethane served as eluent. Using the fraction collector,

only the second out of three fractions was processed, whereas the first and the last fraction containing macromolecules and sulfur, respectively, were discarded. The volume of each fraction was determined with a calibration mixture prepared according to US EPA Method 3640A (US-EPA 1994), and by monitoring the retention time using a UV-detector at a wavelength of 253 nm.

4.2.3.9 Accelerated membrane-assisted clean-up

Accelerated membrane assisted clean-up (AMAC) is a newly developed clean-up method and has recently been described in detail (Schulze et al. 2012a, Streck et al. 2008a, Streck et al. 2008b). Briefly, ASE-extracts were concentrated to 0.5 ml and transferred into pre-cleaned bags prepared from LDPE tubes (Polymer-Synthese-Werk GmbH, Rheinberg, Germany) using a heat-sealing apparatus (Sealboy 2-1038, Audion Elektro, Kleve, Germany). This sealing technique was also applied to completely enclose the extract within the membrane bag. Membranes with the extracts were placed in a 33 ml ASE cell inside an ASE® 200 device, which pressed a 70:30 (v:v) solvent mixture of *n*-hexane and acetone into the cell and, thus, started diffusion of compounds from the extracts across the membrane into the solvent. The device was operated with 16 cycles lasting 10 minutes each, a pressure of 3.45 MPa, a temperature of 40 °C, a flush volume of 60% and a nitrogen purge time of 60 seconds. The high number of automatic solvent exchanges and the elevated temperatures accelerated the dialysis compared to classical dialysis procedures.

4.2.3.10 Direct accelerated membrane assisted clean-up (DAMAC)

Direct accelerated membrane-assisted clean-up (DAMAC) is a method still in development and constitutes a consecutive combination of extraction by ASE® and clean-up by AMAC. 2 g of dried sediment were directly introduced into the membrane bag and dialysed as described for AMAC.

4.2.3.11 Ultrasonic extraction with n-hexane/acetone

An ultrasonic assisted extraction with *n*-hexane/acetone 1:1 (v:v; USE) served as a second extraction step in order to determine the completeness of the first extraction with one of the eight applied methods. Resulting extracts were purified with GPC after exchanging the solvent to dichloromethane.

4.2.3.12 Extraction with methanolic hydrolysis

Extracted samples were submitted to a third extraction step with methanolic hydrolysis according to Eschenbach et al. (1994). With this alkaline saponification of the already treated samples, strongly bound compounds can be extracted. Briefly, sediment material were mixed with methanolic potassium leach (1 M KOH in methanol; Merck, Darmstadt, Germany) and heated for 1 h at 70 °C. Resulting extracts were concentrated to dryness, re-dissolved in dichloromethane and subjected to clean-up by GPC.

4.2.4 Process controls

For each extraction method, blanks were prepared in duplicate according to the specific protocol. Purified sea sand (Merck) was used as a surrogate for sediment. The process controls were tested in parallel to the obtained sediment extracts with all bioassays none revealing elevated background toxicity. All reported chemical analytical results are corrected for blanks of respective method.

4.2.5 Bioassays

4.2.5.1 Cell cultures

The fibroblast-like permanent cell line RTL-W1 (Lee et al. 1993) used for the cytotoxicity and EROD induction bioassays were kindly provided by Drs. N.C. Bols and L. Lee (University of Waterloo, Canada). RTL-W1 cells were maintained in 75 cm^2 plastic culture flasks (TPP, Trasadingen, Switzerland) in Leibowitz's L15 medium (Sigma-Aldrich, Deisenhofen, Germany) supplemented with 9% foetal bovine serum (Sigma-Aldrich) and 1% penicillin/streptomycin solution (10,000 U per 10,000 µg/ml) in 0.9% NaCl (Sigma-Aldrich) at 20 °C.

4.2.5.2 Neutral red retention assay

Acute cytotoxicity of the sediment extracts was determined in the neutral red retention assay (NR) as detailed by Babich and Borenfreund (1992) with modifications 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) to give a final concentration range of 0.78–50 mg sediment equivalent (SEQ) per ml medium (mg/ml). The chemical 3,5-dichlorophenol

(Riedel-de-Haën, Selze, Germany) was used as a positive control at a maximum concentration of 40 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-dimethylamino-phenanzine) for 3 h, and neutral red retention was measured at 540 nm with a reference wavelength of 690 nm using a GENios plate reader (Tecan, Crailsheim, Germany). Dose-response curves expressing viability of the cells compared to controls were plotted and cytotoxic effectiveness was calculated as NR_{50} values.

4.2.5.3 EROD induction assay

The presence of aryl hydrocarbon receptor (AhR) agonists in the sediment extracts was determined in duplicates using the 7-ethoxyresorufin-O-deethylase induction assay (EROD) as described by Behrens et al. (1998) with slight modifications introduced by more recent studies (Gustavsson et al. 2004, Olsman et al. 2007). Briefly, confluent RTL-W1 cells were trypsinized, re-seeded into 96-well microtitre plates (TPP) and exposed to sediment extracts diluted in L15 medium to give 8 dilutions with 6 replicates each covering a range between 0.39 and 50 mg SEQ/ml medium (maximum DMSO concentration below 1%). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was serially diluted to give a final concentration range of 6.25–100 pM on two separate rows of each plate as a positive control. The test plates were incubated at 20 °C for 72 h. EROD induction was terminated by removing the growth medium and freezing at -80 °C to kill and disrupt the cells. After at least 1 h, plates were thawed and 100 µl of 1.2 µM 7-ethoxyresorufin were added to each well. Deethylation was initiated with 0.09 µM nicotinamide adenine dinucleotide phosphate (NADPH) in phosphate buffer, and after 10 min the reaction was stopped by addition of 100 µl of 0.54 mM fluorescamine in acetonitrile. Resorufin was determined fluorometrically at an excitation wavelength of 544 nm and an emission wavelength of 590 nm using a GENios plate reader (Tecan, Crailsheim, Germany). Whole protein was determined fluorometrically using the fluorescamine method (excitation 355 nm, emission 590 nm; (Kennedy and Jones 1994; cf. Hollert et al. 2002). Fluorescent units from the EROD measurement were converted to mass of resorufin and protein via calibration curves. Dose-response curves for EROD induction as the specific enzyme activity were computed, and the concentration of each sample causing 25% of the TCDD-induced maximum EROD activity was defined as EC_{25TCDD} values.

4.2.5.4 Zebrafish egg test

The embryotoxic potential of the sediment extracts was determined with the zebrafish *(Danio rerio)* embryo assay (fish egg test; FET; Hollert et al. 2003b, Nagel 2002), adapted to a high throughput system in 96-well plates. Fish were maintained in a breeding condition and eggs harvested as detailed by Nagel (cf. Braunbeck et al. 2005, Nagel 1986). Sample extracts were diluted in 1.5 ml artificial water (half the volume required for the test), but at twice the highest concentration, i.e. 200 mg SEQ/ml water. Serial dilutions with 5 steps (12.5, 25, 50, 100, 200 mg SEQ/ml water) were prepared in 25 ml beakers for pre-incubation of the fish eggs. Ten fertilized zebrafish eggs were selected per beaker and transferred alongside 150 µl of artificial water each, using a micropipette equipped with a widened 200 µl tip. Thus, the volume of each extract dilution was increased to 3 ml with half of the initial concentration (i.e. 6.25, 12.5, 25, 50, 100 mg SEQ/ml water). Eggs were then transferred to individual wells of 96-well microtitre plates (one egg per well) along with 200 µl of diluted sample. Finally, plates were covered with adhesive film and incubated for 48 h at 26 °C. The developing embryos were inspected after 48 hours post fertilisation. Lethal endpoints were recorded, and mortalities were determined according DIN 38415-6 (cf. Braunbeck et al. 2005). Results from the individual plates were regarded valid, if negative controls showed less than 10% effect. Median effective concentrations (LC_{50} values) for each sample were calculated by plotting dose-response curves.

4.2.6 Instrumental analysis

GC-MS analysis for PAHs and organochlorines was carried out on an HP 6890 GC coupled to a HP MSD 5973 (Agilent, Palo Alto, USA), equipped with a 30 m × 0.32 mm I.D. × 0.25-µm film HP-5 MS fused capillary silica column, a 5-m precolumn (Agilent J&W, Folsom, USA) and a splitless injector with deactivated glass wool. Chromatographic conditions were as follows: 280°C injector temperature, an 1 µl-aliquot was injected in the pulsed splitless injection mode 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, Böhlen, Germany) was 37 cm/s at constant flow. The MS was operated in electron ionization mode (EI+, 70 eV) with a source temperature of 230 °C in single ion monitoring (SIM) for quantification. Concentrations of target analytes were

calculated using an external calibration and corrected using an injection standard (benzo[a]pyrene-D12 or pyrene-D10; Promochem) added to each sample after the clean-up.

4.2.7 Graphical evaluation and statistical analysis

4.2.7.1 Evaluation of effect data

All effect data were plotted against extract concentrations with GraphPad Prism 5.04 (GraphPad 2007). Data points were fitted using sigmoid nonlinear regression as a model equation and effect concentrations were determined by means of this regression curve. Mean values for determined effective concentrations and recoveries for the analyzed compounds were compared statistically by Kruskal-Wallis one-way analysis of variance (ANOVA) with Dunn's posttest using GraphPad Prism 5.04 and Statistica 8.0 (StatSoft 2008). Differences between factors were considered as significant for $p<0.05$.

4.2.7.2 Evaluation of extraction effectiveness

For the evaluation of effectiveness of the different extraction methods, the mean effect concentrations for each biotest and the recovered amounts of each compound class in the first extraction step were compared between the exhaustive and biomimetic methods, respectively. Methods that provided the highest recovery for the respective compound and biotest were assigned the lowest rank number. Calculating the arithmetic mean from assigned ranks led to a rank for each method.

4.2.7.3 Evaluation of extraction repeatability

To determine the repeatability of each extraction/clean-up approach the range of the results of each compound and biotest for the same sediment and method was divided by the associated arithmetic mean values, resulting in a relative range. The relative ranges were then averaged, describing the overall repeatability of the respective method. Finally, an overall rank was calculated using the results of the two sediment samples. As a measure for the repeatability of extraction power, overall mean effect concentration values per ecotoxicological endpoint were determined, based on data for both sediment samples. For every mean value, the range was then expressed in percent, giving a relative range. Finally, corresponding sets of relative range values were averaged resulting in a mean relative range for each approach, with lower values indicating higher repeatability

Furthermore, similarities between the investigated extract types were identified by average linkage hierarchical cluster analysis of the data derived from *in vitro* bioassays using R version 2.9.1 (R Project 2012).

4.2.8 Artificial mixtures and calculation of biological toxicity equivalents for EROD data

Artificial mixtures of the compounds quantified by chemical analysis were prepared in concentrations as found in the extracts and analysed with the EROD assay to confirm the chemical analysis and the observed toxic effects (Grote et al. 2005). For comparison Ah-receptor agonist activities were determined as EC_{25} values for each sample and then were given relative to the positive control 2,3,7,8-TCDD as «biological toxicity equivalent concentration» (Bio-TEQ; Villeneuve et al. 2002, Wölz et al. 2008). Bio-TEQs were calculated using the relation of the EROD-inducing potencies to that of the positive reference (10^{-10} M TCDD) as described by Engwall et al. (1996; Equation $4-1$) as mean values of n=3 independent biotests. TCDD-EC_{25} were determined with each test plate, and mean values of all tests were used for the calculation of Bio-TEQ values. Wölz et al. (2008) gave the mean TCDD-EC_{25} values of n=59 independent tests as 5.3 pg ± 1.8 pg/g. These values were determined with the same cell culture and the same TCDD batch and, thus, can be expected to show the test variation that applies for this study:

$$Bio-TEQ\left[\frac{pgTCDD}{gSEQ}\right]=\frac{TCDD-EC_{25}[pgTCDD/ml]}{sample-EC_{25}[gSEQ/ml]} \quad (4-1)$$

Where Bio-TEQ is the toxic equivalent (in ng TCDD / g SEQ) and TCDD-EC_{25} is the effect concentration of TCDD and sample-EC_{25} is the effect concentration of the sample at the 25% effect level, respectively. The EC_{25} is a more appropriate measure than EC_{50} because it avoids determination of concentrations were the curve flattens and is close to its maximum value (Engwall et al. 1996, Hollert et al. 2002).

4.3 Results

4.3.1 Chemical analysis

4.3.1.1 Total amounts of PAHs and chlorinated compounds – first extraction step

PAHs, PCBs, chlorinated benzenes (CBs), five isomers of HCH, DDT as well as its degradation products DDD and DDE (DDX) were analyzed (Figure 4-2 and Figure 4-3).

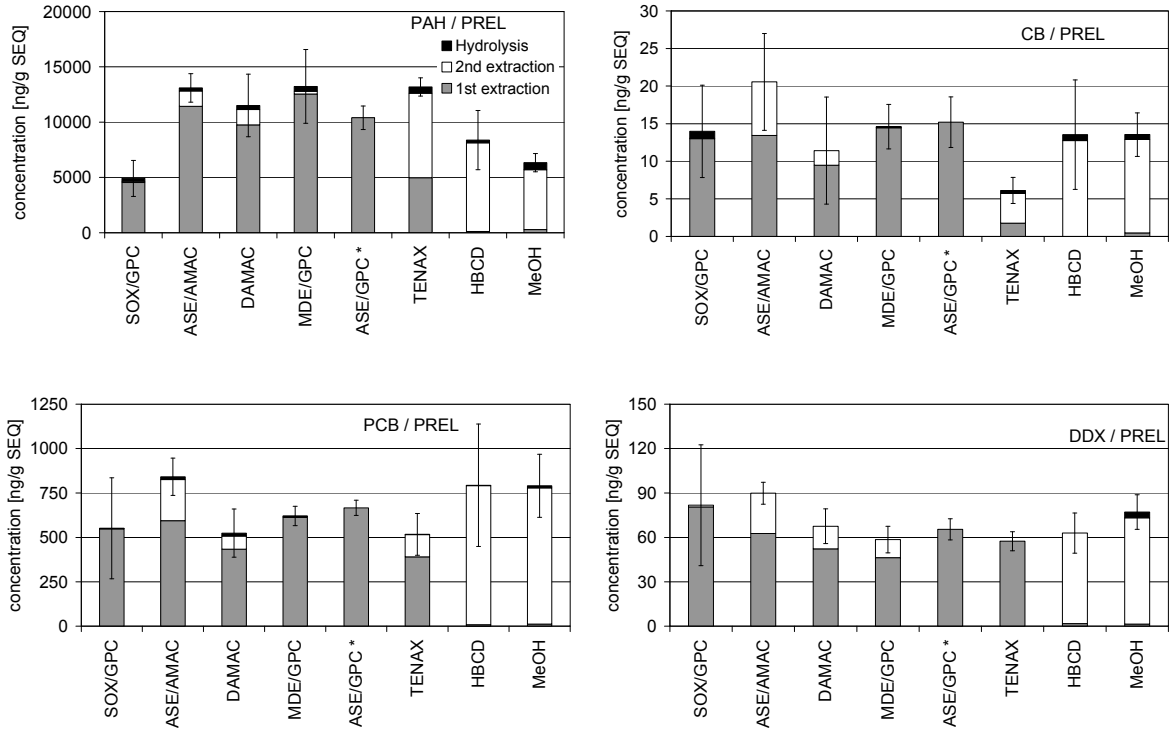


*Figure 4-2 Sum of compound classes analyzed using different extraction and clean-up methods for samples from sampling site Přelouč (PAH = polycyclic aromatic hydrocarbons, CB = chlorobenzenes, PCB = polychlorinated biphenyls, DDX = DDT + DDE + DDD). The error bar indicates the maximum estimated error based on n = 2 independent samples and calculated from the three consecutive treatments for each sample. * = only first extraction step performed*

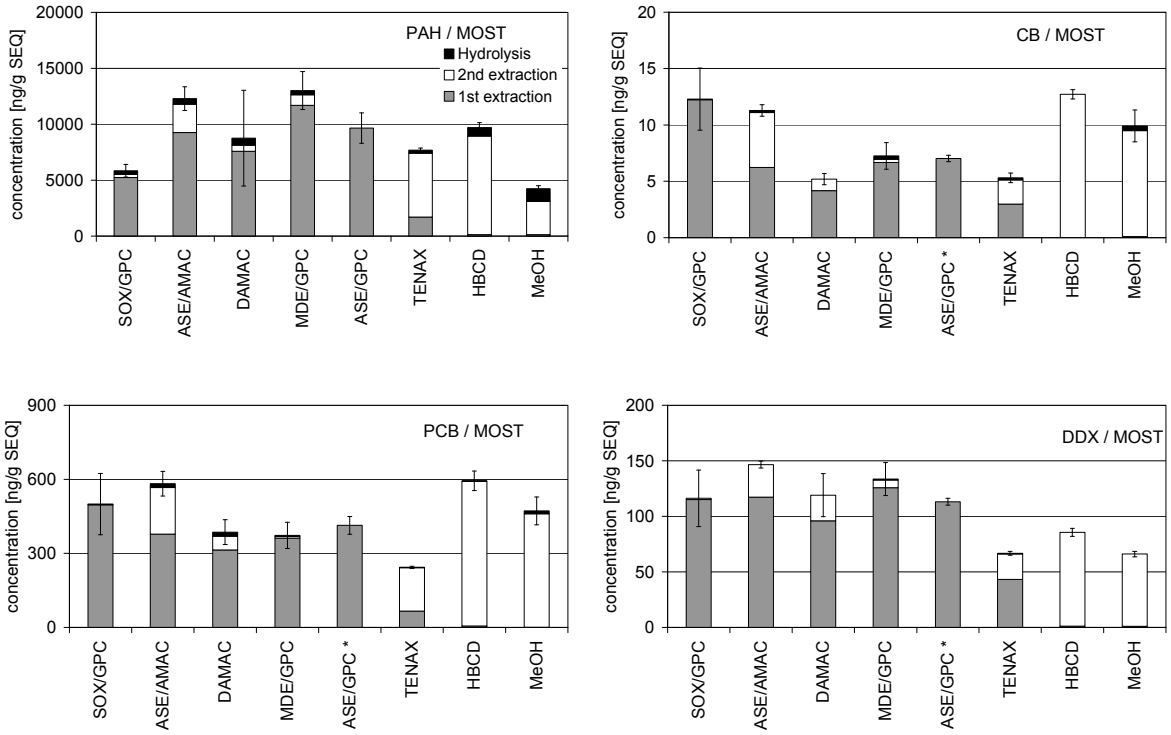


*Figure 4-3 Sum of compound classes analyzed using different extraction and clean-up methods for samples from sampling site Most (PAH = polycyclic aromatic hydrocarbons, CB = chlorobenzenes, PCB = polychlorinated biphenyls, DDX = DDT + DDE + DDD). The error bar indicates the maximum estimated error based on n = 2 independent samples and calculated from the three consecutive treatments for each sample; * = only first extraction performed*

Total amounts of 16 EPA-PAHs ranged between 5230 ng/g SEQ (SOX/GPC) and 11700 ng/g SEQ (MDE) for samples from MOST and 4530 ng/g SEQ (SOX/GPC) and 11430 ng/g SEQ (ASE/AMAC) for samples from PREL when using exhaustive extraction procedures. Amounts of PAHs obtained by SOX/GPC were considerably lower than those gained with MDE or ASE-based methods. Other exhaustive extracts delivered comparable results for both sites. Total concentrations of PAHs received after the first extraction step with mild extraction procedures, which are thought to reflect bioaccessibility, were also lower. TENAX yielded values in the same range as SOX/GPC (1700 ng/g SEQ, MOST; 5000 ng/g SEQ, PREL), while with HBCD and MEOH total concentrations did not exceed 285 ng/g SEQ.

Four of the exhaustive methods, SOX/GPC, ASE/GPC, ASE/AMAC and MDE performed similar when extracting chlorinated compounds. SOX/GPC extracted slightly higher total amounts of PCBs and CBs from the sediment MOST as well as DDX from the sediment PREL. Concentrations of CBs were close to their limit of quantification. Regarding exhaustive methods, extraction power for the three groups of chlorinated compounds was in general lowest for DAMAC.

4.3.1.2 Second and third extraction

After performing the first extraction step, a second and third extraction procedure was applied with the residual sediment. This was done to estimate the completeness of the first extraction step. An ultrasound assisted solvent extraction with acetone/hexane served as second extraction, while the final procedure applied was methanolic hydrolysis. Extracts obtained by the second and third step were purified using GPC. In the case of ASE/GPC, no further extraction was carried out since for this treatment the extraction step was the same as for ASE/AMAC. ASE/GPC served throughout this study only as a benchmark method for the combined first extraction/clean-up process.

Concerning exhaustive methods, substantial amounts of substances leached by ASE/AMAC treated sediments (Figure 4-2 and Figure 4-3). The second extraction step yielded between 11% (PAHs, PREL) and 43% (CBs, MOST) of the total amounts for the respective compound group. However, the extraction power of ASE/AMAC was already comparable to other exhaustive methods for the first step. Thus, the second step added an «extra amount» to the total sum of chlorinated compounds. An explanation for this phenomenon could be that the pressure applied during first extraction filled tiny pores of the sediment particles with solvent (Jansen et al. 2002, Richter et al. 1996), reaching sequestered residues of chlorinated compounds and making them more accessible for the subsequent treatment with the second extraction step.

SOX/GPC showed low extraction power for PAHs in the first extraction step. However, also the second extraction step yielded low amounts of PAHs, indicating that the first step was complete. The third extraction step, performed to extract bound residues from sediment proved comparable to other exhaustive methods. Therefore, losses of substantial amounts of PAHs must have occurred during Soxhlet extraction, maybe by volatilization.

As expected, the second step extracted considerable amounts of compounds from sediments already extracted using TENAX, HBCD or MEOH. These amounts represent – operationally defined by each method – not bioaccessible fractions. In general, aggregated amounts obtained with each of the biomimetic methods and the corresponding two subsequent extraction steps proved to be comparable to the other applied methods.

In third step extracted only low quantities of substances, usually less than 7% of the total amounts. Higher values were achieved for MEOH extracted sediments (especially PAHs from MOST: 26.4%; PREL: 10.0%) may be due to residues of compounds that were not removed with previous extraction steps.

4.3.1.3 Comparison of all three extraction steps

After the first extraction step, sediment residues from all methods (except ASE/GPC) were extracted again with *n*-hexane/acetone in an ultrasonic bath followed by methanolic hydrolysis. Combined results from all three steps should in principle be the same for all approaches. As for the first extraction step, Kruskal-Wallis ANOVA with Dunn's posttest was carried out to prove this hypothesis. Actually, for most clean-up/extraction procedures, the analysis revealed equality of the combined results. However, statistical analysis of the data gave also several differences.

Combined results for sediments extracted with TENAX in the first place generally showed significantly lower values (with the exception of PAHs from the sample PREL). The TENAX method itself consists of two steps: Firstly, the sorbent binds compounds dissolved in an aqueous sediment-TENAX slurry. Then, after removing the sorbent from the slurry, is re-extracted with solvents. It is possible that some of the compounds were not fully re-extracted from the TENAX beads or that losses occurred during evaporation of the solvent leading to lower values for the first of the three extraction steps.

For PAHs, SOX/GPC differed significantly from the other approaches (except TENAX and MEOH) when considering all three extraction steps. Since the ultrasonic extraction with *n*-hexane/acetone extracted only minor amounts of PAHs from sediment residues treated with SOX/GPC, the most probable cause for this observations are losses during application of the first extraction/clean-up procedure.

4.3.2 Biological analysis

4.3.2.1 Comparability with respect to extraction effectively

Biotests were performed with extracts from thirst extraction steps. SOX and MDE were comparable in all investigations (Figure 4-4 and Table 4-1). Extracts from ASE/AMAC were also comparable to SOX/GPC and MDE-derived samples, whereas ASE/GPC produced extracts with overall significantly lower cytotoxic potential than SOX/GPC. DAMAC extracts showed effects in the neutral red retention and the EROD assays comparable to other putatively exhaustive extraction methods. However, regarding embryo toxicity extracts obtained using DAMAC had significantly lower effectiveness than SOX/GPC, MDE and ASE/GPC.

TENAX performed statistically comparable to the vigorous procedures. In contrast, regarding embryo toxicity, TENAX showed even significantly higher effect potentials than DAMAC. The mild approaches HBCD and MEOH showed significant differences to all other methods in almost every comparison. MEOH provided extracts that were principally less toxic than samples obtained using more exhaustive procedures such as SOX/GPC, MDE and ASE/GPC – at least in the neutral red retention and EROD assays. In contrast, statistics for EROD data proved no significance for any comparison with vigorous methods.

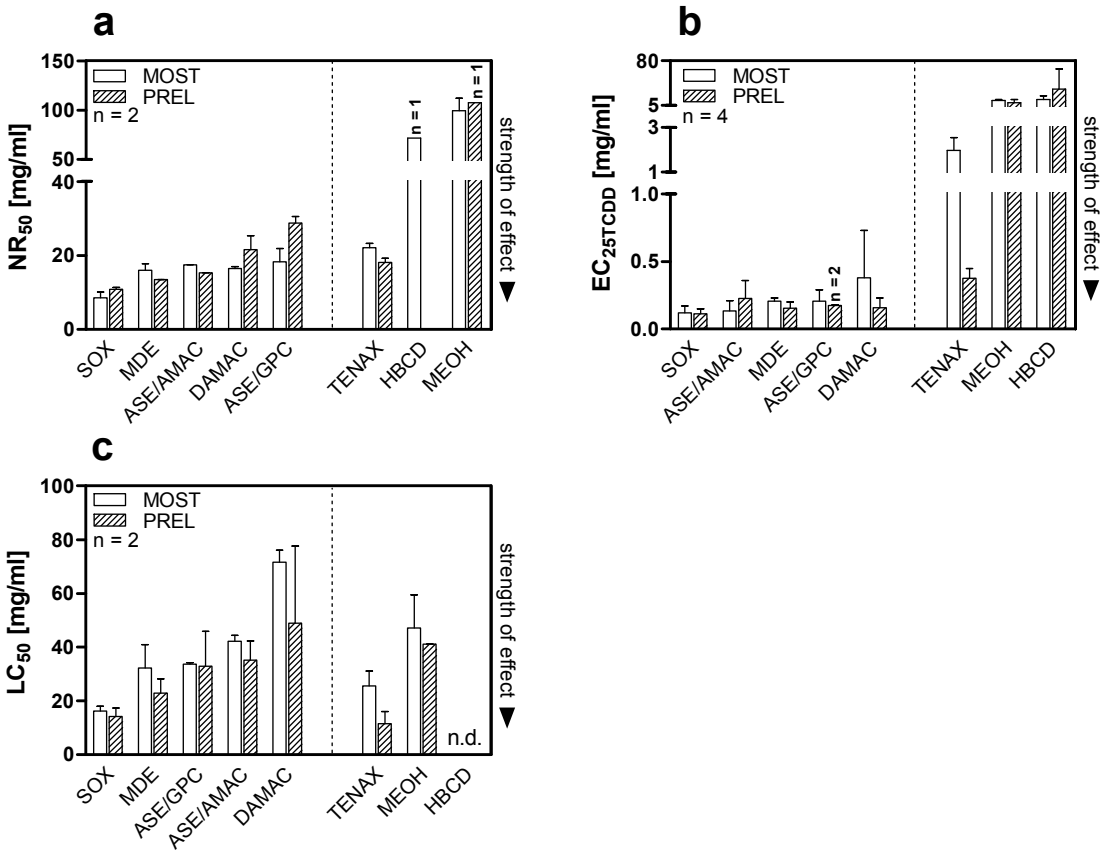


Figure 4-4 Ecotoxicological effects of the different extract types, separated into exhaustive (left group) and biomimetic (right group) methods. Data are given as means of two to four independent biotests ± range. Results were ranked according mean effect values for both sediment samples. Higher bars indicate less effect. a) cytotoxicity (NR), b) dioxin-like activity (EROD), c) fish egg toxicity (FET); SOX=SOX/GPC

Table 4-1 Statistical analysis of pairs of extraction procedures, listed by ecotoxicological endpoints. Order for EROD data: MOST/PREL. For NR and FET, data for MOST and PREL were pooled. Findings in brackets may be questioned from the result graphs. NR: cytotoxicity, EROD: dioxin-like activity, FET: fish egg test (Kruskal-Wallis ANOVA with Dunn's posttest)

NR	**SOX/GPC**	**MDE**	**ASE/GPC**	**ASE/AMAC**	**DAMAC**	**HBCD**	**TENAX**	**MEOH**
SOX/GPC	-	ns	*	ns	ns	-	ns	**
MDE		-	ns	ns	ns	-	ns	ns
ASE/GPC			-	ns	ns	-	ns	ns
ASE/AMAC				-	ns	-	ns	ns
DAMAC					-	-	ns	ns
HBCD						-	-	-
TENAX							-	ns
MEOH								-
EROD	**SOX/GPC**	**MDE**	**ASE/GPC**	**ASE/AMAC**	**DAMAC**	**HBCD**	**TENAX**	**MEOH**
SOX/GPC	-	ns	ns	ns	ns	***	*	***/(ns)
MDE		-	ns	ns	ns	**	ns	*/(ns)
ASE/GPC			-	ns	ns	*	ns	ns
ASE/AMAC				-	ns	***	ns	**/(ns)
DAMAC					-	*	ns	ns
HBCD						-	***	ns
TENAX							-	ns
MEOH								-
FET	**SOX/GPC**	**MDE**	**ASE/GPC**	**ASE/AMAC**	**DAMAC**	**HBCD**	**TENAX**	**MEOH**
SOX/GPC	-	ns	ns	ns	*	-	ns	ns
MDE		-	ns	ns	ns	-	ns	ns
ASE/GPC			-	ns	ns	-	ns	ns
ASE/AMAC				-	ns	-	ns	ns
DAMAC					-	-	**	ns
HBCD						-	-	-
TENAX							-	ns
MEOH								-

*/**/***: significant difference with $p<0.05/0.01/0.001$, ns: not significantly different

4.4 Discussion

4.4.1 Ranking according extraction efficiency

Based on the mean effect concentration values and determined amounts of each compound classes for each sediment sample, all extraction methods were assigned ranks in ascending order of the respective mean (Table 4-2 and Table 4-3).

ASE/GPC achieved the highest overall ranking, followed by ASE/AMAC and MDE, which both performed equally. For the sample MOST, the reverse order could be observed due to the better performance of ASE/AMAC and MDE with respect to the extraction of PAHS from this sample. SOX/GPC and DAMAC reached rank 4 and 5, respectively, of the exhaustive methods. The biomimetic methods ranked in the order TENAX, HBCD and MEOH.

SOX/GPC received the highest ranking for nearly every sample-biotest-combination, followed by MDE and ASE/AMAC. ASE/GPC and DAMAC were the least effective techniques regarding effect potentials of corresponding extracts. With high effectiveness in the neutral red retention assay and especially the fish egg test, TENAX ranked number 1 among the biomimetic approaches. MEOH reached lower rankings in all experiments.

Table 4-2 Ranking of extract types according their average efficiency to all compounds obtained with the first extraction step (in ng/g SEQ)

	PREL		**MOST**		**Both sites**	
	Mean	Rank	Mean	Rank	Overall Mean	Overall Rank
Exhaustive extraction procedures						
ASE/GPC	1.9	1	2.8	3	2.3	1
MDE	2.5	2	2.7	2	2.6	2.5
ASE/AMAC	2.7	3	2.6	1	2.6	2.5
SOX/GPC	3.7	4	3.0	4	3.4	4
DAMAC	4.3	5	4.0	5	4.1	5
Biomimetic extraction procedures						
TENAX	1.2	1	1.1	1	1.1	1
HBCD	2.4	2.5	2.4	2	2.4	2
MEOH	2.4	2.5	2.5	3	2.5	3

Table 4-3 Ranking of extract types according their effects in the cytotoxicity (NR), dioxin-like activity (EROD) and fish egg test (FET) assays

	MOST			PREL			Mean	Overall
	NR	EROD	FET	NR	EROD	FET	Rank	Ranking
Exhaustive extraction procedures								
SOX/GPC	1	1	1	1	1	2	1.2	1
MDE	2	3	3	2	2	3	2.5	2
ASE/AMAC	4	2	5	3	5	5	4.0	3
ASE/GPC	5	4	4	6	4	4	4.5	4
DAMAC	3	5	7	5	3	7	5.0	5
Biomimetic extraction procedures								
TENAX	6	6	2	4	6	1	4.2	1
MEOH	8	7	6	7	7	6	6.8	2
HBCD	7	8	8	8	8	8	7.8	3

Table 4-4 Repeatability of the procedure in order to each measured compound value shown as mean relative ranges and ranks in both sediments (PREL and MOST); no assessment of the repeatability of the methods HBCD and MEOH was performed due to analysis results near to the limit of quantification and due to missing values

	PREL			MOST			Both sites	
	Mean Range (%)	Mean Rank	Rank	Mean Range (%)	Mean Rank	Rank	Mean Rank	Overall Rank
TENAX	17.1%	3.2	2.5	7.4%	1.7	1	1.8	1
ASE/GPC	15.2%	3.2	2.5	11.3%	3.1	3	2.8	2
ASE/AMAC	16.2%	3.3	4	10.5%	2.9	2	3.0	3.5
MDE	12.9%	2.1	1	23.7%	4.1	5	3.0	3.5
DAMAC	48.6%	5.1	6	13.5%	3.5	4	5.0	5
SOX/GPC	23.6%	3.4	5	26.9%	4.9	6	5.5	6

4.4.2 Ranking according repeatability

The effectiveness of an extraction method is not the only relevant measure to evaluate the extraction/clean-up approach. The repeatability of a method, which is the variation of measurements under constant conditions caused by random errors, is of equal importance. The repeatability depends on different factors such as matrix effects and compound properties as well as the concentration – an analysis carried out near the detection limit commonly yields in higher variance between the repeated measurements. Ranking results according repeatability of chemical analysis are shown in Table 4-4 and Table 4-5.

Table 4-5 Repeatability of the procedures in order to each effect values of respective biotests shown as mean relative ranges and ranks averaged for both sediments; NR: cytotoxicity, EROD: dioxin-like activity, FET: embryo toxicity

	Range (%)			**Mean range (%)**	**Rank**
	NR	EROD	FET		
MEOH	24.57	81.67	55.37	53.87	1
SOX/GPC	45.86	86.02	44.66	58.85	2
MDE	28.83	66.21	84.33	59.79	3
ASE/GPC	67.28	61.02	77.86	68.72	4
ASE/AMAC	14.06	154.48	42.39	70.31	5
TENAX	31.63	194.46	130.12	118.74	6
DAMAC	49.74	234.42	94.96	126.37	7

Higher ranking indicates better repeatability of the procedure. Repeatability concerning the chemical results was comparably high for TENAX, ASE/GPC, ASE/AMAC and MDE. The biomimetic approach TENAX showed the best repeatability, followed by ASE/GPC, whereas MDE and ASE/AMAC performed comparable to each other. Higher variability for MDE samples from MOST could be at least attributed to the three low molecular PAHs (acenaphtylene, acenaphthene and fluorine). These compounds could be volatised while concentrating one of the duplicate extracts from MOST during sample preparation. Omitting these three PAHs from the calculations led to an average relative range of 17.1%. DAMAC and SOX/GPC showed for both PREL and MOST the lowest repeatability. The relative average range of 48.6% of DAMAC observed for the sample PREL yielded in the highest rank number of all approaches due to a high variability of the results of PAHs and CBs. With

respect to biotesting, MEOH provided lowest deviations within the entire study, followed by SOX and MDE, forming a group with close repeatability. ASE/GPC and ASE/AMAC resulted in slightly higher variance, whereas TENAX and DAMAC performed comparably poor.

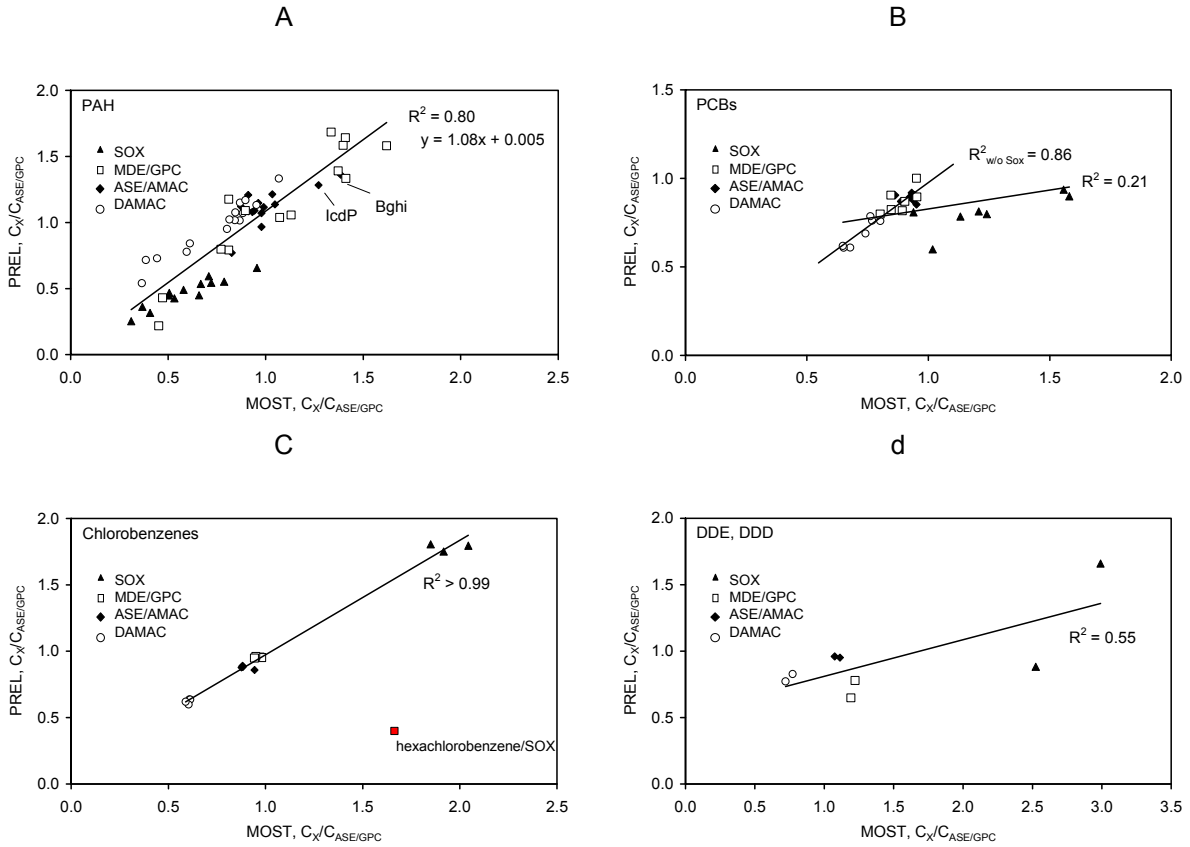


Figure 4-5 Correlation of amounts of target compounds extracted with exhaustive methods normalized to amounts extracted by ASE/GPC for (a) PAHs, (b) PCBs (coefficient of determination R² given with / without data obtained by SOX/GPC), (c) chlorobenzenes (results for hexachlorobenzene received with SOX were excluded from correlation calculations; solid square), and (d) DDE/DDD; MDE/GPC=MDE

4.4.3 Relationship between extraction efficiency as well as sediment and compound specific properties

Sequestration of organic compounds to sediments is dependent on the physicochemical properties of the substances as well as of sediment characteristics. By definition, an exhaustive extraction method should deliver a complete extraction for all compounds independent of the sample (Dean and Xiong 2000). In Figure 4-5 are depicted results from correlation analysis of the exhaustive extraction methods normalized to amounts gained with

the reference method, ASE/GPC, and data from PREL plotted against MOST. The data points of each extraction method should be close to unity if they had similar extraction efficiencies for the specific compound compared to ASE/GPC. If the extraction/clean-up method (as well as the reference method) is truly exhaustive for all compounds and both sediments, then all data points group closely in a cloud point. In this case, deviations are distributed randomly only caused by measurement uncertainties and no observable correlation between the data from PREL and MOST. If one of the methods discriminates only due to the physicochemical properties of the compounds but not due to different sediment composition, data points for the respective method form a linear graph with a slope of one. In the latter case, occurring correlations derivate only from differences between the methods independent from compounds or sediments properties.

The normalised ASE/AMAC data are clustered for all groups of compounds (Figure 4-5 a–d) implying the comparability of ASE/GPC and ASE/AMAC and nonselectivity of both methods without discrimination of specific compounds due to their properties. Three PAHs deviate from the point cloud, i.e. acenaphthene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene, because of their differing physicochemical properties comparing to the other PAHs. Observed deviations might be attributed to the clean-up method (AMAC or GPC). Normalised ASE/AMAC values are in general significantly below unity for chlorinated compounds (Student's t-test, $p<0.05$, values from both sites pooled) and slightly above unity for PAHs. Thus, AMAC appears to have tiny higher clean-up efficiencies for PAHs, but lower ones for PCBs compared to GPC.

In contrast results obtained with DAMAC were not clustered but formed a linear graph with a slope of approximately one and R^2 significantly deviating from zero observed for PAHs and PCBs (Figure 4-5a,b) as well as for pooled data. This result indicates compound selectivity of the current DAMAC procedure leading to low recoveries comparing to ASE/GPC. Further optimization of this method could result in lesser selectivity (Rodil et al. 2009).

Linear relationships were derived from MDE and SOX/GPC regarding PCBs, PAHs and pooled data as well. However, correlation of PREL and MOST data for PCBs was weak and insignificant ($p>0.05$, Students t-test). While the slope of the linear graph for MDE was almost unity (PCBs: 0.85; PAHs: 1.12; pooled data: 1.04), indicating a comparable efficiency regardless of the sediment, results for SOX/GPC were dependent on the extracted sediment (slopes for PCBs: 0.33; PAHs: 0.58; pooled data: 0.56). Chlorobenzenes and DDX showed

virtually the same efficiency for each of the extraction methods (except hexachlorobenzene for SOX/GPC) with differing normalised recoveries. Thus, the scattering of the data is caused by the methodical variance of extractability and not by compound or matrix specific influences (Figure 4-5). The efficiency for SOX/GPC was remarkably higher than ASE/GPC such that the differences are attributed to the extraction method because the clean-up is the same. The chosen Soxhlet extraction with acetone appeared to be much more exhaustive than extraction by ASE, MDE or DAMAC.

4.4.4 Relationship between extraction efficiency and effect potential

Results for all total extracts were evaluated with respect to correlation. Data for the MOST sample were plotted against PREL, points were fitted by means of linear regression and correlation analyses were carried out. Cytotoxic effectiveness could be documented in comparable relative strength by most extracts for both sampling sites (Figure 4-6a). Results for MDE, ASE/AMAC, ASE/GPC and DAMAC were close to each other, whereas SOX/GPC extracts gave stronger toxicity. Dioxin-like activities of total extracts did not correlate significantly ($p = 0.454$; Figure 4-6b). Strong differences between MOST and PREL extracts were found for ASE/AMAC and DAMAC. Data from the fish egg test were highly correlated (Figure 4-6c; $p<0.01$, Pearson r: 0.956). Again, MDE and ASE-based methods (ASE/AMAC and ASE/GPC) formed a separate group of close mean effect concentrations.

For comparison of all biotest results, lowest recorded values for effect concentrations from each biotest and sample were divided individually by every other value for the same test system. Resulting ratios are a measure for the effectiveness relative to the corresponding strongest effect. These values were plotted against each other and analyzed for correlation (Figure 4-6d). A significant correlation ($p<0.05$; Pearson coefficient: 0.894) indicated that all exhaustive extraction procedures were comparable between MOST and PREL. Again, MDE, ASE/AMAC and ASE/GPC grouped close together.

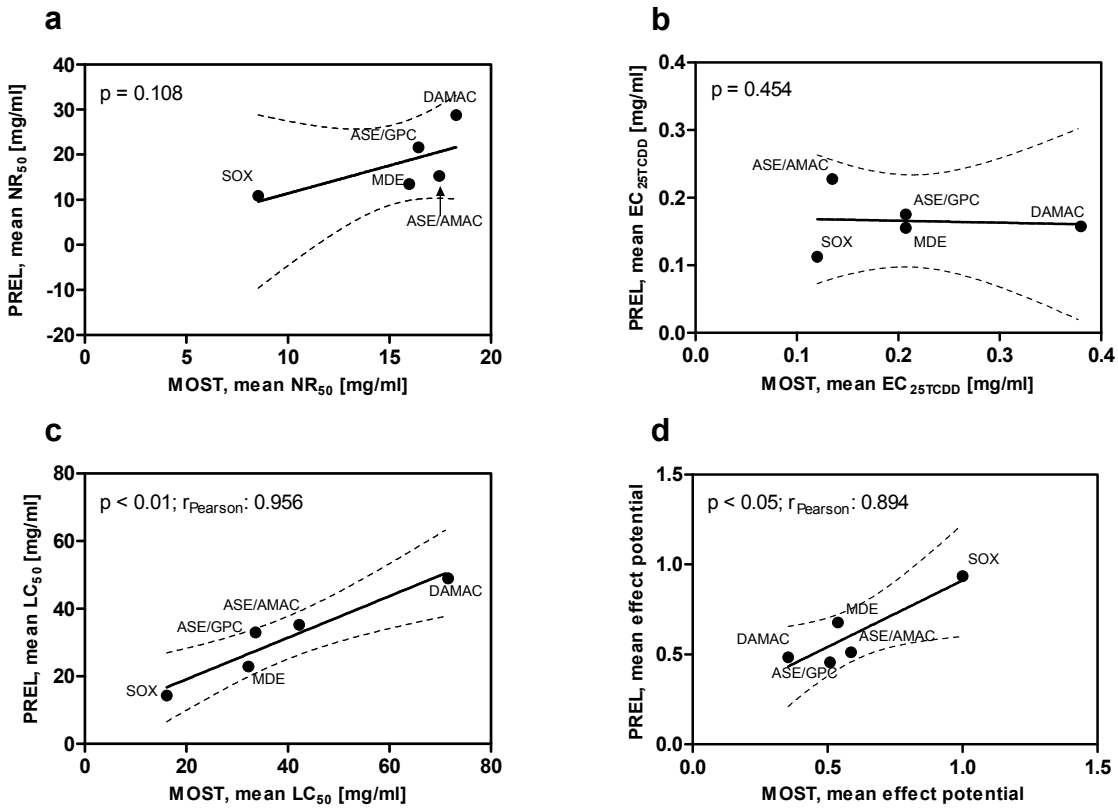


Figure 4-6 Correlations of mean effect data and 95% confidence intervals (dashed lines) for MOST and PREL extracts obtained using the five different exhaustive extraction procedures. a) cytotoxicity, b) dioxin-like activity, c) embryo toxicity, d) pooled data for all three biotests expressed as effect potentials (see text for explanation); SOX=SOX/GPC

A cluster analysis revealed strong relations between MDE, ASE/AMAC and ASE/GPC (Figure 4-7), which supports the observed grouping in the correlation analyses discussed before. Furthermore, the DAMAC approach turned out to be closer to these methods than SOX. As already indicated before, TENAX extracts were at least in part comparable to samples obtained using any of the exhaustive methods.

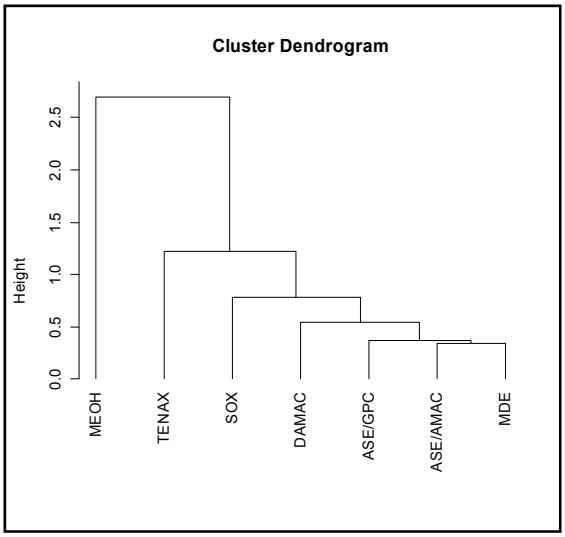


Figure 4-7 Dendrogram of biotest results obtained for each extraction type by average linkage hierarchical cluster analysis. Low height and small distance indicate closer relationship of the samples with respect to toxicological effectiveness

4.4.5 Confirmation of EROD-induction using artificial mixtures

Artificial mixtures representing the concentrations determined by chemical analysis were tested in an EROD assay to verify whether observed discrepancies between the different extraction methods and between chemical and biological analysis could be explained by possible discrimination of certain compound groups (Figure 4-8). Data of HBCD- and MEOH-extracts are not shown, since both methods yielded only very low amounts of targeted compounds (Figure 4-2 and Figure 4-3), which made it difficult to determine an EC_{25}-value in the EROD asssay of the artificial mixture. A comparison appeared especially valuable for extracts treated with Soxhlet, for which chemical analysis showed significantly lower concentrations of PAHs compared to other approaches, while bioanalysis marked this method as the one with the highest effects. Several of the PAHs determined are known to induce EROD activity (Behrens et al. 2001, Billiard et al. 2004, Bols et al. 1999, Bosveld et

al. 2002, Brunström et al. 1991, Hollert et al. 2002). Analyzed PCBs, CBs and DDX are not assigned any dioxin-like activity (Clemons et al. 1997, Clemons et al. 1998, Engwall et al. 1997, van den Berg et al. 1998); however, some of the analyzed compounds may act as suppressor of EROD induction, for example the DDT-isomers (Binelli et al. 2006, Jeong and Kim 2002). Therefore, artificial mixtures contained all analyzed compounds at the concentration levels as determined with the individual extraction/clean-up procedures.

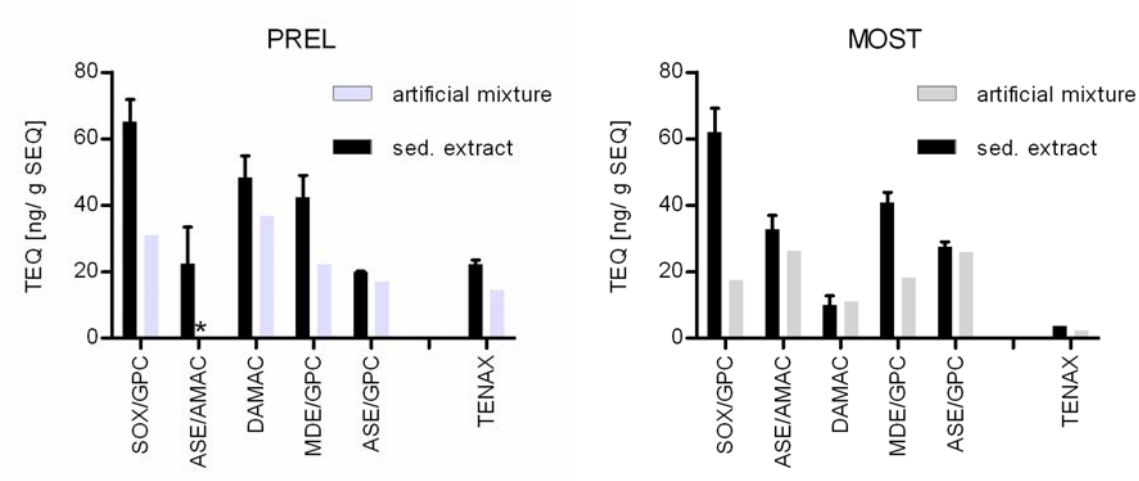


*Figure 4-8 Comparison of extracts and artificial mixtures using EROD data. TEQs were calculated with EC25 values. Error bars indicate range of two independent measurements; artificial mixtures were analyzed without repetition; * = EC25 not calculable; MDE/GPC=MDE*

The various methods applied make use of different solvents for extraction. Thus, it might be hypothesized that differences in polarity of the solvents play a role and different compound classes are extracted. However, solvent used for Soxhlet extraction was acetone, whereas MDE was carried out with *n*-hexane, and DAMAC with a mixture of *n*-hexane/acetone 70:30 (v:v). Hence, it appears to be unlikely that differences in the methods' performance can be attributed solely to different solvent systems. The ASE-based methods are run under elevated temperatures (80 °C and 140 °C for ASE/AMAC and ASE/GPC, 40 °C for DAMAC) and pressure. It is possible, that some compounds degrade under these conditions and thus do not contribute to the total EROD-induction (Seiler et al. 2008). However, further experiments are necessary to clarify what the reason for the observed differences in the methods' performances is.

4.5 Conclusions

SOX, MDE, ASE/GPC and ASE/AMAC showed statistically similar extraction power as well as comparable repeatability and applicability for exhaustive extraction of sediment samples. A major advantage of MDE is the passive principle, which reduces the risk of alteration of the original samples and, thus, adds to veracity of obtained results. AMAC proved to be an alternative to GPC clean-up, possibly reducing the risk of loss of target analytes. DAMAC, the combination of ASE and AMAC in one procedure, is an approach worthwhile but requires further development and optimization. TENAX, MEOH and HBCD produced extracts with highly different effectiveness and therefore do not allow a determination of the direct impact of contaminated sediments at the sampling site. Consequently, it is strongly recommended that investigations in this respect include several mild extraction methods at a time. Moreover, such studies should be accompanied by whole sediment tests using appropriate contact assays (e.g., Feiler et al. 2004, Hollert et al. 2003b, Weber et al. 2006). In that way, comparison of the biotest results can lead to the most compatible extracts for subsequent chemical analysis. Future research needs to provide a better understanding of bioaccessibility and bioavailability as well as the most important influencing parameters. Further development should focus on biomimetic extraction and dosing techniques that reliably separate readily accessible contaminants rather than the operationally defined rapidly desorbing fraction.

4.6 Acknowledgements

The authors greatly appreciate the cluster analysis provided by Markus Brinkmann. We are grateful to Thomas Anger, Joachim Bartels, Sebastian Heger, Marion Heinrich, Erik Leist, Ines Rein, Marion Heinrich, Silke Meyer, Joachim Bartels, Erik Leist and Kerstin Winkens for technical support. We would also like to thank Drs. Niels C Bols and Lucy Lee (University of Waterloo, Canada) for providing RTL-W1 cells. The presented work was partly funded by the European Commission through the Integrated Project MODELKEY (Contract-No. 511237-GOCE), by the German Federation of Industrial Research Associations (Contract-No. KF 0011005 UL6) and by the German Federal Environmental Agency (FKZ 30102018)

Chapter C

Effect potentials and risk assessment of particle-bound contaminants in floodplain soils and suspended particulate matter in the Rhine catchment

5 Risk assessment of river suspended particulate matter and floodplain soils in the Rhine catchment using chemical analysis and *in vitro* bioassays [18] [19] [20] [21]

5.1 Introduction

The European Water Framework Directive (WFD) as well as other related directives and guidelines established a risk-based management of river basins to achieve a good ecological and chemical status by 2015 at a basin-wide scale (Brack et al. 2009a, van Gils et al. 2009). In this context, river-basin wide sediment management was considered as fundamental to reduce risks of particle bound pollutants to the goods and services of river ecosystems (Heise 2009). The functions of sediments and suspended particulate matter (SPM) in aquatic ecosystems were defined as sinks and secondary sources for organic and inorganic pollutants (Power and Chapman 1992, Schulze et al. 2007a).

Besides water pollution due to human activities of urban, industrial or agricultural origin, floods and droughts were identified as main impacts of climate change on water quality (Delpla et al. 2009). Historically contaminated sediments may remobilize during flood events (Förstner 2004), increase the effective potential of SPM (Hollert et al. 2000, Hollert et al. 2003a, Wölz et al. 2008, Wölz et al. 2010b) and might be deposited during flood events in populated areas, floodplains and flood retention areas (Maier et al. 2006, Middelkoop et al. 2010, Schwartz et al. 2006). However, even a disastrous flood event such as the Elbe flood in 2002 does not necessarily cause increased contamination and adverse effects due to

[18] Parts of this section were published as: Ulrich, M; **Schulze, T**.; Leist, E.; Glaß, B.; Maier, M.; Maier, D.; Braunbeck T.; Hollert, H. (2002) Abschätzung des Gefährdungspotenzials für Trinkwasser und Korrelation verschiedener Expositionspfade (Acetonischer Extrakt, Natives Sediment) im Bakterienkontakttest und Fischeitest; Umweltwissenschaften und Schadstoff-Forschung - Zeitschrift für Umweltchemie und Ökotoxikologie 14, 132-137 (DOI: 10.1065/uwsf2002.07.036)

[19] This section contains data that was elaborated during the diploma thesis of Dr. Markus Ulrich (2002): Gefährdung von Trinkwasser durch partikulär gebundene Schadstoffe in den Rheinauen: Vergleich nativer und extrahierter Proben in zwei *in vitro* Biotests; Diplomarbeit Fakultät für Biologie Ruprecht-Karls-Universität; Heidelberg; 78 pp.

[20] This section contains data that was elaborated during the state examination thesis of Volker Garke (2003): Optimierung und Anpassung eines *in-vitro* Bioassays mit RTL-W1- und RTG-2-Zellen zum Nachweis der cytoxischen und Dioxin-ähnlichen Wirkung von komplexen Umweltproben; Staatsexamensarbeit Institut für Zoologie, Ruprecht-Karls-Universität; Heidelberg; 62 pp.

[21] This section is in preparation for submission to «Environmental Science and Pollution Research»: **Schulze, T.**; Ulrich, M.; Garke, V.; Maier, M.; Maier, D.; Terytze, K.; Braunbeck, T.; Hollert H., Risk assessment of river suspended particulate matter and floodplain soils in the Rhine River catchment using chemical analysis and in vitro bioassays.

sediments, but can instead cause a wide distribution of contaminants and thus decrease sediment toxicity due to dilution (Oetken et al. 2005b).

Settling of sediments or SPM to floodplains removes pollutants from the aquatic system temporally (Ensenbach 1998). But deposited contaminated solids might affect terrestrial ecosystems (Klok and Kraak 2008, Tockner et al. 2010), could be remobilized during flood events (Hollert et al. 2000, Hollert et al. 2003a, van Gils et al. 2009) or pose a risk to drinking water resources (Maier et al. 1997, Maier et al. 2006). Hence, in drinking water protection areas there may be a conflict of interests between the potential risk of ground-water contamination during flood events and the construction of natural retention areas due to remobilization of particularly bound pollutants under certain conditions (Busche and Hirner 1997, Kedziorek et al. 1998, Maier et al. 2006, Sauvé et al. 2000).

The purpose of this study was to assess the hazard potentials of contaminated SPM sampled during a flood event for floodplain soils using *in vitro* bioassays and chemical analysis. The samples were characterized for their physico-chemical properties as well as the content of trace metals and organic pollutants. Sediment-contact tests were performed to evaluate the direct exposure of organisms to native soils and SPM at two different trophic levels. For comparison, acetonic extracts were tested using both contact tests and additionally two cell-based biotests. The sediment-contact tests were carried out with the dehydrogenase assay with *Arthrobacter globiformis* and the fish egg test with *Danio rerio* (FET). The cytotoxicity and the dioxin-like effects were determined with the neutral red and the 7-ethoxyresorufin-O-deethylase induction assay (EROD), respectively, using the permanent rainbow trout liver cell line RTL-W1.

Four hypotheses were tested: (1) The exposure of acetonic extracts in the bioassays overestimates the hazard potential comparing to native soils and SPM exposure. (2) The hazard potential of SPM increases during flood events due to remobilization of contaminated sediments. (3) The settling of contaminated SPM in frequently inundated floodplain soils increases the hazard potential comparing to soils from infrequently inundated areas. (4) The priority compounds detected do not fully explain the effects found in the bioassays.

5.2 Material and Methods

5.2.1 Chemicals

Certified standard solutions containing the 16 EPA-PAH were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Certified standards of PCBs and HCB as well as the internal standards acenaphthene-D_{10}, phenanthrene-D_{12}, chrysene-d_{12}, perylene-D_{10}, PCB 53 and PCB 159 were obtained from Promochem (Wesel, Germany). The analytical standards and acids for the inorganic compounds were purchased from Merck (p.a. or suprapur® grade, Merck, Darmstadt, Germany).

5.2.2 Sampling sites, collection of soil and SPM samples and sample preparation

Three different types of samples were investigated, representing several risk scenarios:

- Soil samples from a frequently inundated floodplain riverside of the levee (BT1–BT7; Figure 5-1; Table 5-1; Coordinates: UTM 32U, 447586.26E, 5426588.24N); this area is frequently inundated with potential sedimentation of SPM;
- Soil samples from the land side of the levee, representing an infrequently inundated area (B1–B7; Figure 5-1; Table 5-2; Coordinates: UTM 32U, 447700.12E, 5426453.43N)
- SPM samples from Rhine collected in the fish pass at the upstream location Iffezheim barrage (Rhine km 232; Figure 6-1, p.143; Table 5-3) during different water levels representing a potential source of pollutants entering the planned retention basin (Coordinates: UTM 32U, 434636.80E, 5409283.84N).

The frequently inundated floodplain was flooded from February to October 2001. Therefore, it was possible to analyse the contribution of freshly deposited SPM to the effect potential of the floodplain soil.

The top soil samples (B1, B3–B7, and BT1–BT6; depth: 0–4 cm, recent pollution) were collected using a stainless steel shovel. Coring samples (B2 and BT7; depth: 80–100 cm; reference conditions or potential migration of pollutants to sub ground) were sampled using a corer. The SPM samples were collected using a sedimentation box (Hollert et al. 2000c). The samples for biological and chemical analysis were freeze-dried within 48 h (Christ Beta 1-8, Osterode, Germany), sieved <1.25 mm using a stainless steel mesh and stored at 4 ° C for a

maximum of 8 weeks. Subsamples for grain size distribution were stored wet at 4 °C until analysis.

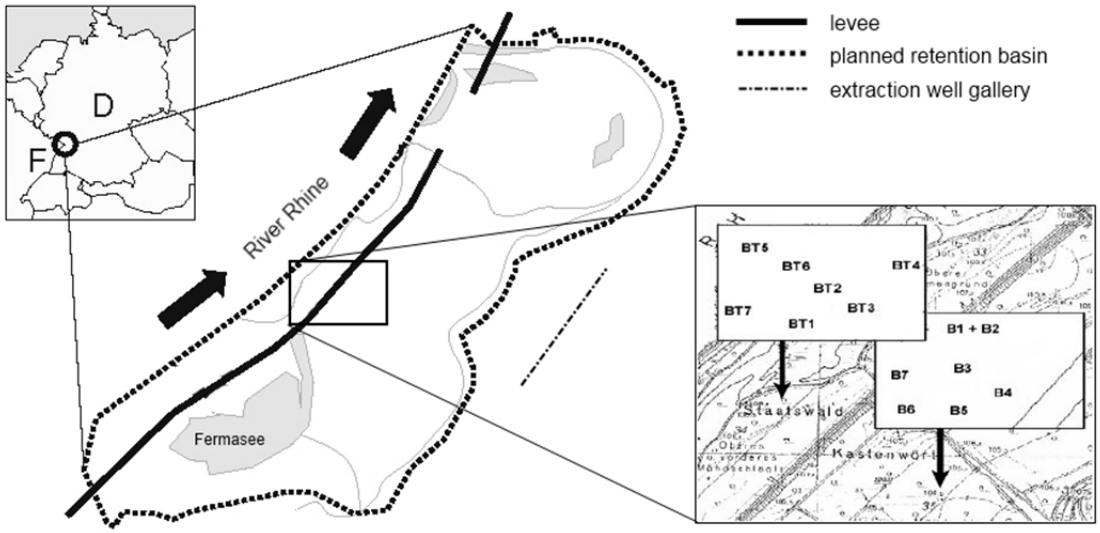


Figure 5-1 Location of the planned retention basin Bellenkopf-Rappenwört near Karlsruhe (Germany; dotted line), the sampling points of the soils samples from frequently inundated floodplain (samples BT1–BT7) river side of the levee (solid black line) and non inundated floodplains (samples B1–B7) land side of the levee, as well as the extraction well gallery (dashed-dotted line); F: France; G: Germany (adapted from Ulrich 2002, Wölz et al. 2011b)

Table 5-1 Sample codes of soil samples from inundated area, the content of TOC, TIC and the grain size distribution (type of soil according Arbeitsgemeinschaft Boden 1996); gray highlighted: core sample; Tt: pure clay, Tu2: poor silty clay, Lu: silty loam

Sample code	BT1	BT2	BT3	BT4	BT5	BT6	BT7
TOC (%)	4.7	3.2	3.9	3.1	1.6	1.6	4.4
Sand (%)	3.7	4.9	8.8	16.4	4.8	4.4	29.6
Silt (%)	20.0	34.6	63.6	63.8	32.1	23.6	18.9
Clay (%)	76.3	60.6	27.6	19.8	72.1	72.0	51.5
Type of soil	Tt	Tu2	Lu	Lu	Tt	Tt	Tl

Table 5-2 Sample codes of soil samples from non inundated area, the content of TOC, TIC and the grain size distribution; type of soil according Arbeitsgemeinschaft Boden (1996); gray highlighted: core sample; Tu4: high silty clay, Su2: poor silty sand, fs: fine sand, ms: middle sand; Ut2; poor clay silt, Tu4: high silty clay

Sample code	B1	B2	B3	B4	B5	B6	B7
TOC (%)	4.6	7.0	5.9	7.2	5.2	3.0	2.6
Sand (%)	1.7	72.9	1.4	3.4	2.6	1.7	4.9
Silt (%)	70.2	22.3	64.4	86.5	67.3	68.6	66.9
Clay (%)	28.1	4.8	34.2	10.1	30.1	29.7	28.2
Type of soil	Tu4	Su2,fs,ms	Ut2	Tu4	Tu4	Tu4	Tu4

Table 5-3 Sample codes and sampling periods of the SPM samples collected at Iffezheim barrage as well as the contents of TOC

Sample code	S1	S2	S3	S4	S5	S6
Start date	01.02.2001	14.02.2001	06.03.2001	07.03.2001	12.03.2001	13.03.2001
End date	14.02.2001	06.03.2001	07.03.2001	13.03.2001	13.03.2001	(1–5 p.m.)
TOC (%)	4.6	7.0	5.9	7.2	5.2	3.0

5.2.3 Grain size distribution and total organic carbon

Standard procedures were used for analysis of grain size distribution of wet soils (ISO 11277; meshes: 2 mm, 630 µm, 200 µm, 63 µm, 20 µm; Retsch GmbH, Haan, Germany). The content of total organic carbon (TOC) was analyzed according to ISO 10694 by means of a C-Mat 500 (Stroehlein Instruments, Juwe GmbH, Viersen, Germany) in soils and SPM sieved <1.25 mm.

5.2.4 Analysis of trace elements

The sieved (<1.25 mm) and dried (105 °C) soil an SPM samples were extracted using aqua regia and a BEROTEST extraction device (Behr, Düsseldorf, Germany) according to DIN ISO 11885. After extraction, the extracts were adjusted to 100 ml using bi-distilled water and filtered using folded paper filters (No. 595½, Schleicher & Schuell, Dassel, Germany). The elements Cr, Cu, Ni and Zn were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-OES Optima 3000, Perkin Elmer, Rodgau-Jügesheim, Germany). As, Cd and Hg were analyzed using graphite oven atomic absorbance spectroscopy with hydride cold vapor technology and Zeeman background correction (Perkin Elmer 4100ZL coupled with Perkin Elmer FIAS 200) according DIN EN ISO 11969 and DIN EN 1483. For quality assurance the certified reference soil BCR CRM 143R was extracted twice (Table S5-1 in Appendix). Element values in simultaneously analyzed blank samples were below detection limits.

5.2.5 Soxhlet extraction and silica gel fractionation

The sieved and freeze-dried soil and SPM samples were extracted using Soxhlet extraction with acetone with six cycles per hour over 24h to obtain the acetone extractable fraction according to Hollert et al. (2000). Rotary evaporation and nitrogen was used to vaporize solvent nearly to dryness. The residues were reconstituted in 0.5 ml *n*-hexane and separated into six fractions by column chromatography (2 g silica gel 60, Merck, Darmstadt, Germany) according to polarity (Ricking and Terytze 1999). Mixtures of *n*-pentane, dichloromethane (DCM) and methanol were used as eluents (Bundt et al. 1991, Franke et al. 1998, Heim et al. 2005). Fraction F1–F4 were vaporized to a final volume of 200 µl using nitrogen and transferred to GC vials that were stored at -20 °C until analysis.

5.2.6 Instrumental analysis of organic compounds

GC-MS analysis was carried out on an HP 5890 II GC coupled to a HP MSD 5971 (Agilent, Palo Alto, USA), equipped with a 60 m × 0.25 mm I.D. × 0.25-µm film DB-XLB fused capillary silica column. The MS was operated in electron ionization mode (EI+, 70 eV) with a source temperature of 180 °C scanning from 50 to 550 amu (full scan mode; scan time: 1.5 sec) or single ion monitoring (SIM) for quantification. The chromatographic conditions for analysis of EPA-PAHs (PAH) and PCBs according to Ballschmiter and Zell (1980) as well as for full scan analyis were as follows: 300 °C injector temperature, 1 µl splittless injection at oven temperature of 80 °C, then programmed to 300 °C at 4 K/min (1 min isotherm) and finally programmed to 310 °C at 0.8 K/min (35 min isotherm). Carrier gas velocity (helium 5.0, Air Liquide, Böhlen, Germany) was 1 ml/min at constant flow.

For quality assurance the internal standards PCB 53 and PCB 139 were spiked to fractions F1 and F2, deuterated PAHs (acenaphthene-D_{10}, phenanthrene-D_{12}, chrysene-D_{12} and perylene-D_{10}) were spiked to fraction F3 and F4 and 4-N-nonylphenole were spiked to fraction F5 and F6 prior to analysis. The recovery values of the internal standards are listed in Table 5-4. The concentrations of the most compounds in simulaneously analyzed blanks (n=2) were below detection limits. The detection limits of the compounds were between 0.5 and 5.5 µg/kg.

Table 5-4 Recoveries of internal standards

Internal standard	Recovery (%)
PCB 53	77.6 ± 11.3
PCB 159	77.6 ± 13.8
acenaphthene-D_{10}	96.5 ± 6.8
phenanthrene-D_{12}	97.0 ± 8.1
chrysene-D_{12}	99.5 ± 7.6
perylene-D_{10}	94.6 ± 7.7
4-n-nonylphenol	101 ± 17.4

5.2.7 Dioxins and furans

Dioxins and furans in samples B1, B2, BT1, BT7 and S2 were analyzed by Analysen Service GmbH (Berlin, Germany) according DIN 38414-24 by purchase order (test report 531-03-1).

5.2.8 Bioassays

5.2.8.1 Neutral red retention assay

Acute cytotoxic effects were determined using the neutral red retention assay (Babich and Borenfreund 1992) according to the method detailed in Seiler et al. (2006). Briefly, cells from CYP1A-expressing cell line RTL-W1 (Bols et al. 1999, Lee et al. 1993) were exposed to serial dilutions of sediment extracts along seven wells in six replicates of a 96-well microtitre plate (TPP, Trasadingen, Switzerland) at a final concentration range of 1.56–100 mg/ml. 80 mg/l 3,5-dichlorophenol was used as a positive control. After incubation at 20 °C for 48 h, cells were stained with neutral red (2-methyl-3-amino-7-dimethylamino-phenanzine) for 3 h, and neutral red retention was determined at 540 nm with a reference wavelength of 690 nm using a microtitre plate reader (Tecan, Crailsheim, Germany). Volker Garke performed the biotests Garke 2003, Garke et al. 2003).

5.2.8.2 EROD induction assay

The dioxin-like inducing potential of sediment extracts was investigated using the 7-ethoxyresorufin-O-deethylase induction assay (EROD; Behrens et al. 1998) according to method given in Keiter et al. (2008). RTL-W1 cells were seeded into 96-well microtitre plates and exposed to sediment extracts in eight dilution steps with six replicates. As a positive control, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was serially diluted on two separate rows of each plate. Following incubation at 20 °C for 72 h, EROD induction was terminated by disrupting the cells by shock freezing in the vapor space of liquid nitrogen. Subsequently, 100 µl of the substrate 7-ethoxyresorufin were added to each well before deethylation was initiated for 10 min with nicotinamide adenine dinucleotide phosphate in phosphate buffer. The reaction was stopped by adding 100 µl of fluorescamine in acetonitrile. The production of resorufin as a metabolite of the substrate was recorded fluorometrically at 544 nm (excitation) and 590 nm (emission) using a GENios plate reader. Whole protein was also determined fluorometrically using the fluorescamine method (excitation 355 nm, emission 590 nm; Hollert et al. 2002, Kennedy and Jones 1994). Fluorescent units were converted to

mass of resorufin and protein with the aid of calibration curves. Volker Garke performed the biotests (Garke 2003, Garke et al. 2003).

5.2.8.3 Fish egg test with Danio rerio

The fish egg test was performed according to draft DIN 38415-6 for 48 h according to Braunbeck et al. (2005) and Hollert et al. (2003b). Markus Ulrich performed the biotest (Ulrich 2002, Ulrich et al. 2002).

5.2.8.4 Bacteria contact test with Arthrobacter globiformis

The bacteria contact test was performed according to modified draft DIN 38412-48. The acetone extracts were tested with 400 mg, 200 mg, 100 mg, 50 mg and 25 mg sediment per test using 24-well micro titer plates. The positive control was 250 mg/l 4-nitrophenol in bi-distilled water. The plates were incubated for 2 h at 30 °C, spiked with 500 µl of the resazurin solution and incubated for 30–120 min at 30 °C until the absorption of resazurin at 595 nm (OD_{595}) of the test wells (with bacteria) were 70% of the OD_{595} of the blank wells (without bacteria) due to metabolic transformation of resazurin to resorufin.

The direct sediment contact test was performed in triplicate with 2 g of each sample using 15 ml polyethylene Falcon-type tubes. Quartz sand (grain-size W4; Merck, Darmstadt, Germany) was used as control sample and spiked with 4 ml of 250 mg/l 4-nitrophenol in bi-distilled water. Three ml bi-distilled water was added to samples and controls followed by 2 ml of DSM medium per 2 ml bacteria suspension. The samples and controls were incubated for two hours at 30 °C using an overhead shaker at 70 rpm.

The OD_{595} was measured photometricaly at 525 nm using a micro-titer well plate reader (Tecan, Crailsheim, Germany). The inhibition of bacteria was computed using Equation 6–1:

$$Inhibition[\%] = 100 - \left[\frac{OD_{595}^{sample} - OD_{595}^{blank\ sample}}{OD_{595}^{control} - OD_{595}^{blank\ control}} \cdot 100\right] \quad (6-1)$$

Markus Ulrich performed the biotest (Ulrich 2002, Ulrich et al. 2002).

5.2.9 Data analysis

5.2.9.1 Concentration-response relationships

Concentration-response relationships were calculated using nonlinear regression with GraphPad Prism® 5.04. A sigmoid function with variable slope was used to fit the EROD and fish egg bioassay data, and a three or four parameter Hill function adapted from Sigma Plot 11 (Systat 2008) was used for fitting the bacteria contact test data. If neither of these fitted, a second order polynomial function was used. If it was not possible to calculate concentration-response curves, the lowest effect concentrations (LOEC) were given.

5.2.9.2 Bio-TEQ values

Bioassay-derived TCDD equivalents (Bio-TEQs) were calculated by relating the biological EROD activities of the samples to the positive control TCDD using the fixed effect level quantification method (cf. Wölz et al. 2008). Mean TCDD-EC_{25} and standard deviation (SD) values were determined using a sigmoid log-logistic model with GraphPad Prism® 5. Bio-TEQs with concentrations in picogram TCDD per gram of sample equivalent (SEQ) were calculated as per Equation 6–2:

$$Bio-TEQ\left[\frac{pgTCCD}{gSEQ}\right]=\frac{TCDD-EC_{25}[\frac{pgTCDD}{ml}]}{sample-EC_{25}[\frac{gSEQ}{ml}]} \quad (6-2)$$

5.2.9.3 Chem-TEQ values

In order to explain the determined Bio-TEQs, chemically derived TEQ values (Chem-TEQs) were calculated using relative potency factors (REP; Bols et al. 1999; Equation 6–3):

$$Chem-TEQ\left[\frac{pgTCDD}{gSEQ}\right]=\sum_i(c_i\times TEF_i) \quad (6-3)$$

where for a given chemical *i*, c_i is the measured concentration in the sample and TEF_i is the toxic equivalency factor for each compound relative to TCDD. TEFs were derived for RTL-W1 cells according to Clemons et al. (1997) and according to World Health Organization (WHO; van den Berg et al. 2006).

5.2.9.4 Significance testing and multivariate explorative analysis

Data was tested for normal distribution (Kolgomorov-Smirnov test; K-S) and for variance homogeneity (Bartlett's test). If data passed K-S and Bartlett's test, one-way analysis of variance (ANOVA) with Tukey's posttest was performed to test significances using GraphPad Prism® 5.04; otherwise, Kruskal-Wallis ANOVA with Dunn's posttest was used. Agglomerated hierarchical analysis (cluster analysis) using complete linkage as cluster rule and 1-Pearson rs as distance measure as well as principal component analysis (PCA) was performed for multivariate explorative data analysis using Statistica 8.0 (StatSoft 2008).

5.3 Results and discussion

5.3.1 Grain size distribution and contents of total organic carbon

The results of analyzes of grain size distribution are listed in Table 5-1 (samples from frequently inundated soils) and Table 5-2 (samples from infrequently inundated area). The samples from infrequently inundated area were silty clays (Tu4; B1, B3, B5–B7) classified according to Arbeitsgemeinschaft Boden (1996). Sample B4 was a clayish silt (Ut2) and sample soil core sample) a silty sand (Su2,fs,ms). The variance between top soil layers and the core samples are due to different sedimentation conditions in the underlying bed. The BT samples were finer grained than the samples from infrequently inundated area due to a settlement of fine-grained settled suspended matter during flood events. The samples were clays (Tt; BT1, BT5, and BT6), silty clays (Tu2; BT2), silty loams (Lu; BT3 and BT4) and a loamy clay (Tl; BT7). The SPM samples were not analyzed for grains size distribution due to low sample amounts. For comparison, SPM samples from the Rhine collected during year 2005 were mainly silty clays (Tu4; Schulze et al. 2007b). The dominant appearance of clay and silt fractions in the top soils is comparable to floodplain soils from the Hessisches Ried (Gocht et al. 2001). Information regarding the grain size distribution and composition of SPM in the catchment is scarcely, however, Lartiges et al. (2001) reported a dominance of clay and silt fractions in SPM samples from the Rhine near Lauterbourg.

The contents of total organic carbon in the soil (B: 9.2% ± 1.2%; BT: 3.0% ± 1.2%) and SPM (S: 5.5% ± 1.6%) samples were relatively homogenous within the different sample types (Figure 5-2). The levels of TOC in the BT and SPM samples were comparable to levels of SPM samples (SPM 2005; 5.0% ± 2.0%; n=157) collected during year 2005 from different

German rivers (Schulze et al. 2007b). Furthermore, Abril et al. (2002) reported TOC contents of SPM samples from the Rhine catchment between 5% and 7%. ANOVA revealed a highly significant difference between the soils from infrequently inundated area (B) and both the soil samples from frequently inundated floodplain (BT) and the SPM samples (S; Figure 5-2).

ANOVA revealed a significant variance between the BT and SPM samples as well. This observation might caused by remobilisation of sediments during the flood event occurred in 2001. This assumption is supported by TOC data in SPM measured during 2005 that showed comparable TOC contents (Figure 5-2) under normal discharge conditions with a short term flood with a recurrence interval of two years (LUBW 2011; Figure 5-13 and Figure S5-1 in Appendix). However, it can be assumed that the TOC in the soil samples from infrequently inundated area (B), located in a forest, mainly originated from litter and the TOC in the soils from inundated area (BT) derived from deposition of SPM on the floodplain.

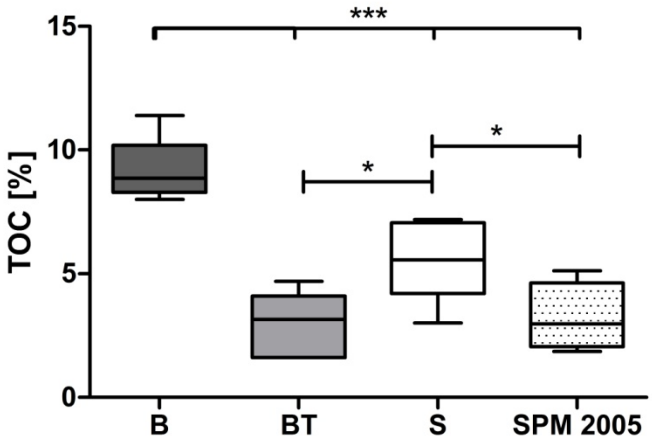


*Figure 5-2 Box-and-whisker plots (boxes: median, 25th-/75th-percentiles; whiskers: minimum, maximum) of the contents of total organic carbon (TOC) in inundated (BT; n=6) and infrequently inundated (B; n=6) top soil layers as well as in suspended particulate matter (SPM) samples (S; n=6) compared with SPM samples collected in year 2005 (SPM 2005; Schulze et al. 2007b); values from borehole samples (B2 and BT7) were not included; *: p≤0.05, *** p≤0.001 (ANOVA with Tukey's posttest)*

5.3.2 Trace elements in whole samples

The results from trace elements analysis are depicted in Figure 5-3. The concentrations of cadmium and nickel were similar in all sample groups (Table S5-2, S5-3 and S5-4 in Appendix). The concentrations of the other elements were found in significantly higher

concentrations in the BT samples than in the S and B samples. The values of the heavy metals in the floodplain soil samples (BT) were not significantly different to concentrations in floodplain soils downstream the Rhine published by Gocht et al. (2001) and Middelkoop (2000; $p \geq 0.05$, ANOVA with Tukey's posttest).

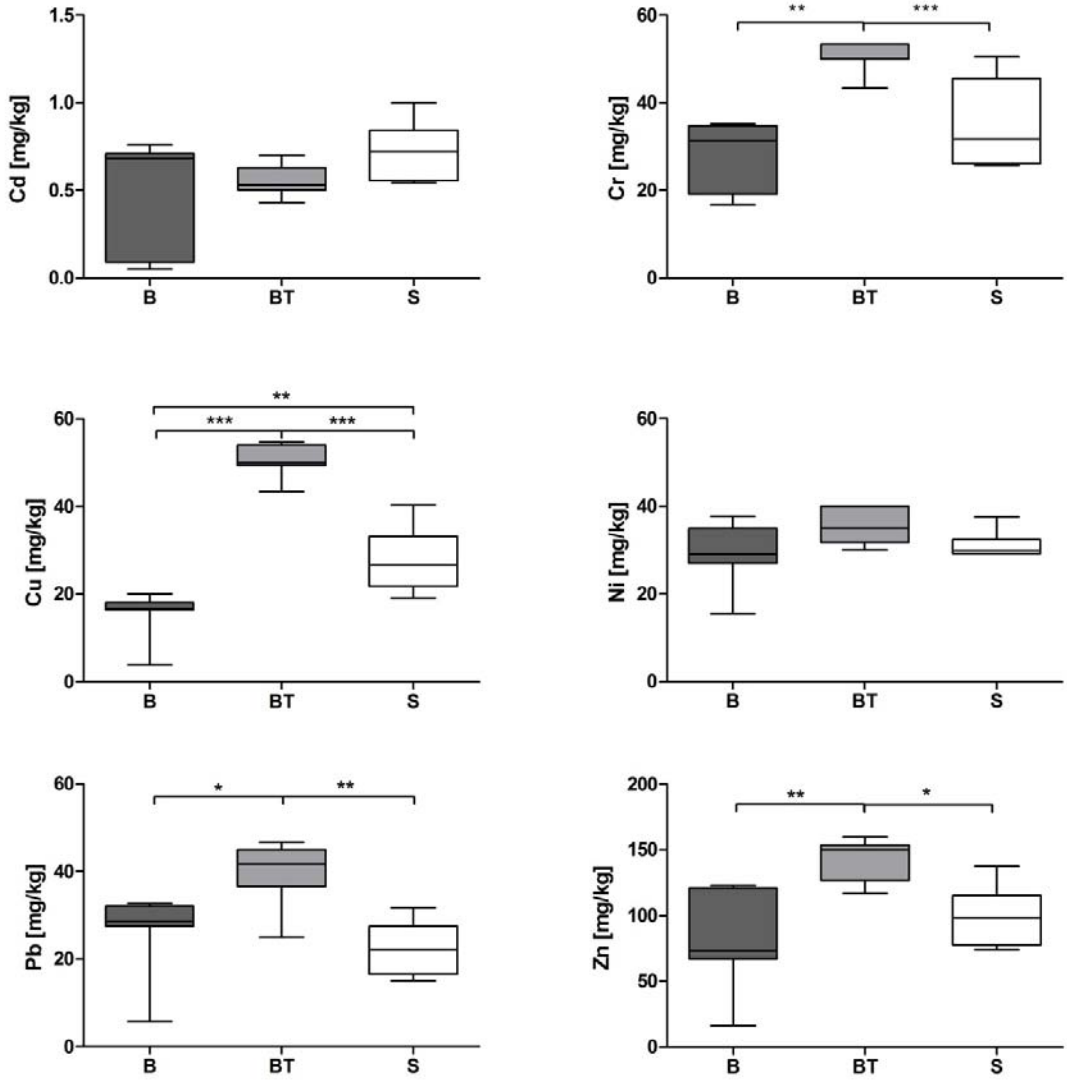


*Figure 5-3 Box-and-whisker plots (boxes: median, 25th-/75th-percentiles; whiskers: minimum, maximum) of the levels of toxic trace elements in inundated (BT; n=6) and infrequently inundated (B; n=6) top soil layers as well as in SPM samples (S; n=6); analysis was performed using the grain size fraction <1.25 mm; *: p<0.05, ***: p<0.001 (ANOVA with Tukey's posttest)*

However, there is only scarce information regarding recent heavy metal pollution of floodplain soils of the Rhine. The mean levels of metals in the SPM samples were not significantly different ($p \geq 0.05$, ANOVA with Tukey's posttest) comparing to average levels in SPM samples collected by the German Environmental Specimen Bank at Iffezheim barrage in 2005 and 2006 (fraction <2 mm; www.umweltprobenbank.de) and by the International Commission for the Protection of the Rhine at Seitz monitoring station between 2001 and 2002 (fraction <20 µm; www.iksr.de).

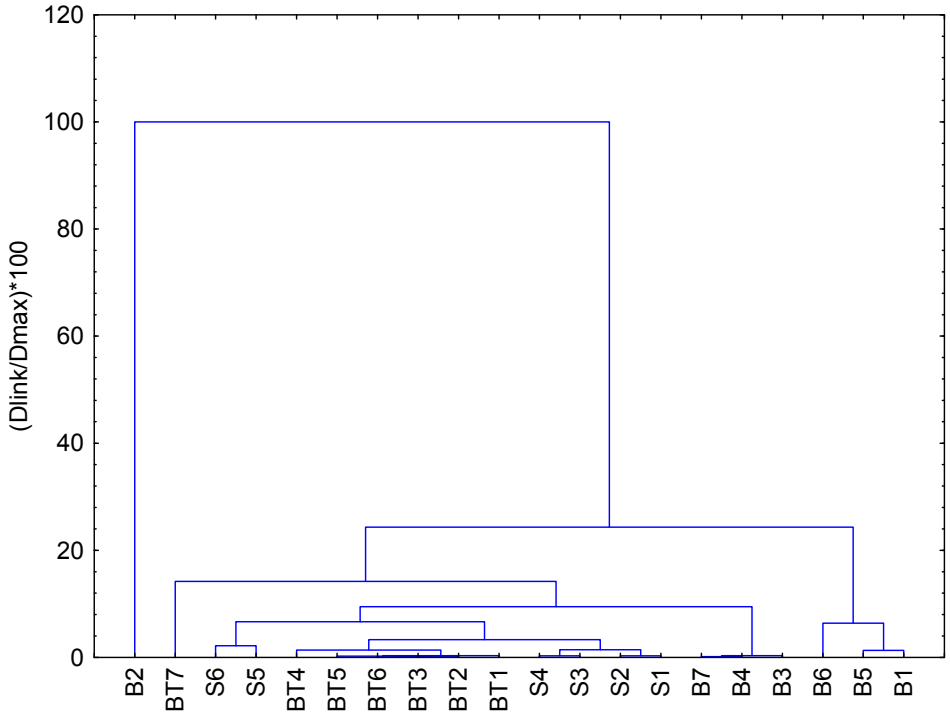


Figure 5-4 *Dendrogram showing the result from complete linkage cluster analysis of the toxic trace elements and the particular samples (fusion rule: complete linkage; distance measure: 1-Pearson r; B: soil from infrequently inundated area; BT: soils from inundated area; S: SPM samples*

Cluster analysis was used to unravel further linkages between the sample types and distributions of trace elements (Figure 5-4). The samples could be assigned to five clusters. Cluster 1 consisted of the SPM samples S5 and S6 collected during the flood event. Cluster 2 included all BT samples from frequently inundated floodplain except the borehole sample BT7. All other SPM samples could be referred to cluster 3. Cluster 4 and cluster 5 comprised two groups of soil samples from infrequently inundated area with exception of the borehole

sample B2. The partitioning could be explained by elevated concentrations of Zn in the samples assigned to cluster 4.

5.3.3 Organic compounds in crude extracts

Figure 5-5 A–C shows the results of organic compounds (PAHs, HCB and PCBs). The ranges of EPA-PAHs were 188–754 µg/kg in the B samples, 345–3034 µg/kg in the BT samples and 474–2318 µg/kg in the SPM samples (S; Figure 5-5A). HCB concentrations were in the ranges of 0.9–1.8 µg/kg in the B samples, 15–53 µg/kg in the BT samples and 25–203 µg/kg in the SPM samples (Figure 5-5B). PCBs were below detection limits in the B samples and were in the ranges of 8–23 µg/kg in the BT samples and of 3–32 µg/kg in the SPM samples (Figure 5-5C).

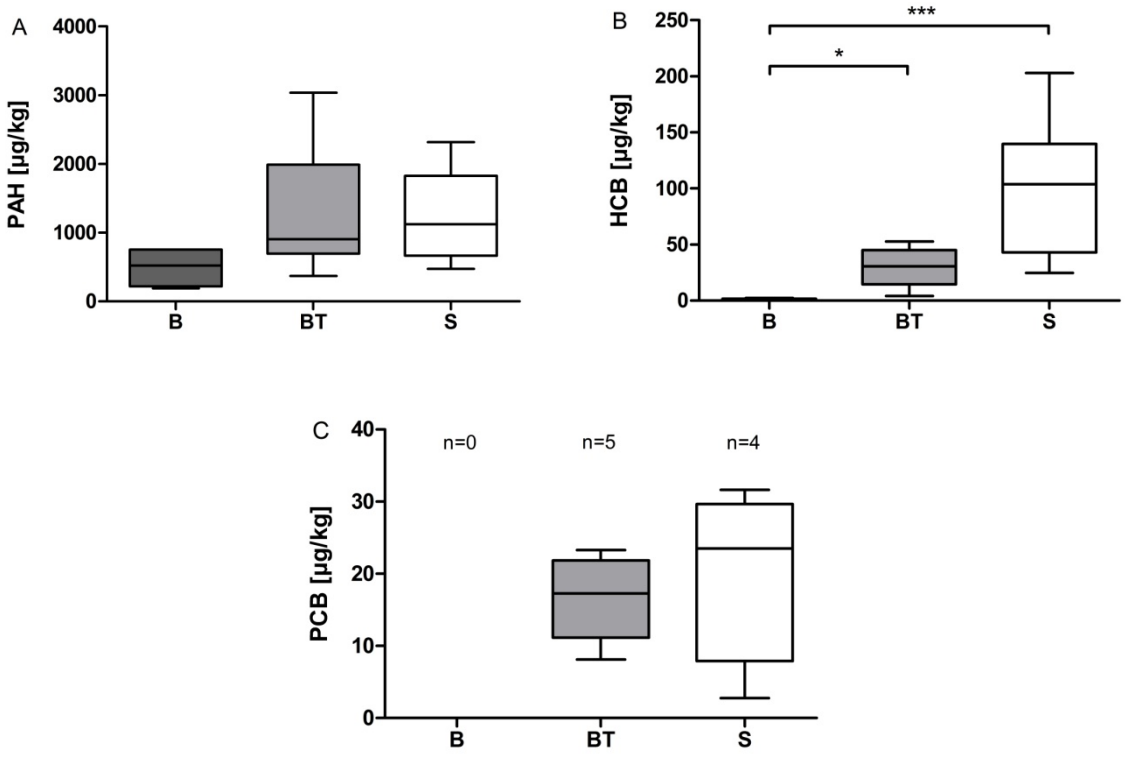


*Figure 5-5 Box-and-whisker plots (boxes: median, 25th-/75th-percentiles; whiskers: minimum, maximum) of the levels of organic compounds (A: PAHs; B: HCB; C: PCB) in inundated (BT; n=6) and infrequently inundated (B; n=6) top soil layers as well as in SPM samples (S; n=6); analysis was performed using the grain size fraction <1.25 mm; values from borehole samples (B2 and BT7) were not included; *: p<0.05, ***: p<0.001 (Kruskal-Wallis ANOVA with Dunn's posttest)*

The summarized concentrations of the 17 analyzed congeners were 118 pg/g in B1, 2017 pg/g in BT1, 2178 pg/g in BT7 and 3304 pg/g in S2 (Table S5–5 in Appendix). The distribution patterns of the respective congeners are depicted in Figure 5-6 (in pg/g WHO-TEQ; van den Berg et al. 2006). The composition in B1 was strongly dominated by PCDFs with a content of more than 90%. In sample BT1 and the borehole sample BT7 still were found about 79% and 58% of PCDFs, respectively. The SPM sample S2 contained more PCDDs (56%) than PCDFs (44%). The contents of PCDD/Fs in this study were at the lower levels of PCDD/Fs analyzed in sediments of the Elbe after the flood in 2002 (3–140 pg/g WHO-TEQ; Stachel et al. 2005).

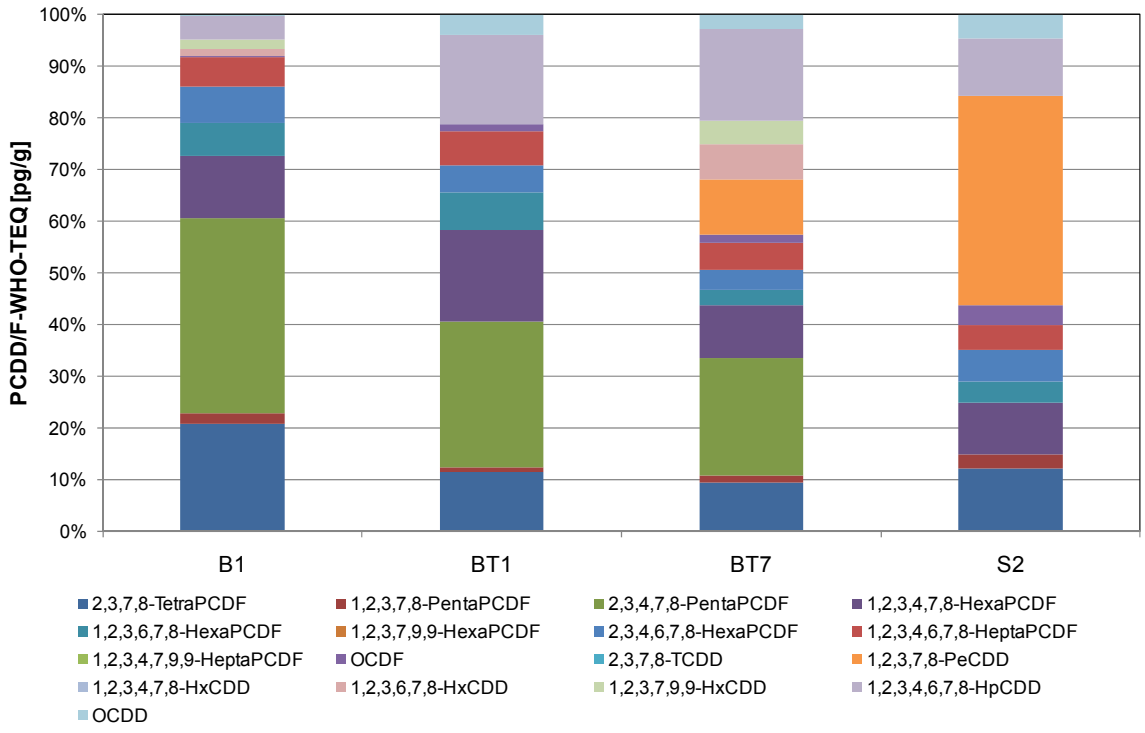


Figure 5-6 Patterns of PCDD/Fs congener distributions (in pg/g WHO-TEQ; van den Berg et al. 2006)

Principal components analysis (PCA) was used to explore the distribution patterns of the PCDD/Fs in the soil and SPM samples. Before analysis, each PCDD/F congener was standardized by calculation of the ratio congener's WHO-TEQ with the total WHO-TEQ of each sample. According to Fiedler et al. (1996b) concentrations below the detection limits were treated as zero to avoid ratios without linear relationship to the true ratio. PC1 explained 65.76% (Eigen value: 2.63), PC2 explained 26.89% (Eigen value: 1.08), PC3

explained 4.96% (Eigen value: 0.2) and PC4 2.4% (Eigen value: 0.1) of the standardized data. The samples were projected at the plane of PC1 and PC2 (Eigen value: 3.71; Figure 5-7). The B1, BT1 and BT7 samples were clearly separated from the S2 sample. BT2 showed some similarity with S2 due to a higher content of PCDDs in these samples compared to B1 and BT1.

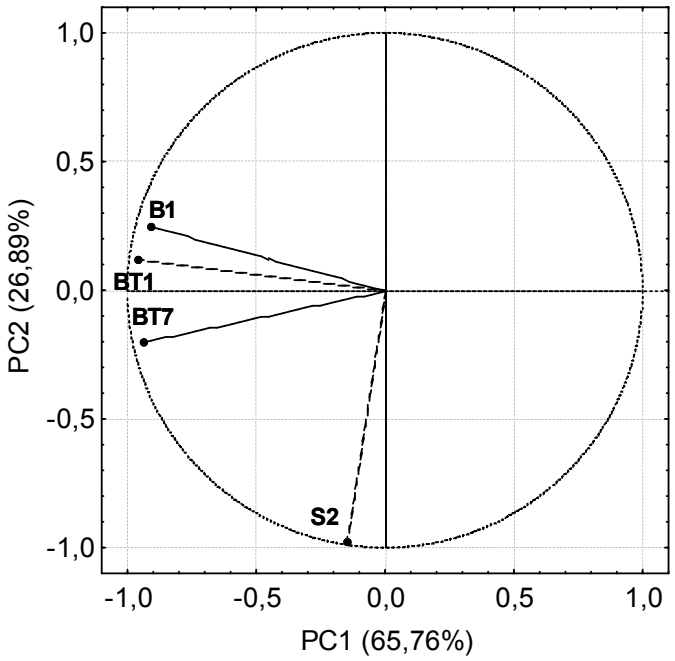


Figure 5-7 Principal component plot of PC1 and PC2 (Cumulative Eigen values: 3.71) of the PCDD/F distribution patterns selected samples in inundated (BT) and infrequently inundated (B) top soil layers as well as in SPM samples (S)

5.3.4 Bioanalysis of crude extracts and whole samples

5.3.4.1 Neutral red retention assay

The results from acetonic extracts of the samples tested in the neutral red retention assay with RTL-W1 cells are shown in Figure 5-8. The half-maximum effective concentrations (NR_{50}) were between 80 and 300 mg/ml in landside soils (B), 70 and 101 mg/ml in floodplain soils (BT) and in the range from 81 to 300 mg/ml in the SPM samples (S). The samples B2 and S6 were not cytotoxic. The observed low NR_{50} value in the soil samples from frequently inundated area (BT samples) may be caused by inputs of cytotoxic compounds during flooding of the area (Garke 2003). However, there was no significant difference in cytotoxicity observed between the different sample groups ($p \geq 0.05$, ANOVA with Tukey's posttest; Figure 5-14A).The values in this study were two to six times lower than in acetonic extracts of sediments from the Danube and the Upper Rhine analyzed by Keiter et al. (2006) and by Kosmehl et al. (2004). ANOVA showed a significant difference between the samples of this study and the samples of the other studies (Keiter et al. 2006).

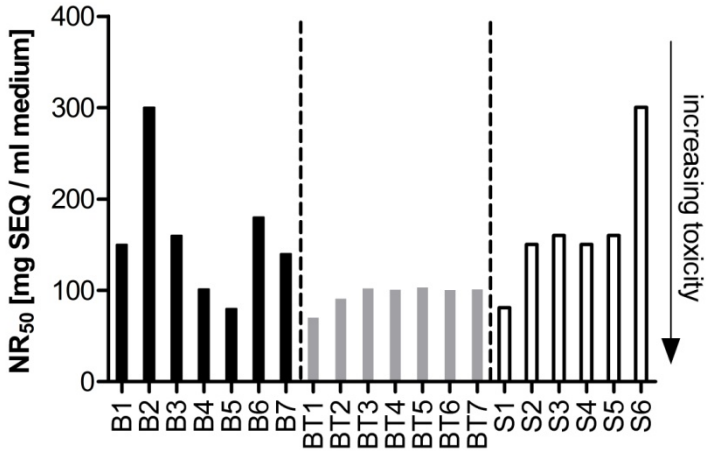


Figure 5-8 Results of acetonic extracts tested with neutral red retention assay (NR) with RTL-W1 cells; given as the half maximum effective concentration NR50 (mg SEQ per ml test medium); B: landside soils; BT: floodplain soils; S: suspended particulate matter; SEQ: sediment equivalent (data source: Garke 2003)

5.3.4.2 EROD induction assay

The EROD induction assay showed dioxin-like effects with sigmoidale concentration-effect curves (Garke 2003) in acetonic extracts of all tested samples except the borehole sample B2 from the landside soils (Figure 5-9). The effect concentrations at the 25th percentile level (EC_{25}) were between 1.6 mg/ml and 40.0 mg/ml in the B samples, between 0.36 mg/ml to 2.1 mg/ml in the BT samples and between 0.5 mg/ml and 0.96 mg/ml in the S samples. For comparison, Rocha et al. (2010) found EC_{25} levels between 0.2 mg/ml SEQ and 28 mg/ml SEQ of different sediments from Tietê River (Brazil). Schulze et al. (2012b; see section 3 p. 58) revealed EC_{25} values of 0.2 mg/ml SEQ and 0.5 mg/ml SEQ, respectively, in two sediments of the Saar.

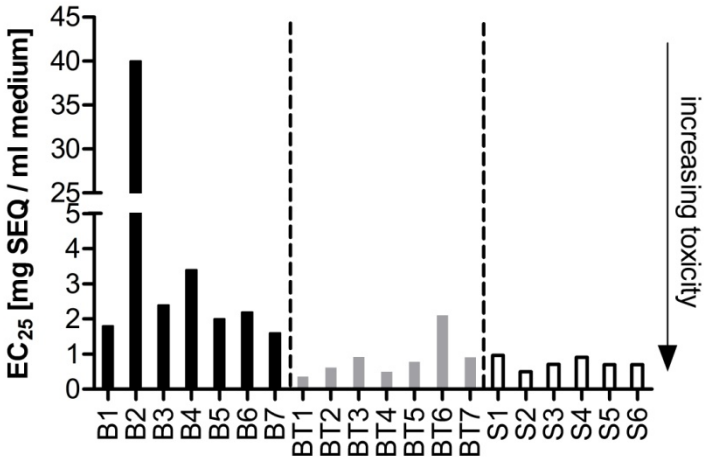


Figure 5-9 Results of acetonic extracts tested with EROD assay ion assay (EC_{50}) with RTL-W1 cells; given as the effective concentration at the 25th percentile level EC_{25} (mg SEQ per ml test medium); B: landside soils; BT: floodplain soils; S: suspended particulate matter; SEQ: sediment equivalent (raw data source: Garke 2003)

5.3.4.3 Fish egg test

The half-maximum effective concentration (EC_{50}) of acetonic extracts tested in the fish egg test with Danio rerio (FET) reached values from 73.3 to 128.7 mg/ml in the landside soils (B), from 7.2 to 50 mg/ml in the floodplain soils (BT) and from 16 to 21.4 mg/ml in the SPM samples (S; Figure 5-10; Table S5–6 in Appendix). The levels of EC_{50} of native samples tested in the FET with Danio rerio were in the ranges from 20.7 to 75.7 mg/ml in the native landside soils (B), from 43.4 to 61.1 mg/ml in the floodplain soils (BT) and between 98.5 and 112.8 mg/ml in SPM (S; Figure 5-10; Table S5–6 in Appendix). The drilling core samples B2 and BT7 showed no effects either. The samples BT3 and BT1 were not toxic in the native sample exposition. S2native, S5native, S6acetonic and BT7native had disrupted concentration-response curves, and no effect concentration values could be derived.

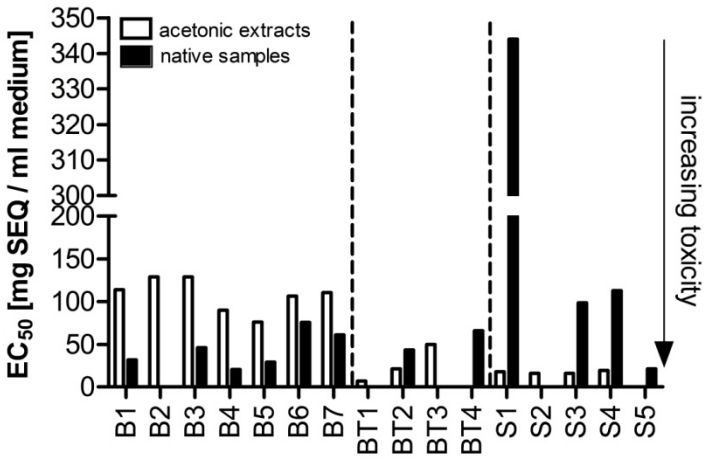


Figure 5-10 Results of acetonic extracts (solid white) and native samples (solid black) tested in the contact assay with Danio rerio; given as the half maximum effective concentration EC_{50} (mg SEQ per ml test medium); B: landside soils; BT: floodplain soils; S: suspended particulate matter; SEQ: sediment equivalent (raw data source: Ulrich 2002)

There are only few studies using raw acetonic extracts for testing in the FET (Hallare et al. 2005, Wu et al. 2010). Seiler (2010) and co-workers achieved EC_{50} levels between 15 and 80 mg/ml at the 48h endpoint in sediments of the Elbe and the Bílina (see section 4, p. 84 in this thesis). Studies using soil samples were not available. Keiter et al. (2006) found EC_{50} levels between 13 and 23 mg/ml in native sediment and native SPM samples of the Danube, Rocha et al. (2011) discovered EC_{50} values from 46.6 to 598.6 mg/ml in native sediments of the Tietê River (Brazil).

5.3.4.4 Bacteria contact assay

Acetonic extracts of the samples tested in the bacteria contact assay with *Arthrobacter globiformis* resulted in half maximum inhibition concentrations (IC_{50}) from 33.1 and 271.5 mg/ml for the landside soils (B), in the range of 70.5 to 132.1 mg/ ml for the floodplain soils (BT) and between 14.8 and 241.8 mg/ml for the SPM samples (S; Figure 5-11; Table S5–7 in Appendix). Native samples revealed IC_{50} values from 118.6 to 1235 mg/ml for the landside soils, between 41.7 and 99 mg/ml for the floodplain soils and in the range 226.5 to 552.9 mg/ml for the SPM (S; Figure 5-11; Table S5–7 in Appendix). Due to an enhanced positive dehydrogenase activity, some of the samples showed negative inhibition that were considered as toxic effects as well (Liß and Ahlf 1997, Ulrich et al. 2002).

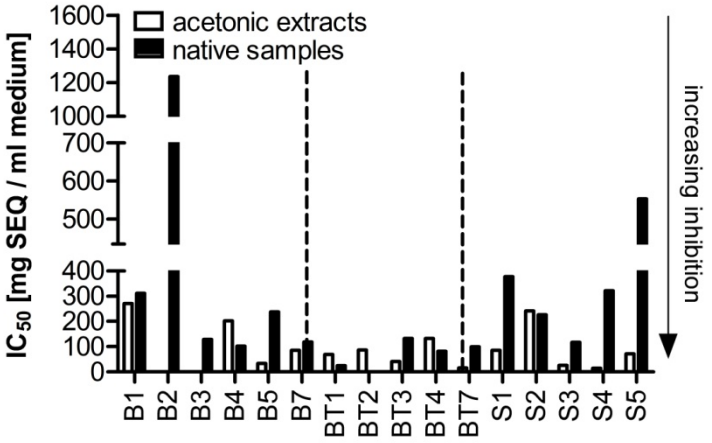


Figure 5-11 Results of acetonic extracts (solid white) and native samples (solid black) tested in the contact assay with Arthrobacter globiformis; given as the half maximum inhibitory concentration IC_{50} (mg SEQ per ml test medium); B: landside soils; BT: floodplain soils; S: suspended particulate matter; SEQ: sediment equivalent (raw data source: Ulrich 2002)

5.3.5 Risk assessment of hazard potentials

5.3.5.1 Comparison of exposition pathway acetonic extract and native sample

Comparing the results from FET with native samples and acetonic extracts thereof revealed a variable picture of the different sample groups. While the soils from infrequently inundated area (B) showed significant higher effect in the native samples (p<0.01, Kruskal-Wallis ANOVA with Dunn's posttest), the soils from frequently inundated area (BT) showed no

difference in effects, while the SPM samples (S) showed a significantly higher effects in the acetonic extracts (p<0.01, Kruskal-Wallis ANOVA with Dunn's posttest; Figure 5-12 A).

Increasing effects of the native B soil samples could be explained by the relative high content of TOC and maybe by dissolved inorganic phosphor, nitrogen or sulfur species. Höss et al. (2010) showed that these parameters were correlated significantly with effects for fish eggs in a comprehensive investigation of the responses of different sediment contact tests with several sediment samples. The comparison of the values from the contact test with *Arthrobacter globiformis* confirmed the higher toxicity of acetonic extracts regarding the SPM samples (Figure 5-12B), a comparable effects for the floodplain soils (BT).

The hypothesis that acetonic extracts overestimate the hazard potential comparing to sediment contact tests with native samples (Rönnpagel et al. 1995) was not supported by our investigation because there were no clear cut difference in effect responses (Ulrich et al. 2002). Due to missing data (i.e. no grain size analysis of the SPM samples, analytical values below the detection limits and a lack of effectivity in different samples), it was not possible to run further statistical analysis such as regression or multivariate analysis. Recent investigations have shown that most adverse effects were not fully explained even if using a battery of different sediment contact tests and a wide range of physico-chemical parameters to characterize the soil or sediment samples (Höss et al. 2010, Tuikka et al. 2011).

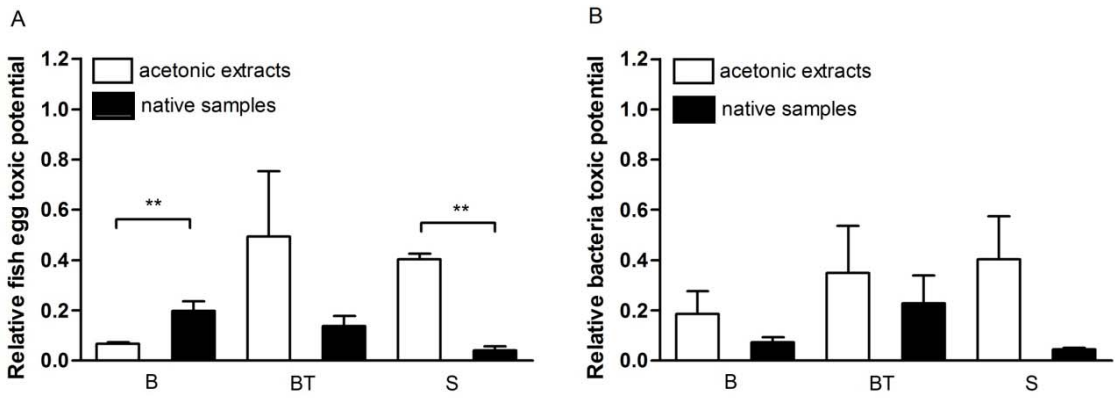


*Figure 5-12 Comparison of acetonic extracts (solid white) and native samples (solid black) tested in the 48 h fish egg test with Danio rerio (A) and in the bacteria contact assay with Arthrobacter globiformis (B) given as relative effect potentials; data shown as means with standard deviations; B: infrequently inundated soils; BT: frequently inundated soils; S: suspended particulate matter; **: p<0.01 (Kruskal-Wallis ANOVA with Dunn's posttest)*

5.3.5.2 Change of hazard potential of SPM during a flood event due to sediment remobilisation

Figure 5-13 shows the Bio-TEQs derived from EROD assay as well as the PAHs and PCDD/Fs related Chem-TEQs according to Clemons et al. (1997) for the SPM samples in comparison with the discharge during the sampling periods of the SPM. Samples S1 and S2 with Bio-TEQs of 2389.6 pg/g and 4553.7 pg/g, respectively, were collected during mean to low discharge conditions (Figure 5-13A). S3 was collected during increasing discharge with a Bio-TEQ of 3213.7 pg/g. S4 represents a higher discharge plateau level with a Bio-TEQ of 2533.6 pg/g. Samples S5 and S6 were collected while discharge increased further nearly to the two-yearly flood level (3100 m3/s, LUBW 2011) each with a Bio-TEQ of 3014 pg/g. The levels of Bio-TEQs in this study were comparable of those found by Wölz et al. (2010b) at the same location.

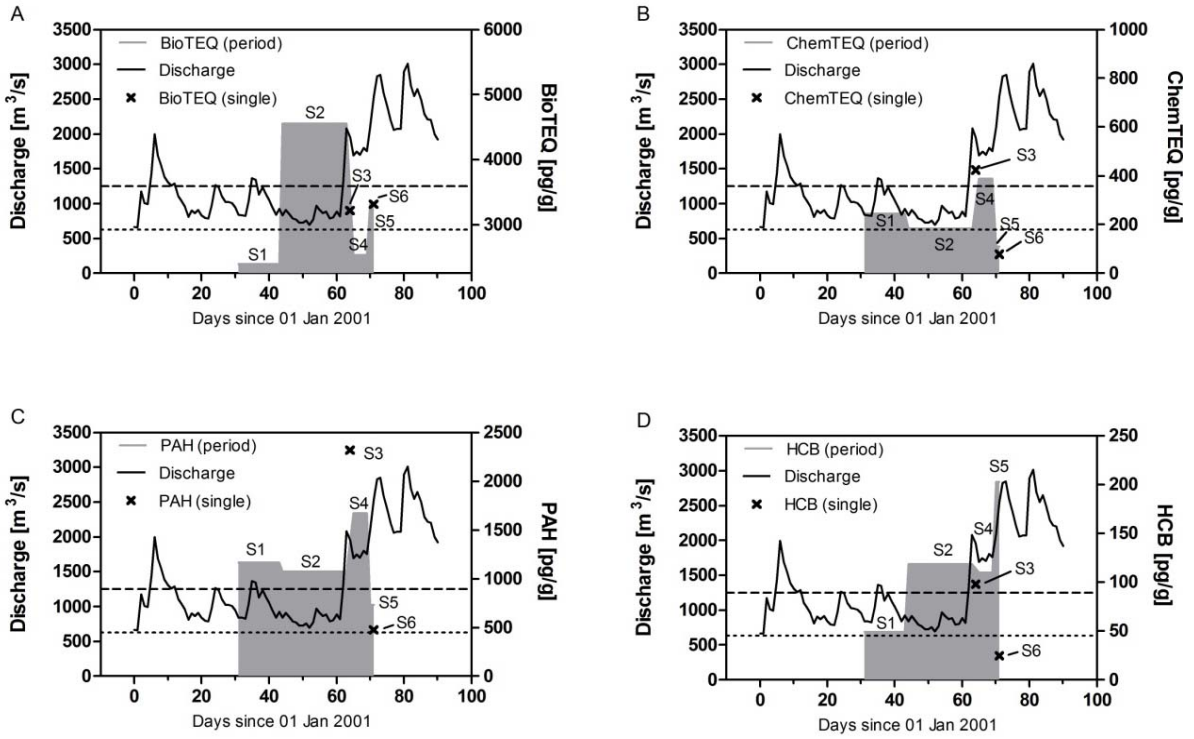


Figure 5-13 Discharge at gauging station Maxau (Rhine km 362.2; solid line; data provided by Rheinkraftwerk Iffezheim GmbH, Iffezheim Germany, Bio-TEQs, Chem-TEQs (B) according to Clemons et al. (1997), PAHs (C) and HCB in the SPM samples; data are given as pg/g; S1, S2, S4 and S5 are shown as sampling periods (gray areas); S5 and S6 are single samples (x); Doted line: average low water discharge 1998–2007; Dashed line: mean water discharge 1998–2007 (LUBW 2011)

The pattern of the Chem-TEQs according to Clemons et al. (1997) Figure 5-13B) and of the related PAHs (Figure 5-13C) were inverse to that of the Bio-TEQs. The HCB pattern followed the Bio-TEQs except of sample S6 (Figure 5-13D). However, there was no significant correlation between HCB levels and Bio-TEQs (Pearson r = 0.24, $p \geq 0.05$). In contrast, Wölz et al. (2010) revealed a good concordance between HCB content and Bio-TEQs. From the results could be assumed that (1) PAHs and HCB had a moderate mixture toxicity effect on the Bio-TEQs during steady state discharge levels (S2 and S4), that (2) the PAHs had a predominant effect potential during moderate discharge peaks (S1), and (3) HCB was more hazardous than the PAHs at flood like discharges (S5). The reason for the latter case could be the remobilization of HCB contaminated sediments due to the flood event. HCB is one of the Rhine specific compounds (IKSR 2011) and present in contaminated sediments at Iffezheim Barrage (Boettcher and Klose 2003, Hollert et al. 2007b).

5.3.5.3 Increased hazard potential of frequently inundated soils compared with infrequently inundated soils due to settlement of contaminated SPM or sediments

The accumulation of trace elements and organic contaminants in flood plain soils due to settlement of SPM and suspended sediments is a widely known and investigated issue (Baborowski et al. 2007, Fiedler et al. 1996b, Förstner 2004, Gocht et al. 2001, Japenga et al. 1990, Malmon et al. 2002, Martin 2009, Martin 1997, Middelkoop 2000, Pies et al. 2007, Umlauf et al. 2005, Witter et al. 1998). Most of the studies have investigated the input and fate of contaminants but not the potential adverse effects of such settlement. However, there some studies have shown effects of floodplain soils *in vitro* and *in vivo* (e.g., de Jonge et al. 1999, Hamers et al. 2006, Hobbelen et al. 2004, Klok and Kraak 2008, Rader et al. 1997, Schwartz et al. 2006, Wölz et al. 2011b). Figure 5-14 contains box-and-whisker plots of biotest results of this study to compare the relative effect potentials of the different sample groups. A significantly increased hazard potential regarding the dioxin-like potential ($p<0.05$; ANOVA with Tukey's posttest) was found (Figure 5-14B). The effects in the FET of the acetonic extract of frequently inundated soil (B samples) were present comparing to those of infrequently inundated soils (BT samples; Figure 5-14C). The cytotoxic potential (Figure 5-14A) of the BT samples was higher and less variable than that of the B samples, but not significantly different. The bacterial effect potentials (Figure 5-14D) in both acetonic extracts and native samples of the BT samples were insignificantly higher than those of the B samples. However, the results of the BT samples were more variable.

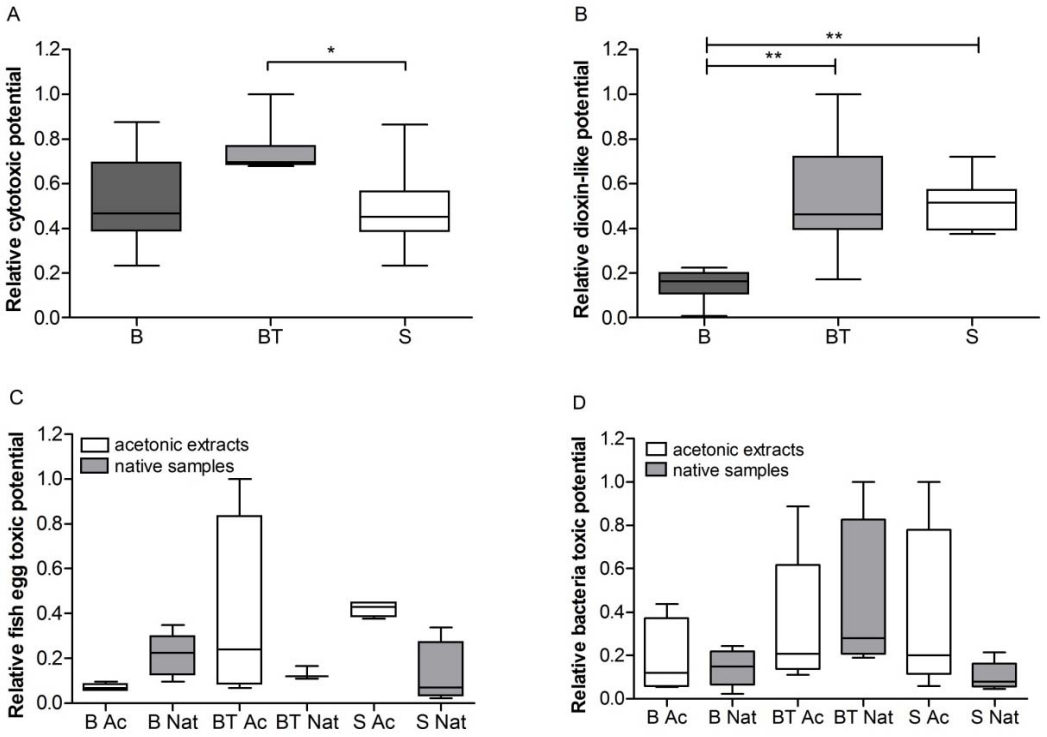


*Figure 5-14 Box-and-whisker plots (boxes: median, 25th-/75th-percentiles; whiskers: minimum, maximum) of acetonic extracts tested in the neutral red assay (A) and EROD assay (B) as well as of acetonic extracts (solid white) and native samples (solid gray) tested in the FET with Danio rerio (C) and in the bacteria contact assay with Arthrobacter globiformis (D); given as relative effect potentials; B: infrequently inundated soils; BT: frequently inundated soils; S: suspended particulate matter; Ac: acetonic extract; Nat: native sample; *: p<0.05 (ANOVA with Tukey's posttest or Kruskal-Wallis ANOVA with Dunn's posttest)*

The cytotoxic effect potentials of the BT samples were significantly higher (p<0.05; ANOVA with Tukey's posttest) compared with the S samples (Figure 5-14A). The dioxin-like potential of the BT and S samples were comparable (Figure 5-14B) as well as the bacterial effect potentials of the acetonic extracts (Figure 5-14C). The fish egg effect potential was determinable in only a few native BT samples (n=3) and thus no strength deduction is possible. The bacterial effect potentials in the BT samples were above those of the S samples, but there was no significant difference between the groups. The hypothesis that the settlement of contaminated SPM increases the hazard potential of the frequently inundated soils compared with the infrequently inundated is supported by the results of this investigation. Although, the results of the FET were ambiguous due to absent effects and

disrupted concentration-response relationships. However, the test performance was considered as valid (Ulrich 2002).

5.3.5.4 Ecotoxicological effects vs. analyzed pollutants

Linking ecotoxicological effects in biotests to the pollutants detected in the samples is often a problem in environmental risk assessment studies. Synergistic, antagonistic, or additive mixture toxicity effects as well as varying modes of toxic actions (MOAs) at different effect concentration levels are in contrast to a commonly relative low number of identified and quantified compounds in the respective samples or sub fractions thereof (Brack 2003, Brack et al. 2008, Grote et al. 2005, Kammann et al. 2005).

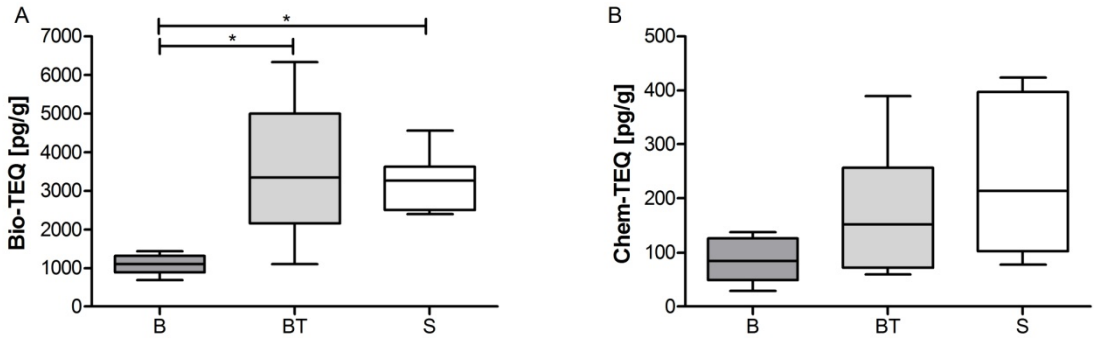


*Figure 5-15 Box-and-whisker plots (boxes: median, 25th-/75th-percentiles; whiskers: minimum, maximum) of Bio-TEQs (A) and Chem-TEQs (B) derived from EROD assay as well as PAH and PCDD/Fs analysis; data given as pg/g; borehole samples (B2 and BT7) were not included; B: infrequently inundated soils; BT: frequently inundated soils; S: suspended particulate matter; *: p<0.05 (ANOVA with Tukey's posttest or Kruskal-Wallis ANOVA with Dunn's posttest)*

Box-and-whisker plots of Bio-TEQs derived from EROD assay and the Chem-TEQs derived from the PAHs' and PCDD/Fs' analysis are shown in Figure 5-15. Chem-TEQs explained in average 7.9% ± 5.9% of the effects expressed by the Bio-TEQs. This result is in concordance with related studies (Kammann et al. 2005, Schulze et al. 2012b, Wölz et al. 2010a; see section 3, p. 58, and section 6, p. 141, in this thesis). Hence, the analysis of a few priority pollutants such as PAHs, PCBs and PCDD/Fs is not sufficient to achieve real cause-effect relationships of adverse effects of anthropogenic environmental pollution.

Numerous known and unknown chemicals that may cause adverse effects are present in soils and sediments (Brack et al. 2008, Schwarzbauer 1997; see section 2, p. 8, in this thesis).

5.4 Conclusions

The results of this study have shown that the native samples could be significantly more effective then the respective extracts in the bacteria contact assay and in the fish egg test. These results contradict the common concept that acetonic extracts might overestimate the toxicity of soil and SPM samples. Furthermore, the priority organic compounds analyzed did not fully explain the toxic potential of the samples. The outcomes of this study revealed the insufficient knowledge regarding the relationship between the different exposition pathways. Further investigations are necessary including a higher count of samples and effect-directed analysis in combination with a chemical-analytical nontarget screening to identify toxic compounds and to get more insight of the bioavailability of particularly bound pollutants. Furthermore, there are concerns about adverse effects of settling suspended particulate matter and remobilized sediments in frequently inundated floodplain soils due to an increase of the hazard potential comparing to infrequently inundated floodplain soils.

5.5 Acknowledgements

This study was supported by the Stadtwerke Karlsruhe (Germany) during the project «Ecotoxicological assessment of the Rhine sediments and suspended particulate matter in inundated areas». We are grateful to Mr. Beiser (Stadtwerke Karlsruhe) for technical support of the sampling as well as Beate Kemink, L. Dunne, H. Johannsen, Dr. Anne Seebach, und Manuela Demirci-Scholz for technical support of the laboratory analysis. Dr. Emma Schymanski (Eawag, Dübendorf, Switzerland) gave valuable comments to a previous version of the manuscript.

6 Impact of contaminants bound to suspended particulate matter in the context of flood events [22] [23]

6.1 Introduction

Investigation of contaminants bound to suspended particulate matter (SPM) is critical to a sound understanding of hazard potentials caused by flood events. Contaminants impacting aquatic systems in flood events originate primarily from sediment erosion and subsequent translocation (Köthe 2003). Since sediments serve as contaminant sinks, but also as important secondary sources, increasing contaminant loads are expected with more extreme floods in the near future (Heise and Förstner 2006). Remobilized particle-bound pollutants are trans-located with SPM that has been recognized as the carrier of contaminants and hazard potentials (Schulze et al. 2007a).

At present, extreme flood events such as the Elbe flood in 2002 are still hydrological outliers causing considerable economic and ecological damage with high recurrence intervals (Ikeda et al. 2005, Klok and Kraak 2008). Nevertheless, as a consequence of climate change, these events are expected to increase in frequency and intensity in many regions worldwide (IPCC 2007, Scheurer et al. 2009). Furthermore, it is assumed that changes in precipitation may even be amplified in river runoff (Chiew and McMahon 2002), and there is evidence that the magnitude of peak flows is increasing (Middelkoop et al. 2001). Thus, recurrence intervals of floods comparable to that of the Elbe in 2002 will become shorter (Bronstert 2003)

Strategies to manage flood impact often include the operation of flood water retention areas with clearly higher retention volumes than available at present (Disse and H. 2001, Hooijer et al. 2004) However, required retention basins can be situated close to or within wellhead protection areas, that are needed for the operation of public well fields which are often situated close to rivers and, thus, may cause conflict of interests (Maier et al. 2006).

[22] The original publication is available at http://www.springerlink.com: Wölz, J.; Fleig, M.; **Schulze, T.**; Maletz, S.; Lübcke-von Varel, U.; Reifferscheid, G.; Kühlers, D.; Braunbeck, T.; Brack, W.; Hollert, H. (2010): Impact of contaminants bound to suspended particulate matter in the context of flood events; Journal of Soils and Sediments 10, 1174-1185 (DOI: 10.1007/s11368-010-0262-y)

[23] This section is part of the PhD thesis of Dr. Jan Wölz (2009): Impact of contaminants on aquatic systems and inundated sites with respect to flood events: *in vitro* biotests, chemical target analysis and fractionation methods; Ruperto Carola University; Heidelberg; 194 pp.

To date, many studies have shown high contaminant loads of sediments and suspended particulate matter resulting from floods (Hollert et al. 2003a, Oetken et al. 2005a, Wölz et al. 2008). Further studies showed elevated contamination of floodplain soils (Hilscherova et al. 2007, Pies et al. 2007, Yang et al. 2008). However, so far, there has been only one study of the (eco-)toxicological hazard potentials of sediments, suspended particulate matter, and floodplain soils to drinking water production, indicating an increase of the (eco)toxicological hazard potential of near-surface soil samples at frequently inundated sites (Ulrich et al. 2002) in a wellhead protection area. Since an elevated impact was observed in the study, an interdisciplinary follow-up project was initiated to investigate this hazard potential in more detail: «Flood retention and drinking water supply – preventing conflict of interests» (RIMAX-HOT). The results detailed in the present study are part of the outcomes of the joint research project (Kühlers et al. 2009, Maier et al. 2006). The project investigated the possible conflict of interests at the planned flood water retention area Bellenkopf-Rappenwoert and the nearby planned public well field, 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. Samples were taken at monthly intervals in 2006 and more frequently during a flood event with a recurrence interval of 10 years in August 2007 and investigated using in vitro biotests. Dioxin-like and aryl hydrocarbon receptor (AhR)-mediated activities were determined with the 7-ethoxyresorufin-O-deethylase assay (EROD) and the rainbow trout liver cell line RTL-W1. Furthermore, mutagenic potentials were assessed with the Ames fluctuation assay and the two bacterial tester strains TA 98 and TA 100. A recently developed fractionation method was applied in effect-directed analysis to receive further insight into contaminant loads in SPM sampled in the context of the flood event assessed. The 18 factions obtained were investigated using the biotests listed above to identify effective compound classes. Chemical analysis was focused on PAH that were defined as priority hazardous by the U.S. Environmental Protection Agency (EPA-PAHs) with higher molecular weight, since low-molecular PAHs are known not to induce EROD.

Thus, the present study aimed

(a) to identify in vitro hazard potentials of SPM sampled on a long-term scale and in a flood event,

(b) to discover effective compound classes using effect-directed analysis,

(c) to chemically identify and quantify effective com-pounds in inducing fractions, and

(d) to highlight contamination hazard of inundated sites and retention basins by flood events.

6.2 Materials and methods

6.2.1 Chemicals used

Chemicals were at least reagent grade and have been provided by Sigma-Aldrich (Deisenhofen, Germany). All solvents used for clean-up and HPLC fractionation procedure as well as GC-MS analysis of PAH were obtained from Merck (Darmstadt, Germany) with Suprasolv® or LiChrosolv® quality. Certified high purity standards for GC-MS analysis of PAH were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and Supelco (Seelze, Germany).

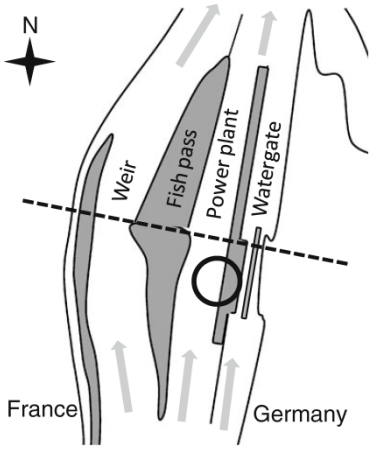


Figure 6-1 Location of the continuous-flow centrifuge and the passive sedimentation boxes at the Rhine barrage at Iffezheim, Germany (circle). River flow direction is shown by light gray arrows; the dashed line represents the road across the river

6.2.2 SPM sampling

In this study, SPM was sampled at a site that has been used for years. Thus, the available data allowed verification of the results of SPM samples in this study. SPM collection took place monthly in 2006 using a continuous-flow centrifuge that was installed just above the hydro-power plant at the Rhine barrage at Iffezheim, Germany (Figure 6-1), at a depth of 0.8 m according to the method described by (Baborowski et al. 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.

Table 6-1 Periods of SPM sampling at the Rhine barrage at Iffezheim, Germany, in the course of the flood from August 2007 are given; Samples selected for effect-directed analysis are marked (X)

Sample no.	Sampling				Fractionated samples
	Start		Ende		
	(Date)	(Time)	(Date)	(Time)	
1	14.07.07	12:00 AM	31.07.07	12:00 AM	X
2	31.07.07	12:00 AM	09.08.07	21:00 PM	
3	09.08.07	21:00 PM	10.08.07	10:40 AM	
4	10.08.07	10:40 AM	10.08.07	12:00 AM	
5	10.08.07	12:00 AM	11.08.07	14:50 PM	X
6	11.08.07	14:50 PM	14.08.07	09:00 AM	X
7	14.08.07	09:00 AM	17.08.07	14:00 PM	
8	17.08.07	14:00 PM	31.08.07	12:00 AM	X

Furthermore, SPM was sampled at the same site (Figure 6-1) with higher frequency (Table 6-1) in the course of a flood event (Figure 6-1) with a recurrence interval of 10 years in August 2007 using two passive sedimentation boxes (Schulze et al. 2007a). SPM was transferred to glass bottles, protected from light and transported at 4 °C. SPM was then treated according to DIN 38414-22. Samples were freeze-dried in two steps using a BETA 2–16 (Christ, Osterode, Germany). Initially, SPM was dried for 2 days at 0.6 to 1 mbar and a temperature of 20–25 °C. Subsequently, SPM was post-dried for 2 days and at least 0.001 mbar to lower the residual moisture to <0.5%. SPM was than sieved at a mesh size of 600 µm for 15 min using a Sonorex RK 255 H ultrasound bath (Bandelin, Berlin, Germany). SPM was stored at 4 °C in darkness until extraction.

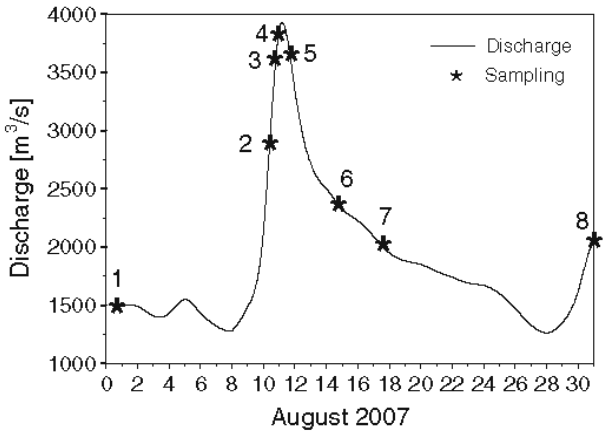


Figure 6-2 Discharge at the gauge at Maxau, Germany, close to the sampling site and removal times of SPM from sediment traps in the course of the flood in August 2007 are shown. Sampling times of SPM in August 2007

6.2.3 Preparation of crude extracts

In a first step, 10 g of each freeze-dried SPM was weighed in 200-ml extraction thimbles (Schleicher & Schuell, Dassel, Germany), plugged 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 redissolved in dimethylsulfoxide (DMSO) and stored at 4 °C until biotesting. Empty extraction thimbles were subjected to extraction and processed in two parallel experiments to serve as process controls.

6.2.3.1 Clean-up of extracts

Suspended matter was extracted using a slightly modified method in order to satisfy fractionation requirements. Ten grams of each freeze-dried SPM were 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 redissolved in *n*-hexane/acetone (70:30; v/v). Accelerated membrane-assisted clean-up (AMAC) was used for purification of SPM extracts (Streck et al. 2008b). 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). Solvents,

temperature, pressure, number, and duration of cycles were chosen as described previously by Lübcke-von Varel et al. (2008). Extracts were collected in ASE glass vials closed by PTFE-coated screw caps, reduced in volume, evaporated under a gentle N2-stream and redissolved in *n*-hexane/DCM (90:10; v/v) to a final concentration of 10 g/ml for subsequent fractionation.

6.2.3.2 Automated fractionation of the extracts

AMAC-purified extracts were fractionated using an automated fractionation method (Lübcke-von Varel et al. 2008). Compounds of the AMAP extracts were loaded on three types of columns: polar compounds were trapped on a cyanopropyl (CN) silica column with *n*-hexane as mobile phase. Nonpolar polyaromatic substances were retained on a nitrophenylpropyl-silica (NO) column and PCBs, PCDD/ Fs and other small halogenated aromatic hydrocarbons on porous graphitized carbon (PGC). Subsequently, compounds were sequentially eluted from the columns starting with halogenated diaromatic compounds from PGC using *n*-hexane and toluene as mobile phases. Compounds trapped on the NO phase were 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 redissolved in *n*-hexane (for GC-MS) and DMSO (for biotesting) to a final concentration of 10 g/ml.

6.2.4 Chemical analysis of HCB and PCBs

The following PCB isomers were analyzed using GC: #28, #52, #101, #118, #138, #153, #170, #180, and #194. Analysis was performed with a Perkin Elmer Autosystem XL (Waltham, MA, USA) equipped with 63Ni electron-capture detector. The two columns used for analysis were: column A (CLP, 30 m × 0.5 mm × 0.32 µm; Restek Corp., Bellefonte, PA, USA) and column B (DB5, 30 m × 0.25 mm × 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 (=TCX tetrachloroxylol/P 209 polychlorinated biphenyl), 1 ng/µl was 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. The limit of quantification (LOQ) of HCB and PCBs is <2 ng/g dry weight (dw).

6.2.5 GC-MS analysis for PAHs

GC-MS analysis was carried out on an HP 6890 GC coupled to a HP MSD 5973 (Agilent, Palo Alto, USA), equipped with a 30 m × 0.25 mm I.D. × 0.25-µm film HP-5 MS fused capillary silica column, a 5-m precolumn (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-1 to 150 °C, at 6 K min^{-1} to 186 °C, and finally at 4 K min^{-1} to 280 °C (16.5 min isotherm). Carrier gas velocity (helium 5.0, Air Liquide, Boehlen, Germany) was 1 ml/min at constant flow. The MS was operated in electron 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 SIM using the PAH Mix No. 9 purchased from Dr. Ehrenstorfer (Augsburg, Germany). The results were corrected for errors due to injection and matrix effects using an internal standard mixture (46955U) containing deuterated PAH obtained from Supelco (Seelze, Germany) with recoveries from 88% to 118%. The limit of detection (LOD) was 0.1-0.7 ng/g dw, and the LOQ was 0.6–2 ng/g dw for the PAH. The LOD was defined as three times the signal-to-noise ratio (S/N) and the LOQ as ten times the S/N of the analyte's peak, respectively.

6.2.6 EROD induction assay

Induction of 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. 2009). 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 2–3% 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 plates were incubated in the dark at room temperature for 10 min. Subsequently, NADPH was supplemented to start the deethylation 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; (Kennedy and Jones 1994, Lorenzen and Kennedy 1993). The concentration–response curves for EROD induction in the RTL-W1 bioassay were computed by nonlinear regression using GraphPad Prism 4 (GraphPad, San Diego, USA) and classic sigmoid or Boltzmann curves as model equations (Seiler et al. 2006). Variability of EC_{50} was more generally given to be ±35% by Keiter et al. (2009), using a dataset of n=59 positive controls. The enzyme-inducing potential of the samples was converted to biological toxic equivalents (Bio-TEQs) as described below.

Bio-TEQ values: Ah receptor agonist activities were determined as EC_{25} values of each sample and were given relative 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 as mean values of n=3 independent biotests. TCDD-EC_{25} were determined with each test plate and mean values were used for the calculation of Bio-TEQ values. (Wölz et al. 2008) gave the mean TCDD-EC_{25} values as 5.3 pg ± 1.8 pg/g (n=59 independent tests). These values were determined with the same cell culture and the same TCDD batch and, thus, can be expected to show the test variation that applies for this study. Subsequently, Bio-TEQs with concentrations in picograms TCDD/gram of SEQ will be given as picograms per gram:

$$Bio-TEQ\left[\frac{pgTCDD}{gSEQ}\right] = \frac{TCCD - EC_{25}[\frac{pgTCDD}{ml}]}{sample - EC_{25}[\frac{gSEQ}{ml}]} \quad (7-1)$$

6.2.7 Ames fluctuation assay

The Ames fluctuation assay is a modification of the plate incorporation Ames test (Maron and Ames 1983) according to the method described by (Reifferscheid et al. 2005). In contrast to the classic test, bacteria were exposed 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 and 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 (l=595 nm) to a standard (10 g/l hexamethylenetetramine, 1 g/l hydrazinesulfate; equals 1,800 FAU) according to the method described by Hawe and Friess (Hawe and Friess 2008). For testing, overnight cultures were adjusted to 1,800 FAU (TA 98) and 450 FAU (TA 100).

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, a liver homogenate S9-fraction (RCC Rossdorf, Germany) from phenobarbital/ß-naphthoflavon-treated rats (protein concentration, 30.5 mg/ml S9) was added in a buffered cofactor mixture to each well.

For each test ±S9 mix 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 ≤10 per 48 wells (TA 98 and TA 100) at all testing conditions with both strains ±S9. Positive controls were valid when the number of revertants was ≥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 TA98 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).

In contrast, Fisher's exact binomial test 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 ISO standard (International Organization for Standardization) of the Ames fluctuation assay which is currently in preparation. Fisher's exact test allows

calculating 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 (IFmax) were computed that give the induction of the highest inducing sample concentration, referred to the negative control induction.

6.3 Results

6.3.1 SPM sampled in 2006

Whereas various contaminants were analyzed (e.g., HCHs, DDT, and metabolites), elevated concentrations were only determined for HCB and selected PCBs, which showed concentrations of 7.4 to 29 µg/kg (HCB) and 4.7 to 28 µg/kg (PCBs) as given in Figure 6-3a. These contaminants showed minor variations in concentration throughout 2006. Seasonal- or discharge-dependent influences could not be observed. Whereas the reasons for the increased effects in 2006 remain unclear, it is evident that Ah receptor-mediated activities showed a Bio-TEQ range of 1160 to 6640 pg/g (Figure 6-3b). In contrast to PCB and HCB concentrations, Bio-TEQs of SPM showed a seasonal variation with highest inductions in June and the following months and comparably lower inductions in spring and winter. The elevated activities were not correlated with discharge.

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

Consistent with the data presented for SPM sampled in 2006, HCB and PCBs were the most highly concentrated contaminants in SPM sampled 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 Figure 6-4a. HCB concentrations were more than twice as high in SPM 2 compared with the concentrations determined before the flood at the end of July (SPM 1). Maximum concentrations of 110 µg/kg were measured at the peak discharge of the flood event. HCB concentrations decreased markedly after the flood peak. However, SPM sampled subsequently still indicated elevated concentrations that were about twice as high as those of the SPM from the end of July. In contrast, PCBs indicated an increase only at the beginning of the flood (67 µg/kg). Subsequently sampled SPM indicated lower concentrations (5 to 25 µg/kg) and showed no relation to river discharge. Measured Ah receptor-mediated activities, given as Bio-TEQs, indicated a clear-cut increase of activity in accordance to the increasing

discharge (Figure 6-4b). TEQs indicated decreasing AhR-agonist activities about one day post-flood peak (SPM 5) in accordance to HCB analysis. However, the maximum Bio-TEQ was measured with the following sample SPM 6 (6140 pg/g). SPM sampled subsequently indicated decreased, but still high Bio-TEQs that were comparable to end of July SPM 1.

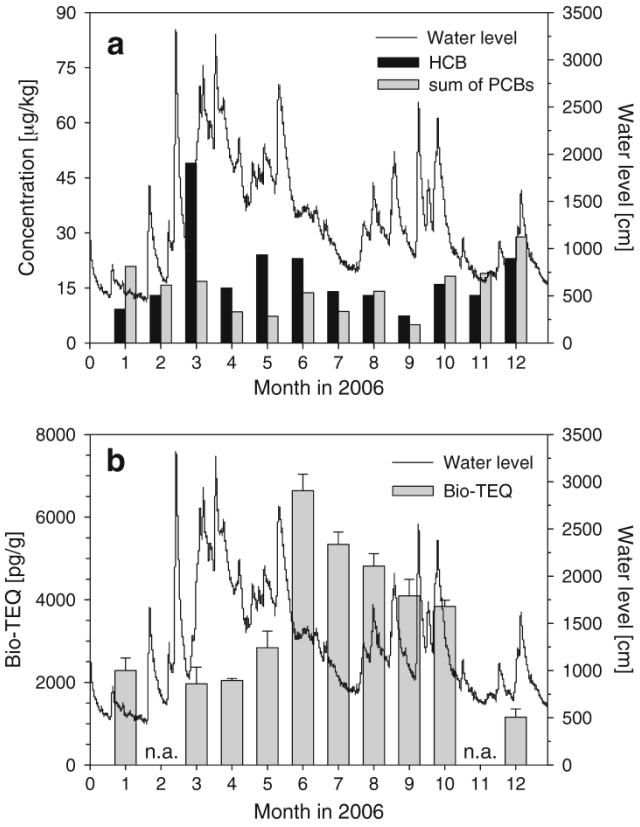


Figure 6-3 *(a) HCB and PCB contents were determined in SPM that was taken as a mixed sample over the period of a month each in 2006. SPM was sampled using a centrifuge at the Rhine barrage at Iffezheim, Germany, and is presented in the context of the water level at Maxau, Germany, which is close to Iffezheim. Furthermore, (b) AhR-mediated activity is shown that was determined with the same SPM samples and n=3 independent replicates each, as reflected by the error bars. na not assessed*

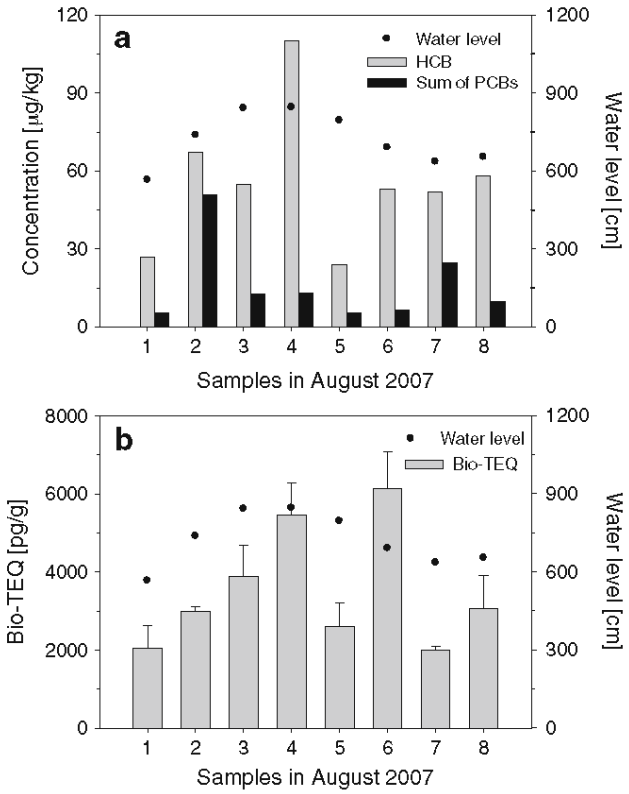


Figure 6-4 *(a) HCB and PCB concentrations for SPM of August 2007, sampled at the Rhine barrage at Iffezheim (Germany) using a sediment trap. (b) Ah receptor-mediated activities for the corresponding SPM sample, given as Bio-TEQ values in picograms per gram (n=3)*

6.4 Identification of effective fractions

For a more thorough analysis and identification of effective compound classes, EDA samples were fractionated, providing 18 distinct fractions (Figure 6-5). 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, coeluting with mononitro-PAHs, was less inducing, while fraction F13, (e.g., quinones, hydroxy-PAHs), was highest inducing. Fraction 14, coeluting with (hydroxyl-)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, coeluting, e.g., with 2,6-diisopropylnaphthalin, octylphenol, and 2-hydroxyanthraquinone, showed decreasing but nevertheless elevated Bio-TEQ.

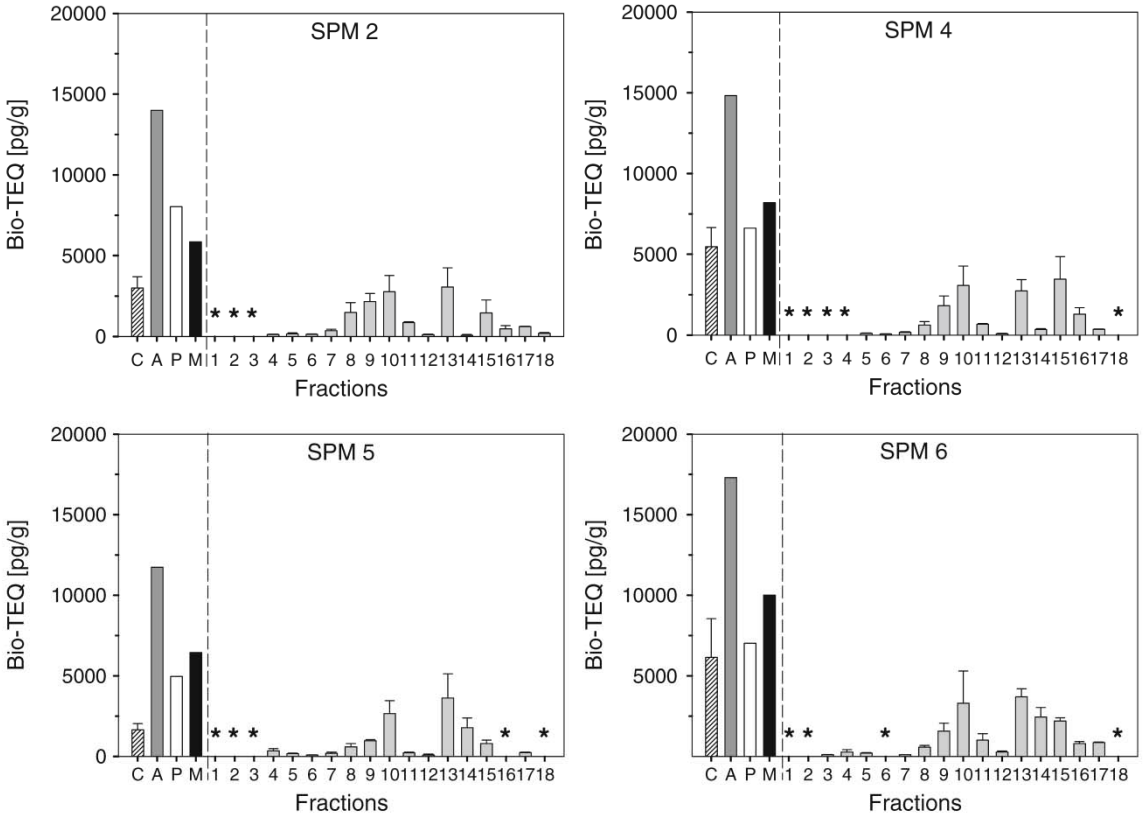


*Figure 6-5 Ah receptor-mediated activity, given as Bio-TEQ values for SPM crude extracts (C), added fractions F1 to F18 (A), added PAH fractions F5 to F12 (P), added fractions with more polar-to-polar compounds F13 to F18 (M) as well as for each single fraction (n=3). *No EROD induction detected*

The fraction sample substances listed above were taken from Lübcke-von Varel et al. (2008). In order to determine contributions of compound groups to the total effect, as a first step, Bio-TEQs of all 18 fractions were added yielding 11800 pg/g (SPM 5) to 17300 pg/g (SPM 6). Furthermore, fractions contain PAHs (F5 to F12) and fractions containing more nonpolar compounds (F13 to F18) were added. Most importantly, added Bio-TEQs of all 18 fractions showed a three- to sevenfold increase of enzyme inductions compared with crude SPM extracts with all SPM assessed. Added PAH fractions were the highest inducing compound group (maximum Bio-TEQ=8028 pg/g; SPM 2).With each other sample, sums of Bio-TEQs were higher with fractions containing more polar-to-polar compounds and a maximum Bio-TEQ of 10012 pg/g (SPM 6).

6.4.1.1 Mutagenic potentials of fractions

Mutagenic activity was measured with each SPM sample and with fractions. SPM crude extract sampled in 2006 and 2007 indicated no significant mutagenic potentials. However, fractions of SPM sampled in August 14, 2007, at 9 a.m. (SPM 6), caused significantly elevated effects. Fisher's exact binominal test showed significant NOEC values ≤ maximum concentration with fractions containing more polar-to-polar compounds (Table 6-2). 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. Furthermore, mutagenic activity was highly increased with TA 100 without S9 metabolism in F13, showing that compounds causing base pair mutations were most highly concentrated in this fraction. Furthermore, maximum induction factors were computed for fractions that were determined to show significant elevated mutagenic potentials. With respect to IFmax, highest mutagenic activity was determined accordingly in F15 and TA 98 without S9 metabolism (IFmax=14.7) and in F18 and TA 100 with S9 metabolism (IFmax=9.3).

Table 6-2 Mutagenic potential of SPM 6 fractions in the Ames fluctuation assay using bacterial strains TA 98 and TA 100, determined with n=1 and 48 replicates per test

Fraction no.	NOEC (mg/ml)				Fraction no.	Induction factor (IFmax)			
	TA98 –S9	TA98 +S9	TA100 – S9	TA100 +S9		TA98 –S9	TA98 +S9	TA100 –S9	TA100 +S9
1	a	a	a	a	1	a	a	a	a
…	…	…	…	…	…	…	…	…	…
13	a	16.67	4.17	a	13	a	3.0	3.7	a
14	a	a	a	a	14	a	a	a	a
15	<2.08	a	a	a	15	14.7	a	a	a
16	8.33	8.33	a	16.67	16	4.3	1.7	a	2.0
17	a	a	a	a	17	a	a	a	a
18	a	16.67	a	33.33	18	a	5.3	a	9.3

Mutagenic potentials are given as NOEC value and maximum induction factor (IFmax)

Maximum concentration in test, 66.67 mg/ml; lowest concentration in test 2.08 mg/ml, ±S9 mix–metabolic activation using rat liver homogenate of the S9 fraction in the liver centrifugate and cofactors

a NOEC>66.67 mg/ml and no IFmax determined

6.5 Discussion

6.5.1 Chemical loads of crude extracts

Chemical analysis showed that PCBs were detectable at low concentrations in SPM sampled in 2006 and not particularly 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 (maximum, 339 µg/kg) indicated concentrations in SPM to be comparatively low (Mai et al. 2003, Samara et al. 2006, Zhang et al. 2007, Zhang et al. 2004).

In contrast, the HCB concentrations in SPM stood at 4.8 to 85 µg/kg (median=16 µg/kg) in 2006 and 24 to 110 µg/kg (median=53 µg/kg) in the August flood. Thus, the median concentration increased 3.3-fold during the flood. The highest concentrations coincided with the highest discharges recorded during the flood, which is believed to stem from HCB bound to riverbed sediments. The maximum displacement of the contaminated riverbed sediments occurs at maximum discharge. (Ulrich et al. 2002) found maximum HCB concentrations of 203 µg/kg in SPM sampled in the fish ladder at the Iffezheim barrage, twice the level of the levels observed in 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 the surface (Alcock et al. 2003). Using the chemistry toxicity test approach (Heise and Förstner 2006, Heise et al. 2004), the action level for HCB (=20 µg/kg) is clearly exceeded. Thus, e.g., dumping of Rotterdam port sediment at sea, which can be the way to treat sediment which is fulfilling specific criteria, would no longer be allowed and disposal at specialized landfills, at considerably higher cost would be necessary (Netzband 2007). However, HCB in detected concentrations is worrying as this compound is classified as «substance of concern», since it frequently exceeds regulatory criteria for suspended matter (Heise and Förstner 2006). Furthermore, due to its persistence, HCB is listed as one of the «dirty dozen» in the Stockholm convention on persistent organic pollutants (UNEP 2001) and as a priority hazardous substance in the water framework directive (European Community2000). HCB is therefore a significant hazardous compound and has to be included in measure strategies, since successful management 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 bound to SPM

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 thus 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 rapidly increasing contaminant (re)mobilization and exposure, while, in 2006, effects seemed to increase more slowly, beginning with SPM sampled in May, but lasted for months, showing 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=2300 pg/g, whereas SPM sampled in the winter months (November 2003 to February 2004) induced Bio-TEQs=3700 pg/g (Wölz et al. 2008). In the present study, Bio-TEQs were 2.7 times higher than these 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 sediments of the Hyeongsan River, Korea, using H4IIE-luc cells. Hilscherova et al. (2003) used the same cell line and found Bio-TEQ of 1860 pg/g in sediment of the Tittabawassea River, Michigan, USA. Furthermore, Hollert et al. (2002) used RTL-W1 cells and the EROD induction assay to assess sediments of the catchment area of the Neckar in Germany and determined Bio-TEQs of about 1000 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 contaminants 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 polar compounds were found to be the most inducing. Chemical analysis was performed with respect to 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 substance (c.f. 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 (Barron et al. 2004, Brack et al. 2005b, Brack et al. 2002). Furthermore, more polar substances that were highest inducing in this study and that are usually not in the focus of research were, e.g., shown to be of major relevance (Karlsson et al. 2008).

Next to AhR-agonists, mutagenic potentials of SPM crude extracts and fractions were assessed. No significantly elevated bacterial reversions were determined with crude SPM extracts but flood SPM fractions were mutagenic with SPM sampled after the peak of discharge during the flood event. Sediment and SPM extracts of a flood with a recurrence interval of 1 year at the Neckar in 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 IFmax=3.2 (Hollert et al. 2003a). 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 (IFmax=14.7). Elevated mutagenic potencies of compounds have been shown before (Eisenträger et al. 2008, Kataoka et al. 2000, Schuetzle et al. 1981) and, therefore, indicate the high relevance of more polar and polar compounds in exposure and risk assessment of floods.

Furthermore, crude SPM extracts of 2006 were also investigated using the Comet assay as detailed by (Singh et al. 1988) in the modification of Schnurstein and Braunbeck (2001; details not shown). These SPM extracts showed up to 13-fold increased IFmax. Kosmehl (2004) assessed sediment cores from the Rhine in Germany, and gave IFmax=90.5 with RTG 2 cells and IFmax=47.0 with RTL-W1 cells. Ranking these findings with the present study indicates lower, but nevertheless elevated, genotoxic potentials with the SPM assessed. Kosmehl et al. (2006) showed that genotoxic 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 into retention basins during floods may result in deposition of highly toxic compounds on inundated soils.

6.6 Conclusions

The investigation of SPM sampled continuously over months and with higher frequency during flood events, allows evaluating dioxin-like and Ah receptor agonist activities as well as mutagenic potentials. 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. At the Rhine, HCB is a known and ubiquitous 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 erosion and subsequent deposition at inundated sites. 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. Furthermore, 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 and possibly new compounds are more relevant. Thus, more polar-to-polar compounds should be investigated with greater emphasis in future studies that investigate hazard potentials of contaminant loads in floods with high recurrence intervals. Dioxin-like and AhR-mediated, as well as mutagenic activity are valuable endpoints to determine hazard potentials, since worrying activities in crude extracts and fractions with more polar compounds have repeatedly been shown.

In conclusion, here, the shown study supports the need for an increased regulatory awareness with respect to contaminants that are bound to SPM. In this context, the daughter directive of the EU WFD requires that concentrations of so-called priority pollutants may not increase in both sediments and organisms. In detail, an initial characterization undertaken by the German states revealed that only about 14% of all surface waters are considered to meet the WFD objectives that were intended to be reached by 2015 at all European catchment areas. Approximately 60% of the water bodies assessed are at risk of failing the WFD objectives, if not systematic efforts are made to improve the quality also of SPM. Screening

of sources and paths of exposure for «priority substances» and «priority hazardous substances» according to the WFD identified the «historical pollution from sediments» as one distinct pollution source for surface waters. Because of industrial emissions in the past, several river catchment areas are expected to fail the standards demanded by the WFD, due to a risk of remobilization of contaminants from sediments during floods. This holds true for the Rhine with high loads of HCB as well as for the Elbe, where contaminated sediments can be a severe problem. Therefore, integration of sediments and SPM into the holistic river basin management approach and their consideration within the «programs of measures» is highly recommended.

With respect to conflict of interests between flood management and drinking water supply, it has to be considered that SPM translocated during flood events, may cause increasing contaminant charges and biological mechanism-based activities correlated with increasing discharge. However, the extent of contaminant charge and intensity of biological activities cannot be predicted from these results since these are depending on the catchment area and the intensity of the specific flood event. Nevertheless, operators of retention basins have to be aware of SPM that will be introduced into the basins those are flooded just before the peak of extreme flood events with recurrence intervals of 100 years and higher (using present benchmarks). Thus, pollution of soils in flooded areas including retention basins can be safely assumed.

6.7 Acknowledgements

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7 Investigation on soil contamination at recently inundated and noninundated sites [24] [25]

7.1 Introduction

Floodplain soils are often loaded with many contaminants because of inundation during flood events (Zonta et al. 2005) In general, floods cause increasing sediment erosion in correlation with water discharge. Erosion can reach deep and remobilize older sediment layers, which are often likely to be more heavily contaminated than younger sediment layers (Hollert et al. 2007a, Stronkhorst and van Hattum 2007). 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, being deposited in considerable amounts since flow velocities are lower along flat river banks that are usually abundantly covered with vegetation (Jeffries et al. 2003).

SPM is initial matter for soil genesis and appears to be an important nutrient source (Wassen et al. 2002). However, SPM and 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 (Förstner 2004, Kosmehl et al. 2004). Thus, matter deposited on floodplains provides a potential to contaminate affected sites, especially given the intensified erosion during extreme floods (Weber et al. 2008). Typical contaminants at the Rhine are polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) as well as hexachlorobenzene (HCB; Heise and Förstner 2006, Klok and Kraak 2008, Wölz et al. 2010b).

Deposited matter accumulates preferably in surface depressions and water basins, which, as a result, often contain the highest contaminant loads (Asselman and Middelkoop 1995). These contaminants can impact adjacent areas by aeolian transport, but the bulk remains on floodplains (Baborowski et al. 2007). The contaminants may be retained in the topsoil layers

[24] The original publication is available at http://www.springerlink.com: Wölz, J.; **Schulze, T.**; Lübcke-von Varel, U.; Fleig, M.; Reifferscheid, G.; Brack, W.; Kühlers, D.; Braunbeck, T.; Hollert, H. (2011): Investigation on soil contamination at recently inundated and non-inundated sites; Journal of Soils and Sediments 11, 82–92 (DOI: 10.1007/s11368-010-0267-6)

[25] This section is part of the PhD thesis of Dr. Jan Wölz (2009): Impact of contaminants on aquatic systems and inundated sites with respect to flood events: *in vitro* biotests, chemical target analysis and fractionation methods; PhD thesis, Ruberto Carola University of Heidelberg; Heidelberg; 194 pp.

that provide humic substances and clay minerals providing huge adsorption capacities (Allen-King 2002).

The findings presented in this study were generated as part of the joint research project «Flood retention and drinking water supply – Preventing conflicts of interest» (RIMAXHOT; Kühlers et al. 2009, Maier et al. 2006). This project, funded by the Federal Ministry of Education and Research (BMBF), aimed to identify potential conflicts of interest at the planned retention basin Bellenkopf–Rappenwoert and the nearby planned public well field Kastenwoert, both located near Karlsruhe, Germany.

Chemical loads as well as hazard potentials of SPM sampled during a flood close to the soil sampling site of this study were previously detailed by Wölz (2010a). PAH and HCB concentrations were determined to be increased, and elevated AhR-agonist activities and mutagenic potencies were determined. Therefore, the present study aimed to assess whether compound concentrations and biological activities in soil cores sampled at inundated sites were elevated compared to noninundated sites that are located behind a levee. Chemical analysis was used to identify loads of PAHs, PCBs, and HCB. 7-Ethoxyresorufin-o-deethylase induction assay (EROD) and Ames fluctuation assay showed biological hazard potentials with respect to in vitro biotest systems. An automated fractionation procedure (Lübcke-von Varel et al. 2008) was used to identify effective compound classes. Target analysis was used to determine contribution of priority EPA-PAHs (defined by the United States Environmental Protection Agency) to the overall EROD induction. Furthermore, fractions were assessed with the Ames fluctuation assay (Reifferscheid et al. 2005), and mutagenic potencies were detected as caused by compounds in each fraction.

Thus, the present study aimed:

1. To measure chemical loads and biological responses in soil core layers from inundated and noninundated sites
2. To use automated fractionation, biological and chemical analysis to identify effective fractions and shares of target analytes
3. To assess whether frequently inundated sites show elevated in vitro toxicity effects and contaminations when compared with noninundated sites

7.2 Materials and methods

7.2.1 Chemicals used

Chemicals were at least reagent grade and have been provided by Sigma-Aldrich (Deisenhofen, Germany).

7.2.2 Description of methods

The methods below have already been described in detail by (Wölz et al. 2010b) and will therefore be presented only briefly here.

7.2.3 Soil sampling

In this study, soil was sampled in August 2006 at the planned retention basin Bellenkopf–Rappenwoert. Soil was sampled at six locations in the basin area (north, middle, and south), three of them at inundated sites close to the river and three at sites behind a levee that are not influenced by flooding (Figure 6-5).

Soil was sampled from the surface down to a depth of 90 cm using a viscoplastic standard stainless steel soil corer according to Pürckhauer with a diameter of 2.8 cm (Schierholz et al. 2000) and a maximum drilling depth of 1 m. Each sample was further separated into three subsamples of 30 cm depth (0–30, 30–60, 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. Freeze-dried samples were sieved using a mesh size of 600 µm for 15 min using an ultrasound bath type Sonorex RK 255 H (Bandelin, Berlin, Germany). Sieved samples were stored at 4 °C in darkness until extraction.

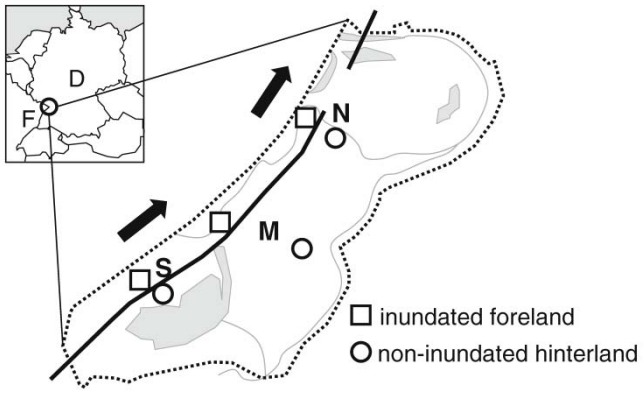

*Figure 7-1 Location of the planned retention basin Bellenkopf–Rappenwoert near Karlsruhe, Germany. Inundated foreland and noninundated hinterland are separated by a levee (*straight black line*). Soil was sampled in the north (N), middle (M), and south (S).* Gray lines *and* filled areas *represent water courses and basins at site.* Black arrows *show the Rhine flow direction.* D *Germany,* F *France*

7.2.4 Soil extraction for assessment of total samples

Total soil samples were treated according to DIN 38414-24. Samples were freeze-dried fast as possible and sieved as described in Section 7.2.3. Ten grams of each freeze-dried soil sample were extracted with 250 ml dichloromethane (Sigma-Aldrich) 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 redissolved 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.

7.2.5 Soil extraction and cleanup for fractionation

Ten grams of each freeze-dried soil layer was Soxhlet extracted using dichloromethane/acetone (3:1; v/v) solvent mixture. Accelerated membrane-assisted cleanup (AMAC) step technique was used for purification of soil extracts according to the protocol by Streck et al. (2008b). To this end, 1 ml extract with a concentration of 10 g soil equivalent per millilitre was transferred to polyethylene dialysis membranes and extracted using an accelerated solvent extraction (ASE) 200 device (Dionex, Sunnyvale, CA) as detailed by Lübcke-von Varel et al. (2008). Extracts were reduced in volume, evaporated under a gentle

N_2-stream, and redissolved in *n*-hexane/dichloromethane (DCM; 90:10; v/v) to a final concentration of 10 g/ml for subsequent fractionation.

7.2.6 Automated fractionation procedure

AMAC-purified extracts were fractionated using an automated fractionation method (2008) loading them on three types of columns (cyanopropyl, nitrophenylpropyl-silica, and porous graphitized carbon). Fractions were collected in glass vessels, separated in two aliquots with equal volumes, reduced in volume, evaporated under a gentle N_2-stream, and redissolved in *n*-hexane (for gas chromatography – mass spectrometry (GC-MS) analysis) and dimethylsulfoxide (DMSO; for biotesting) to a final concentration of 10 g/ml. Model compounds for each fraction are detailed by Lübcke-von Varel et al. (2008).

7.2.7 GC–MS analysis of fractions

GC–MS analysis were carried out on a HP 6890 GC coupled to a HPMSD 5973 (Agilent, Palo Alto, USA), equipped with a 30 m×0.25 mm ID×0.25-µm film HP-5MS fused capillary silica column, a 5-m precolumn (Agilent J&W, Folsom, USA), and a splitless injector with deactivated glass wool. The MS was operated in electron 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 SIM. The results were corrected with an internal standard containing deutered PAH (Mix 35, Promochem, Wesel, Germany).

7.2.8 EROD induction assay

Induction of EROD was measured in the CYP1A expressing cell line RTL-W1. Cells were seeded in 96-well plates (TPP, Trasadingen, Switzerland) and 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 (3.13–100 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. After lysing the cells, 7-ethoxyresorufin and reduced nicotinamide adenine dinucleotide phosphate were supple-

mented to start deethylation of the exogenous substrate. EROD activity and protein content was determined fluorometrically. The concentration–response curves for EROD induction in the RTL-W1 bioassay were computed by nonlinear regression using GraphPad Prism 4 (GraphPad, San Diego, USA) and classic sigmoid or Boltzmann curves as model equations (Seiler et al. 2006). The enzyme-inducing potential is given as EC_{25} (effective concentration for 25% AhR-mediated activity in the EROD assay compared to positive controls exposed to TCDD) of the samples was converted to biological equivalent concentration (Bio-TEQ) as described below.

7.2.9 Bio-TEQ values

Ah-receptor agonist activities were determined as EC_{25} values for each sample and then were given relative to the positive control 2,3,7,8-TCDD as «biological toxicity equivalent concentration» (Bio-TEQ; Villeneuve et al. 2002, Wölz et al. 2008). Bio-TEQs were calculated as given in Equation 8–1 as mean values of n=3 independent biotests. TCDD-EC_{25} were determined with each test plate, and mean values of all tests were used for the calculation of Bio-TEQ values. Wölz et al. (2008) gave the mean TCDD-EC_{25} values of n=59 independent tests as 5.3 pg ± 1.8 pg/g. These values were determined with the same cell culture and the same TCDD batch and, thus, can be expected to show the test variation that applies for this study. Subsequently, Bio-TEQs with concentrations in picogram TCDD per gram of SEQ will be given as picogram per gram:

$$Bio - TEQ\left[\frac{pgTCDD}{gSEQ}\right] = \frac{TCCD - EC_{25}[\frac{pgTCDD}{ml}]}{sample - EC_{25}[\frac{gSEQ}{ml}]} \quad (8-1)$$

7.2.10 Ames fluctuation assay

The Ames fluctuation assay is a modification of the plate incorporation Ames test (Maron and Ames 1983) according to the method described by Reifferscheid et al. (2005). In contrast to the classic test, bacteria were exposed 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 and Ames (1983). Bacteria were cultured overnight in Oxoid Nutrient Broth no. 2 and ampicillin (50 µg/ml) at 37 ± 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 (λ=595 nm) to a standard (10 g/l hexamethylenetetramine, 1 g/l hydrazinesulfate; equals 1,800 FAU) according to the method described by Hawe and Friess (2007). For testing, overnight cultures were adjusted to 1,800 (TA 98) and 450 FAU (TA 100).

Bacteria were preincubated with exposure medium, containing low concentrations of histidine (6.45 µm per well), in 24-well microtitre plates (TPP) for 90 min at 37 °C to allow some cell divisions. Preincubated bacteria were six fold diluted in histidine-deficient reversion indicator medium, containing bromocresol purple as pH indicator. 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, a liver homogenate S9-fraction (RCC Rossdorf, Germany) from phenobarbital/β-naphthoflavon treated rats (protein concentration 30.5 mg/ml S9) was added in a buffered cofactor mixture to each well.

For each test, ±S9 mix 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 ≤10 per 48 wells (TA 98 and TA 100) at all testing conditions with both strains ±S9. Positive controls were valid when the number of revertants was ≥25 per 48 wells as mean values for both bacterial strains ±S9 at all testing conditions. DMSO was added as solvent/negative control with a maximum of 1% per well at which no toxic effect was observed. Positive controls were 4-nitroo-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).

Fisher's exact binomial test for low numbers of tests was chosen to identify significant mutagenic activity with $p<0.05$. Next to no observed effect concentration (NOEC) values, maximum induction factors (IFmax) were computed, which give the induction of the highest inducing sample concentration referred to the negative control induction.

7.3 Results

7.3.1 AhR-mediated activities and identified compounds

Soil sampled at inundated and at noninundated sites was assessed as 30-cm-thick layers down to a depth of 90 cm in order to determine concentrations of PAHs, PCBs, and HCB as well as EROD-inducing potential (Figure 7-2). At each location, the highest concentrations of EPA-PAHs were identified in the top soil layer (0-30 cm). Highest concentration was determined in ground swale soil sampled at the inundated area giving a maximum of 43 mg EPA-PAHs/kg in topsoil (NF sample). Furthermore, this sample showed elevated concentrations of PCBs (0.19 mg/kg) as well as of HCB (0.049 mg/kg). Deeper soil layers at this site gave decreasing concentrations with only EPA-PAHs being detectable below a depth of 60 cm. At the other sites, only EPA-PAHs were detected but were shown to be limited to topsoil (0–30 cm). Concentrations ranged between 0.083 and 0.13 mg/kg at the inundated foreland and 0.07 and 0.14 mg/kg at the noninundated area behind the levee.

EROD inductions indicated ground swale soil to cause the highest Ah-receptor mediated effects, and maximum induction was measured in the topsoil layer with a Bio-TEQ = 43000 pg/g. The AhR-agonists were less active at the other inundated sites, where mean concentrations stood at 153 ± 0.7 pg/g and at noninundated sites behind the levee with mean Bio-TEQs of 129 ± 77 pg/g. At each site, lower soil layers showed decreasing Bio-TEQs.

7.3.2 EROD-inducing potential by soil fractions

In order to identify active substance classes in the EROD assay, the highest inducing soil layer (0–30 cm) of the ground swale was selected for effect-directed analysis processing in automated fractionation procedure (Figure 7-2). Resulting fractions F1 to F5 containing PCBs and PCDD/Fs indicated no or minor activities (ΣF1–F5 Bio-TEQ = 24.2 pg/g). In contrast, PAH fractions (F6 to F13) were highly inducing and showed highest Bio-TEQ in fraction 10 (13000 pg/g), which contains PAHs with five aromatic rings (e.g., benzo[a]pyrene). Fraction 12, containing, for example coronene, resulted in negligible inductions; F13, containing mono-nitro-PAHs, was not inducing. In total, PAH fractions F6–F13 gave a total Bio-TEQ of 31848 pg/g. In contrast, F14–F17, with more polar compounds, indicated elevated activities of about one-fourth EPA-PAH fraction activities. Induction of F18 containing most polar compounds was negligible. In sum, fractions with more polar and polar substances (F14–F18) gave a Bio-TEQ = 6021 pg/g.

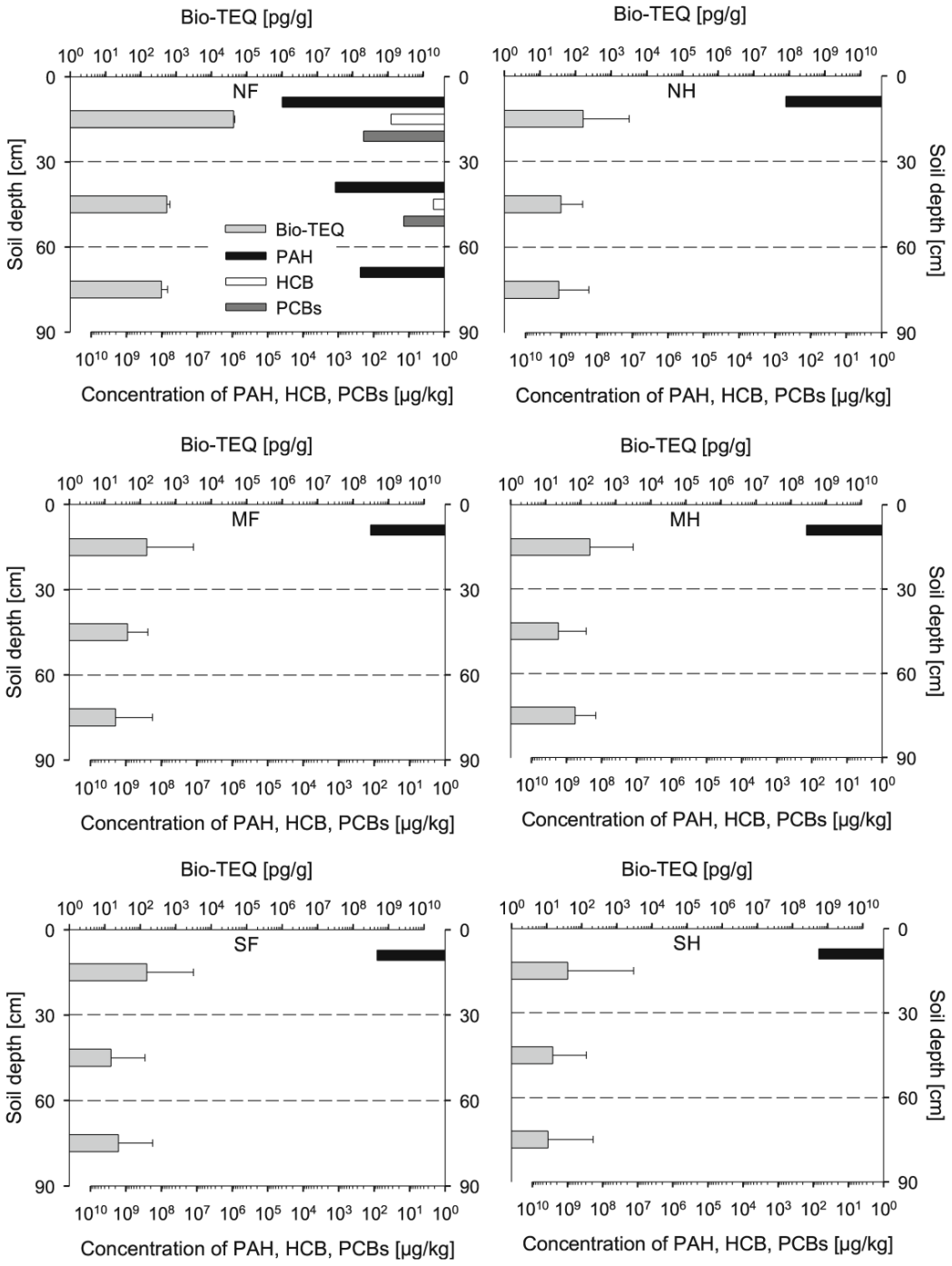

Figure 7-2 Concentrations of chemically analyzed HCB and selected PCBs and PAHs, as well as bioanalytically determined Bio-TEQs are shown for distinct soil layers (0–30, 30–60, 60–90 cm). These allow the comparison of samples from the north (N), middle (M), and south (S) of the inundated foreland (F) and the noninundated hinterland (H) within the planned retention area, which are separated by a levee

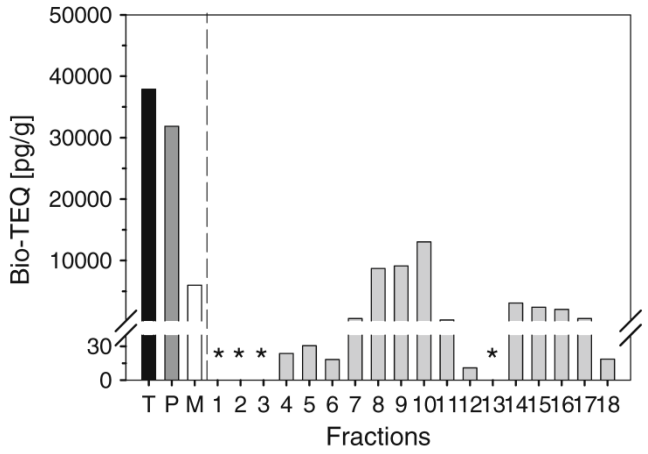

*Figure 7-3 EROD induction given as Bio-TEQ and determined with HPLC fractions of the topsoil layer sampled at the NF site. T (total)=ΣF1–F18, P (PAH fractions)=ΣF6–F13, M (more polar and polar fractions)=ΣF14–F18, *=no EROD induction and, thus, no Bio-TEQ determined*

7.3.3 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 ground swale soil sample showed elevated potentials. Significantly decreased NOECs and increased IFmax were measured in all soil fractions except for fraction F8 containing PAHs with four aromatic rings (e.g., pyrene). Fraction F17, containing more polar compounds (e.g., 2-hydroxyanthraquinone), showed highest potentials in strain TA 98 (frameshift mutations) with S9 metabolism and a NOEC=0.03 mg/ml. Fractions containing PAHs that need metabolic activation to express their DNA-damaging potential showed mutagenicity even without supplementation of S9 mix. However, in nine of 16 cases, fractions treated with TA 98 and metabolic activation showed higher mutagenicity. Tester strain TA 100 (base pair substitution) indicated fractions to be less active. Rather low activities were determined in the approach without exogenous S9 supplementation. Fractions were nonactive in the Ames fluctuation test with S9 mix, except F16 that was the highest inducing fraction with TA 100 (IFmax=5.7) as well as one of the highest inducing fractions at all (Table 7-1).

Table 7-1 Mutagenic activity of HPLC fractions 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

Fraction no.	NOEC (mg/ml)				Induction factor (IFmax)			
	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9
1	a	1.04	a	a	a	4.3	a	a
2	a	16.7	a	a	a	6.7	a	a
3	8.3	8.3	a	a	6.7	3.9	a	a
4	8.3	a	a	a	5.7	a	a	a
5	a	2.1	a	a	a	7.1	a	a
6	4.2	4.2	16.7	a	3.6	5.7	8.0	a
7	a	16.7	a	a	a	3.3	a	a
8	a	a	a	a	a	a	a	a
9	1.0	1.04	a	a	16.1	13.3	a	a
10	4.2	2.1	a	a	8.6	19.0	a	a
11	2.1	2.1	16.7	a	4.3	12.3	3.3	a
12	2.1	2.1	16.7	a	4.3	1.9	2.4	a
13	a	2.1	a	a	a	2.9	a	a
14	2.1	a	a	a	6.7	a	a	a
15	a	a	16.7	a	a	a	4.3	a
16	2.1	a	a	1.0	16.7	a	a	5.7
17	0.07	0.03	a	a	19.0	29.0	a	a
18	0.1	0.3	a	a	19.0	14.3	a	A

Maximum induction factors (IFmax) were only computed for fractions with significantly reduced NOECs. Data are given as no observed effect concentration in milligram soil equivalent per millilitre test medium and as maximum induction factor a NOEC>33.3 mg/ml (maximum test concentration)

7.4 Discussion

7.4.1 Chemical contamination of crude extracts

EPA-PAHs were measured in each topsoil sample at inundated and noninundated sites. However, soil sampled in a ground swale in the inundated area showed elevated contaminant concentrations down to a depth of 90 cm. In this depth, concentrations were still comparable to those determined in topsoil at inundated and noninundated sites. Ranking the maximum EPA-PAH, topsoil concentrations of 43 mg/kg with concentrations reported in other studies highlights an elevated chemical load of ground swale topsoil. Gocht et al. (2001) measured PAH concentrations, including the EPA-PAHs, of 3.7 mg/kg in topsoil of floodplains. Hilscherova et al. (2007) reported of up to 20 mg PAHs/kg in floodplain soil of the rivers Morava and DÍevnice, Czech Republic, and their tributaries measured after a 100-year flood. Pies et al. (2007) investigated on bank soil along the rivers Mosel and Saar, Germany,

that shows evidence of coal mining activities and which is frequently inundated. PAH concentrations, including EPA-PAHs, 1-methylnaphthalene, 2-methylnaphthalene, and perylene, reached up to 81 mg PAHs/kg down to depth of 2 m. Thus, concentrations measured in the present study are in the same order of magnitude than soil sampled at other inundated sites and even of soil sampled in an area that can be expected to show elevated background levels of PAHs due to coal mining activity. PAHs in soil may be introduced via aeolian processes or accidental releases. However, sediments are known to substantially be contaminated with substances of this compound category. Sediment erosion, translocation as SPM and deposition at inundated sites seems at least to be a considerable source for floodplain contamination.

PCBs were detected in ground swale soil down to a depth of 60 cm and showed a maximum concentration of 0.19 mg/kg in topsoil. Compared to the findings given by, e.g., Hilscherova et al. (2007), concentrations determined in the present study indicate a comparable or increased contamination: about 0.1 mg/kg (1997) and about 0.01 mg/kg (2005) along different reaches of the rivers Morava and DÍevnice. PCBs are known to be major constituents of at least older sediments and thus detection on ground swale floodplain soil indicates deposition of contaminated matter during inundation as plausible origin of recent PCB contamination.

Furthermore, soil was analyzed for HCB, which is a specific the Rhine contaminant, in this study and was measured in ground swale topsoil with a maximum concentration of 0.05 mg/kg. Alcock et al. (1998) reported that sediment sampled at the Rhine barrage at Iffezheim, which is situated upstream and close to the soil sampling site of the present study, showed HCB concentrations of 0.22 µg/kg at a depth of 1.2 m and 0.04 µg/kg at a depth of 0.2 m. SPM that was sampled during a flood with a recurrence interval of 10 years in August 2007 close to the sampling site of this study was determined to contain up to 0.11 mg/kg SPM at the peak of the flood (Wölz et al. 2010b). Thus, HCB concentration measured with ground swale topsoil of an inundated site and concentrations and flood SPM, sampled in the same river section, were in the same order of magnitude, while sediment concentrations were lower. HCB is an exclusive particle-bound contaminant, and at present, there are no other sources of HCB than contaminated sediment. The only plausible origin of HCB contamination of ground swale soil seems to be the erosion of contaminated sediments in flood events that is translocated with SPM and deposits at inundated site (Heise et al. 2004).

Further, the elevated concentrations of contaminants down to90 cm depth in the swale soil core could be an evidence of accumulation of contaminated soil due to erosion of top soils in the surrounding area.

The findings of the presented study also have to be discussed with results of Ulrich et al. (2002) who investigated on few topsoil samples in the area of the present study and whose results acted as precursor study. In this study EPAPAH concentrations varied between 0.19 and 0.76 mg/kg at rarely inundated sites and 0.37 to 1.64 mg/kg at frequently inundated sites. HCB concentrations varied between <0.001 and 0.002 mg/kg at rarely inundated sites as well as 0.015 and 0.053 mg/kg at frequently inundated sites. Thus, maximum EPA-PAH concentrations of the present study were about 50 times increased compared to Ulrich et al. (2002) while HCB concentrations were in the same range. In order to be complete, it should be added that Ulrich et al. (2002) detected HCB which was not reproduced in the present study (sampling sites differed). Thus, heterogeneities of soil contamination were determined in the present and the precursor study which highlights the need to carefully select sampling sites and to discuss results in the context of sampling sites and methods. In the present study, elevated contamination was exclusively shown with soil sampled in a ground swale that would not have been detected with sampling strategies excluding swales as, e.g., not representative.

Three contaminant categories were assessed in the present study, and each can be assumed to originate from contaminated sediment as discussed above. Thus, there is a line of evidence that cannot be assumed to be the only true origin of measured contaminants but which seems to be the most plausible.

7.4.2 Biological hazard potentials by crude extracts

Next to chemical analysis and contaminant concentrations measured with soil samples, biological testing approaches should be used to allow the detection of hazard potentials on a biological scale and, thus, to determine whether contaminants impact biological systems or not. Therefore, the in vitro EROD assay was used to determine Bio-TEQs and showed elevated Ah-agonistic activities of at least crude topsoil extracts. In accordance with chemical analysis, ground swale samples showed effects down to 90 cm depth and maximum Bio-TEQs of 43000 pg/g with topsoil. Lower Bio-TEQ in the same order of magnitude of about 10000 pg/g was determined by Keiter et al. (2008) with sediment from the Danube,

Germany. SPM sampled in a flood with a recurrence interval of 2 years at the Rhine showed Bio-TEQs of about 2300 pg/g, and SPM of a flood at the the Neckar with the same recurrence interval showed maximum Bio-TEQ of 8,300 pg/g (Wölz et al. 2008).

EROD assay-derived Bio-TEQs determined with ground swale soil were higher than sediment and SPM-derived TEQs given above. However, published studies providing data on EROD induction with sediment and in particular with flood SPM are rare, and the few above detailed Bio-TEQs may only provide snap-shots that cannot give an overview of the total range of possible Bio-TEQs. Nevertheless, biological effect monitoring using the same in vitro test system that was used in the present study showed that sediment and flood SPM contamination may cause biological effects lower or in the same order of magnitude of floodplain soil that showed elevated effects in the present study. Thus, there is another although less solid line of evidence for the origin of the soil contamination.

7.4.3 Identification of active fractions

While fractions with PAHs in sum caused the highest EROD activity, in particular, fractions containing compounds of four to five aromatic rings, EPA-PAHs only contributed with ≤1% to the total EROD activity (therefore, data not given in detail). This percentage was surprisingly low since other studies reported that EPA-PAH at least contributed some percent of the overall EROD induction. EPA-PAHs once were prioritized as most relevant representatives of their substance category to environmental contaminants. In contrast to this prioritization, the present and further studies indicate that EPA-PAHs are not necessarily the PAHs that should be addressed (Brack et al. 2005b, Wölz et al. 2008).

However, pattern of EROD-inducing fractions of the present study are in line with pattern determined using the same automated fractionation method with flood SPM sampled at the Rhine barrage of Iffezheim upstream and close to the present soil sampling site (Wölz et al. 2010b). Furthermore, these findings are consistent with pattern determined with flood SPM sampled at the Rhine in another study (Wölz et al. 2010a) that used a precursor fractionation method (Brack 2003). In order to be complete, it should be given that only pattern of EROD inducing fractions are comparable with ground swale soil of the present study while total height of each fractions Bio-TEQ varied.

Nevertheless, analog pattern of EROD inductions with sediment, flood SPM, and ground swale soil act as another line of evidence that soil contamination at inundated sites may originate from eroded sediment that finally deposits at inundated sites.

Next to EPA-PAHs, automated fractionation highlighted further fractions to cause elevated Ah-agonist activity; fractions containing more polar and polar compounds, for example characterized by (hydroxyl-)quinones, keto-, dinitro-,hydroxy-PAHs, and N-heterocycles with rising polarity. More polar and polar compounds were perceived to cause elevated effects with sediment by (Keiter et al. 2008) and with flood SPM by Wölz et al. (2010b), however, only as a total. The above detailed results presented in this study show in more detail the distribution of activity among fractions of more polar and polar substances and, thus, allow encircling possible effect causing contaminants to be addressed in future.

More polar and polar compounds which were often given less attenuation should be in the focus of upcoming research on environmental pollution, in particular, as higher polarity indicates elevated water solubility and mobility as well as bioavailability than, e.g., nonpolar HCB, PCBs, and most PAHs. Likewise, Petrovi et al. (2003) discussed emerging contaminants such as surfactant degradation products, pharmaceuticals, and polar pesticides.

Next to EROD induction, mutagenic potential of ground swale soil crude extract and fractions were assessed using the Ames fluctuation assay. Crude extract showed no mutagenicity while increased fractions containing polar as well as nonpolar substances showed significant effects. Mutagenic effects following fractionation might be explained with the removal of masking or inhibiting substances by means of the fractionation procedure. Following fractionation, masking compounds were separated partially or completely in less or nonactive fractions (Brack et al. 2005b). Cytotoxicity of the crude extracts of the bacterial test strains was screened and can be excluded as the reason for absence of mutagenic effects (Chenon et al. 2003).

However, most mutagenic activity was related to tester strain TA98 and frameshift mutations, and most fractions caused metabolic activation after S9 treatment. Activities were increased in fractions containing PAHs, and highest NOECs and IFmax were determined with fractions containing more polar and polar substances. However, there is no clear trend toward direct or indirect mutagenic activity. It can only be stated that mutagenic potentials were increased

in fractions containing PAHs, which are well-known inducers of mutagenic effects (Brack et al. 2005b, Perez et al. 2003, White 2002) as well as in fractions with moderately polar and polar substances (Marvin and Hewitt 2007, Villalobos-Pietrini et al. 2007).

Thus, in accordance with EROD induction, mutagenic potentials were shown to be increased with ground swale topsoil and, furthermore, that more polar and polar substances are major contaminants causing the highest mutagenic effect.

7.4.4 Regulatory aspects

In the discussion chapters above, elevated soil contamination at a floodplain was discussed versus contamination of sediment and flood SPM and given as results of chemical and biological analysis as well as of fractionation methods. The focus was on the effect and hazard assessment. However, the findings of the presented study should also be discussed with respect to a possible risk of soil contamination.

Initially, the question shall be discussed on whether the measured EPA-PAH concentration raises a concern. There are different possibilities to discuss this question with the German Bundesbodenschutzgesetz (BBodSchG 1998), the Federal Soil Protection Act, being one of them. Ranking the maximum EPA-PAH concentration of 43 mg/kg soil with threshold for children playgrounds of 5 mg/kg clearly indicates a concern. Children playgrounds show the highest protection level and thus legal contaminant thresholds are lowest. The measured EPA-PAH concentrations are even close to the threshold of industrial real estate soil which is set to be 100 mg/kg soil. Thus, according to BBodSchG (1999), ground swale soil could not be used for children playground purposes but only as industrial real estate.

Furthermore, HCB concentrations shall be related to legal thresholds for soil. Soil contamination measured in the present study with 0.032 mg HCB/kg does not exceed the threshold of 4 mg HCB/kg soil as set for children playground in BBodSchG (1998). Thus, HCB concentration determined in the present study could, in principle, be used as soil for children playgrounds.

With these examples, possible risks of soil contamination to human interests shall be highlighted and allowed to rank the level of contaminant concentration that was determined at the highest contaminated site within the assessed floodplain.

7.5 Conclusions

The present study used biological and chemical analysis in combination with fractionation methods and advised a heterogeneous dispersion of soil contamination at site. Comparison of soil cores sampled at inundated and non inundated sites allowed to identify that soil sampled in a ground swale at an inundated site showed elevated contamination. Several substance categories were analyzed, and contaminated sediments and flood SPM were discussed as source of the detected soil contamination. There is no direct proof of the origin of detected soil contaminants. However, lines of evidence suggest eroded sediment and deposited flood SPM to cause elevated soil contamination, at least at specific sites such as ground swales in inundated areas. Furthermore, automated fractionation methods allowed the determination of PAHs as well as more polar and polar substances to contribute significantly to determined biological in vitro effects. These give another line of evidence since pattern of biological activity among the fractions was comparable between soil and flood SPM.

Discussing the origin of the soil contamination is of relevance since the presented study was carried out as part of project that aimed to investigate on possible conflicts of interest between retention basins and close located water works. Since soil contamination by deposition of contaminated flood SPM appears to be given, increased soil contamination has to be anticipated when retention basins are inundated during flood events. Retention basins keep flood water if necessary up to weeks which allows SPM to deposit and to remain on the soil when water is released to the river after the flood. Operators of retention basins should take actions to avoid or reduce SPM deposition while flood water is kept in the basin.

Recently planned retention basins are destined to be operated during extreme flood events with recurrence intervals of 100 years and more. Sediment erosion will be extensive and reach deep into highly contaminated older sediment layers during extreme flood events and thus flood SPM will be highly contaminated. Deposition of flood SPM could result in elevated soil contamination on other sites then ground swales.

Furthermore, it was discussed that at present, highly contaminated soil exceeds legal thresholds, however, only shown for the highest contaminated site and with respect to EPA-PAHs. Deposition of highly contaminated SPM during extreme floods might result in the exceedance of further thresholds, and larger areas might be impacted.

As given above, more polar and polar contaminants were shown to contribute significantly to biological effects. These substances are characterized by elevated water solubility and mobility and thus can be assumed to be easily transported with water flow. These substances could raise concerns for the use of river bank water that is often used as drinking water resource.

The results of this study highlight that origin and legacy of river bank soil contamination have to be discussed in a broader sense. Prospective, river bank soil contamination has additionally to be discussed in the context of hazards emerging with extreme flood events as well as with protection measures, such as the operation of retention basins that are intended to control the risk of extreme floods. Retention basins may cause hazards themselves when in conflict with further interests such as drinking water supply. Thus, while planning extreme flood control measures, impacts of these measures to further interests should be considered.

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10 Scientific dissemination

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Appendix

11 Appendix

Section 2 Screening and identification of organic compounds in sediments and suspended particulate matter of the Saar and the Rhine

Table S2–1 Comparison between the Rhine and the Saar (Data source: Eckoldt 1998, IKSMS 2005a, IKSR 2005)

	Rhine	**Saar**
Length	1.320 km	227 km
Spring	Helvetian Alps	Vosges Mountains – sandstone area
River catchment	200.000 km²	7.431 km² (93% in the Saarland area)
River mouth / estuary	North Sea near Hoek van Holland (NL)	Moselle near Konz
Inhabitants in the catchment	50 Mio.	1.08 Mio. (Saarland)
Countries	Switzerland, Liechtenstein, Austria, Germany, France, The Netherlands	France, Germany
Main tributaries	Ill, Aare, Neckar, Main, Nahe, Mosel, Ruhr, Lippe	Blies, Nied, Prims
Main annual discharge (m³/s)	Constance: 338; Karlsruhe-Maxau: 1.260; Rees (border Germany / The Netherlands): 2.270	Konz: 80
Navigability	International waterway from Rotterdam (The Netherlands) to Schaffhausen (Switzerland) with 21 locks between Iffezheim (Germany) and Schaffhausen (Switzerland)	International waterway from Konz to Saarbrucken with 6 locks between Kanzem and Saarbrücken (Germany)
Connection to waste water treatment plants	96% (+ 2% small industrial waste water treatment facilities)	89% (Saarland, Germany)[a]

[a] including industrial waste water treatment plants

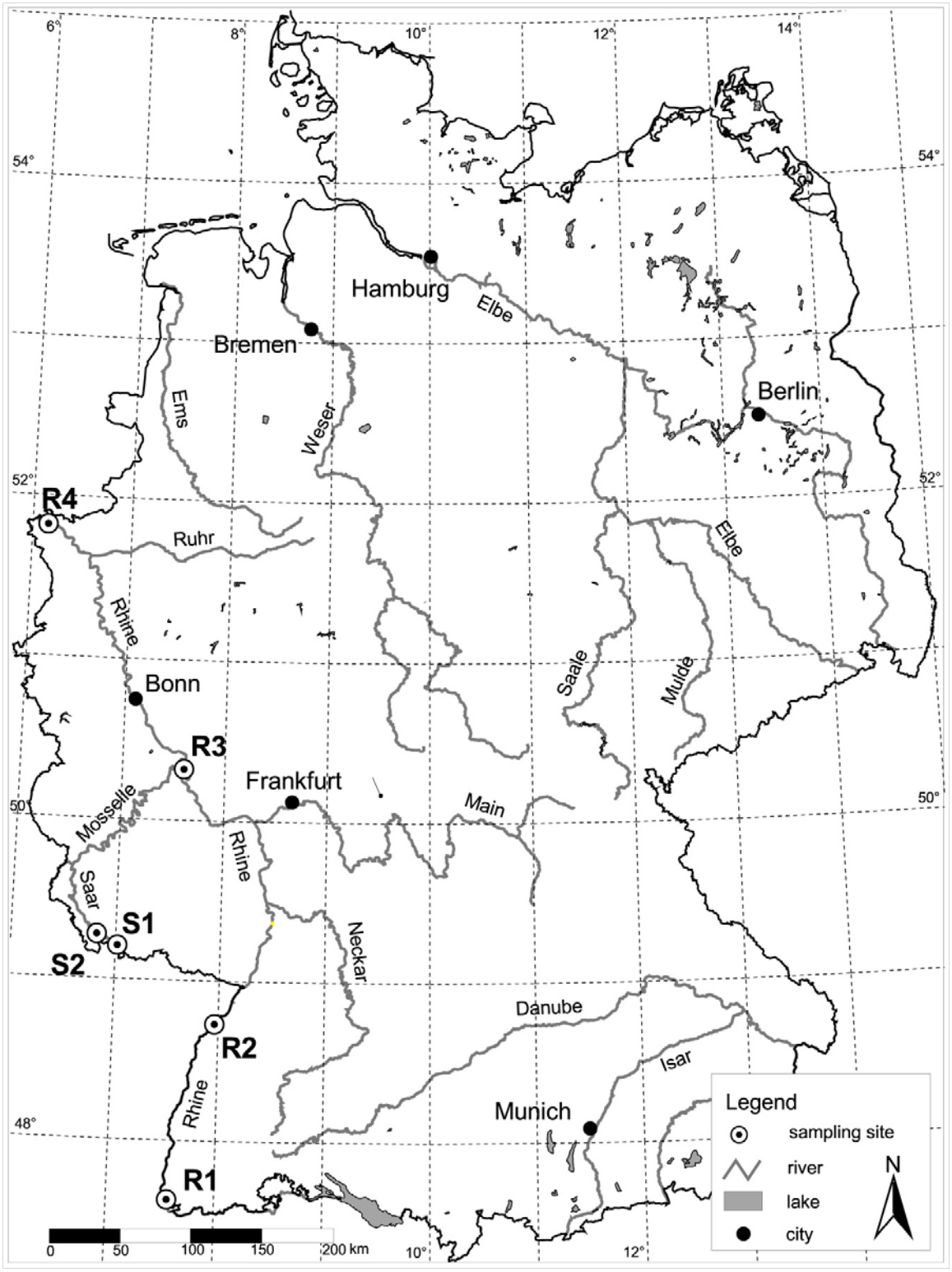

Figure S2–1 Map of the sampling locations at the Saar (S1: Güdingen; S2: Rehlingen) and the Rhine (R1: Weil am Rhein; R2: Iffezheim; R3: Koblenz; R4: Bimmen); adapted from Schulze et al. (2007a)

Table S2–2 Quality measures of the SOMs obtained with different three normalizations (QE: quantization error; TE: topographic error)

	Normalization	QE	TE	Map Size
Rhine locations	Logistic	0.073	0.000	4x4
	Range	0.118	0.100	4x4
	Var	0.343	0.100	4x4
	Log	1.949	0.100	4x4
	histD	0.408	0.000	8x2
	histC	0.297	0.000	8x2
Rhine parameters	Logistic	0.277	0.000	7x3
	Range	0.383	0.000	7x3
	Var	1.519	0.000	4x4
	Log	1.455	0.000	7x3
	histD	0.462	0.000	7x3
	histC	0.417	0.000	7x3

Table S2–3 Results of suspect screening in sediment core samples from locations S1 (Saar, Güdingen) and R3 (Rhine, Koblenz) (sieved <2 mm)

	Fraction	Isomers	S1 0-10	S1 20-25	S1 35-40	S1 44-49	S1 54-59	R3 0-5	R3 10-15	R3 20-25	R3 35-40	R3 55-59
Technical / industrial compounds												
Dimethyl phthalate	5		x	x	x	x	x	x	x	x	x	x
Di-iso-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x
Di-n-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x
Bis(2-ethylhexyl) phthalate	5		x	x	x	x	x	x	x	x	x	x
Tri-iso-butyl phosphate	5		x	x	x	x	x	x	x	x	x	x
Tri-n-butyl phosphate	5		x	x	x	x	x	x	x	x	x	x
Tris(2-ethylhexyl) phosphate	5		x	x	x	x	x	x	x	x	x	x
Tris(chloropropyl) phosphate	5		x	x	x	x	x	x	x	x	x	x
Dioctyldiphenylamine	4											
N-Phenylnaphthylamine	4		x	x	x	x	x			x	x	x
Linear alkyl benzenens (LAB)												
C10-LABs	1,2		x	x	x	x	x	x	x	x	x	x
C11-LABs	1,3		x	x	x	x	x	x	x	x	x	x
C12-LABs	1,4		x	x	x	x	x	x	x	x	x	x
C13-LABs	1,5									x	x	
Polycyclic aromatic compounds (PAC)												
Naphthalene	2,3		x	x	x	x	x	x	x	x	x	x
C1-Naphthalenes	2,3	4	x	x	x	x	x	x	x	x	x	x
C2-Naphthalenes	2,3	6	x	x	x	x	x					
C3-Naphthalenes	2,3	15	x	x	x	x	x					
Biphenyl	2,3		x	x	x	x	x	x	x	x	x	x
Acenaphthylene	2,3		x	x	x	x	x	x	x	x	x	x
Acenaphthene	2,3		x	x	x	x	x	x	x	x	x	x
Fluorene	2,3		x	x	x	x	x	x	x	x	x	x
C1-fluorenes	2,3		x	x	x	x	x					
C2-fluorenes	2,3	5	x	x	x	x	x					
1-phenylnaphthalene	2,3		x	x	x	x	x	x	x	x	x	x
2-phenylnaphthalene	2,3		x	x	x	x	x	x	x	x	x	x
9-Vinylanthracene	3.4		x	x	x	x	x	x	x	x	x	x
Phenanthrene	3.4		x	x	x	x	x	x	x	x	x	x
Anthracene	3.4		x	x	x	x	x	x	x	x	x	x
C1-phenanthrenes	3.4	4	x	x	x	x	x	x	x	x	x	x
C2-phenanthrenes	3.4		x	x	x	x	x	x	x	x	x	x
C3-phenanthrenes	3.4		x	x	x	x	x	x	x	x	x	x
Ethylphenanthrene	3.4		x	x	x	x	x					
4H-Cyclopenta(def)phenanthrene	3.4		x	x	x	x	x	x	x	x	x	x
Fluoranthene	4		x	x	x	x	x	x	x	x	x	x
Acephenanthrylene	4		x	x	x	x	x					
Pyrene	4		x	x	x	x	x	x	x	x	x	x
Benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x
C1-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x
C2-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x
Ethylfluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x
o-terphenyl	4		x	x	x	x	x	x	x	x	x	x
m-terphenyl	4		x	x	x	x	x	x	x	x	x	x
p-terphenyl	4		x	x	x	x	x	x	x	x	x	x
Benzo[c]phenanthrene	4		x	x	x	x	x	x	x	x	x	x
Cyclopenta[cd]pyrene	4		x	x	x	x	x	x	x	x	x	x
Benzo[a]anthracene	4		x	x	x	x	x	x	x	x	x	x
Triphenylene	4		x	x	x	x	x	x	x	x	x	x
Chrysene	4		x	x	x	x	x	x	x	x	x	x
C1-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x					
C2-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x					
1,1´-binaphthyl	4		x	x	x	x	x	x	x	x	x	x
1,2´-binaphthyl	4		x	x	x	x	x	x	x	x	x	x
2,2'-binaphthyl	4		x	x	x	x	x	x	x	x	x	x
Phenylphenanthren, -anthracen	4		x	x	x	x	x					
Benzo[b,j]fluoranthene	4		x	x	x	x	x	x	x	x	x	x
Benzo[k]fluoranthene	4		x	x	x	x	x	x	x	x	x	x
Benzo[e]pyrene	4		x	x	x	x	x	x	x	x	x	x
Benzo[a]pyrene	4		x	x	x	x	x	x	x	x	x	x
Perylene	4		x	x	x	x	x	x	x	x	x	x
C1-benzo[e]pyrenes/perylenes	4		x	x	x	x	x					
Indeno[1,2,3-cd]pyrene	4		x	x	x	x	x	x	x	x	x	x
Benzo[ghi]perylene	4		x	x	x	x	x	x	x	x	x	x

Table S2–3 continued

	Fraction	Isomers	S1 0-10	S1 20-25	S1 35-40	S1 44-49	S1 54-59	R3 0-5	R3 10-15	R3 20-25	R3 35-40	R3 55-59
Polycyclic aromatic compounds (PAC)												
Dibenzanthracene	4		x	x	x	x	x	x	x	x	x	x
Benzo[b]chrysene	4		x	x	x	x	x	x	x	x	x	x
Picene	4											
Dibenzo[def,mno]chrysene	4		x	x	x	x	x	x	x	x	x	x
Coronene	5		x	x	x	x	x	x	x	x	x	x
S-PAC												
Dibenzothiophene	3		x	x	x	x	x	x	x	x	x	x
C1-dibenzothiophenes	3	3	x	x	x	x	x					
C2-dibenzothiophenes	3	6	x	x	x	x	x					
Benzo[b]naphtho[2,1-d]thiophene	2,3		x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]thiophene	2,3		x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]thiophene	2,3		x	x	x	x	x	x	x	x	x	x
S-PAC												
Dibenzofuran	2		x	x	x	x	x	x	x	x	x	x
C1-dibenzofuran	2		x	x	x	x	x					
Benz[b]naphtho[2,1-d]furan	2	4	x	x	x	x	x	x	x	x	x	x
Benz[b]naphtho[1,2-d]furan	2							x	x	x	x	x
Benz[b]naphtho[2,3-d]furan	2							x	x	x	x	x
N-PAC												
9H-carbazole	3		x	x	x	x	x	x	x	x	x	x
C1-carbazoles	3	4	x	x	x	x	x			x	x	x
C2-carbazoles	3	9	x	x	x	x	x			x		
Benzocarbazole	3		x	x	x	x	x	x	x	x	x	x
Aromatic ketones												
9H-fluorene-one	2,3		x	x	x	x	x	x	x	x	x	x
Methyl-fluorene-one	2,3		x	x	x	x	x					
9,10-anthraquinone	2,3		x	x	x	x	x	x	x	x	x	x
Sulfones												
a,a -Dinaphthylsulfon	6								x	x	x	x
a,b -Dinaphthylsulfon	6								x	x	x	x
b,b-Dinaphthylsulfon	6								x	x	x	x
Personal care products												
Galaxolide	5		x	x	x	x	x	(x)	x			
Tonalide	5		x	x	x	x	x	(x)	x			
α-tocopherol	6											
α-tocopherol acetate	6											
Halogenated compounds												
1,3,5-trichlorobenzene	1									x	x	
1,2,4-trichlorobenzene	1									x	x	
1,2,3-trichlorobenzene	1									x	x	X
1,2,3,5-/1,2,4,5-tetrachlorobenzene	1									x	x	X
1,2,3,4-tetrachlorobenzene	1									x	x	
Pentachlorobenzene	1									x	x	X
Hexachlorobenzene	1							x	x	x	x	X
Polychlorinated biphenyls (PCBs)	2,3		x	x	x	x	x	x	x	x	x	X
2,4,5-Trichloroanilin	4								x	x	x	X

Table S2–4 Results of suspect screening in SPM samples from location S1 (Saar, Güdingen; sieved <2 mm)

Sample	Fraction	Isomers	S1 02/05-1	S1 03/05-1	S1 04/05-1	S1 05/05-1	S1 06/05-1	S1 07/05-1	S1 08/05-1	S1 08/05-2	S1 09/05-1	S1 10/05-1	S1 11/05-1	S1 12/05-1
Technical / industrial compounds														
Dimethyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x	x
Di-**iso**-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x	x
Di-**n**-butyl **phthalate**	5		x	x	x	x	x	x	x	x	x	x	x	x
Bis(2-ethylhexyl) phthalate	5		x	x	x	x	x	x	x	x	x	x	x	x
Tri-**iso**-butylphosphate	5		x	x	x	x	x	x	x	x	x	x	x	x
Tri-**n**-butyl **phosphate**	5		x	x	x	x	x	x	x	x	x	x	x	x
Tris(2-ethylhexyl) phosphate	5													
Tris(chloropropyl) phosonate	5		x	x	x	x	x	x	x	x	x	x	x	x
Cresyl-**diphenyl** phosphate	5													
Methyldiphenylmethane	4	3												
Methylbisdiphenylmethane	4	12												
Dioctyldiphenylamine	4													
HCB	1		x	x	x	x	x		x	x		x		
PCBs	2,3		x	x	x	x	x	x	x	x	x	x	x	x
Linear alkyl benzenes (LAB)														
C10-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x	x
C11-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x	x
C12-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x	x
C13-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x	x
Polycyclic aromatic compounds (PAC)														
Naphthalene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
C1-naphthalenes	2,3	4	x	x	x	x	x	x	x	x	x	x	x	x
C2-naphthalenes	2,3	6	x	x	x	x	x	x	x	x	x	x	x	x
C3-naphthalenes	2,3	15	x	x	x	x	x	x	x	x	x	x	x	x
Biphenyl	2,3		x	x	x	x	x	x	x	x	x	x	x	x
C1-biphenyl	2,3		x	x	x	x	x	x	x	x	x	x	x	x
Acenaphthylene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
Acenaphthene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
Fluorene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
C1-fluorenes	2,3		x	x	x	x	x	x	x	x	x	x	x	x
C2-fluorenes	2,3	5	x	x	x	x	x	x	x	x	x	x	x	x
Benzo[a]fluorene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]fluorene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
1-phenylnaphthalene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
2-phenylnaphthalene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
9-vinylanthracene	3,4		x	x	x	x	x	x	x	x	x	x	x	x

Table S2–4 continued

Sample	Fraction	Isomers	S1 02/05-1	S1 03/05-1	S1 04/05-1	S1 05/05-1	S1 06/05-1	S1 07/05-1	S1 08/05-1	S1 08/05-2	S1 09/05-1	S1 10/05-1	S1 11/05-1	S1 12/05-1
Phenanthrene	3.4		x	x	x	x	x	x	x	x	x	x	x	x
Anthracene	3.4		x	x	x	x	x	x	x	x	x	x	x	x
C1-phenanthrenes	3.4	4	x	x	x	x	x	x	x	x	x	x	x	x
C2-phenanthrenes	3.4	9	x	x	x	x	x	x	x	x	x	x	x	x
C3-phenanthrenes	3.4	9	x	x	x	x	x	x	x	x	x	x	x	x
Ethyl phenanthrene	3.4		x	x	x	x	x	x	x	x	x	x	x	x
4H-cyclopenta(def)phenanthrene	3.4		x	x	x	x	x	x	x	x	x	x	x	x
Fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x	x
Acephenanthrylene	4		x	x	x	x	x	x	x	x	x	x	x	x
Pyrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x	x
C1-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x	x
C2-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x	x
Ethylfluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x	x
o-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x	x
m-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x	x
p-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[c]phenanthrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Cyclopenta[cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[a]anthracene	4		x	x	x	x	x	x	x	x	x	x	x	x
Triphenylene	4		x	x	x	x	x	x	x	x	x	x	x	x
Chrysene	4		x	x	x	x	x	x	x	x	x	x	x	x
C1-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x	x
C2-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x	x
1,1'-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x	x
1,2'-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x	x
2,2'-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x	x
Phenylphenanthren, -anthracen	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b,j]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[k]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[e]pyrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[a]pyrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Perylene	4		x	x	x	x	x	x	x	x	x	x	x	x
C1-benzo[e]pyrenes/perylenes	4		x	x	x	x	x	x	x	x	x	x	x	x
Indeno[1,2,3-cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]perylene	4		x	x	x	x	x	x	x	x	x	x	x	x
Dibenzanthracene	4		x	x	x	x	x	x	x	x	x	x	x	x
11H-benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x	x
11H-benzo[b]fluorene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]chrysene	4		x	x	x	x	x	x	x	x	x	x	x	x
Picene	4		x	x	x	x	x	x	x	x	x	x	x	x
Dibenzo[def,mno]chrysene	4		x	x	x	x	x	x	x	x	x	x	x	x
Coronene	4,5		x	x	x	x	x	x	x	x	x	x	x	x

Table S2–4 continued

Sample	Fraction	Isomers	S1 02/05-1	S1 03/05-1	S1 04/05-1	S1 05/C5-1	S1 C6/05-1	S1 07/05-1	S1 08/05-1	S1 08/05-2	S1 09/05-1	S1 10/05-1	S1 11/05-1	S1 12/05-1
S-PAC														
Dibenzothiophene	3		x	x	x	x	x	x	x	x	x	x	x	x
C1-dibenzothiophenes	3	3												
C2-dibenzothiophenes	3	6												
Benzo[b]naphtho[2,1-d]thiophene	1,2		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]thiophene	1,2		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]thiophene	1,2		x	x	x	x	x	x	x	x	x	x	x	x
C1-benzonaphthothiophenes			x	x	x	x	x	x	x	x	x	x	x	x
O-PAC														
Dibenzofuran	2		x	x	x	x	x	x	x	x	x	x	x	x
C1-dibenzofuran	2		x	x	x	x	x	x	x	x	x	x	x	x
C2-dibenzofuran	2		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,1-d]furan	2		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]furan	2		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]furan	2		x	x	x	x	x	x	x	x	x	x	x	x
C1-benzonaphthofuranes	2		x	x	x	x	x	x	x	x	x	x	x	x
C2-benzonaphthofuranes	2		x	x	x	x	x	x	x	x	x	x	x	x
Aromatic ketones														
9H-fluorene-one	2,3		x	x	x	x	x		x	x		x		(x)
9,10-anthraquinon	2,3		x	x	x	x	x		x	x		x		(x)
7H-benzo[de]anthracene-7-one	2,3		x	x	x	x	x	(x)	x	x		x		(x)
11H-benzo[b]fluorene-11-one	2,3		x	x	x	x	x	(x)	x	x		x		(x)
Personal care products														
Galaxolide	5													
Tonalide	5													
α-tocopherol	6		x	x	x	x	x		x	x	x	x	x	x
α-tocopherol acetate	6		x	x	x	x	x		x	x	x	x	x	x

Table S2–5 Results of suspect screening in SPM samples from location S2 (Saar, Rehlingen; sieved <2 mm)

Sample	Fraction	Isomers	S2 02/05-1	S2 03/05-1	S2 04/05-1	S2 05/05-1	S2 06/05-1	S2 07/05-1	S2 08/05-1	S2 08/05-2	S2 09/05-1	S2 11/05-1	S2 12/05-1
Technical / industrial compounds													
Dimethyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Diiso-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Di-n-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Bis(2-ethylhexyl) phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Tri-iso-butyl phosphate	5		x	x	x	x	x	x	x	x	x	x	x
Tri-n-butyl phosphate	5												
Tris(2-ethylhexyl) phosphate	5												
Tris(chloropropyl) phosphate	5		x	x	x	x	x	x	x	x	x	x	x
Cresyl-diphenyl phosphate	5		x	x	x		x	x	x	x		x	
Methyldiphenylmethane	4	3						(x)	(x)	(x)	x	(x)	
Methylbisphenylmethane	4	12	x		x	x	x	x	x	x	x	x	x
Dioctyldiphenylamine	4		x	x	x	x	x	X	x	x	x	x	x
HCB	1		x	x	x	x	x	x	x	x	x	x	X
PCBs	2,3		x	x	x	x	x	x	x	x	x	x	x
Linear alkyl benzenes (LAB)													
C10-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x
C11-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x
C12-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x
C13-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x
Polycyclic aromatic compounds (PAC)													
Naphthalene	2,3		x	x	x	x	x	x	x	x	x	x	x
C1-naphthalenes	2,3	4	x	x	x	x	x	x	x	x	x	x	x
C2-naphthalenes	2,3	6	x	x	x	x	x	x	x	x	x	x	x
C3-naphthalenes	2,3	15	x	x	x	x	x	x	x	x	x	x	x
Biphenyl	2,3		x	x	x	x	x	x	x	x	x	x	x
C1-biphenyl	2,3												
Acenaphthylene	2,3		x	x	x	x	x	x	x	x	x	x	x
Acenaphthene	2,3		x	x	x	x	x	x	x	x	x	x	x
Fluorene	2,3		x	x	x	x	x	x	x	x	x	x	x
C1-fluorenes	2,3		x	x	x	x	x	x	x	x	x	x	x
C2-fluorenes	2,3	5	x	x	x	x	x	x	x	x	x	x	x
Benzo[a]fluorene	2,3		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]fluorene	2,3		x	x	x	x	x	x	x	x	x	x	x
1-phenylnaphthalene	2,3		x	x	x	x	x	x	x	x	x	x	x
2-phenylnaphthalene	2,3		x	x	x	x	x	x	x	x	x	x	x
9-vinylanthracene	3,4		X	x	x	x	x	x	x	x	x	x	x

Table S2–5 continued

Sample	Fraction	Isomers	S2 02/05-1	S2 03/05-1	S2 04/05-1	S2 05/05-1	S2 06/05-1	S2 07/05-1	S2 08/05-1	S2 08/05-2	S2 09/05-1	S2 11/05-1	S2 12/05-1
Phenanthrene	3,4		x	x	x	x	x	x	x	x	x	x	x
Anthracene	3,4		x	x	x	x	x	x	x	x	x	x	x
C1-phenanthrenes	3,4	4	x	x	x	x	x	x	x	x	x	x	x
C2-phenanthrenese	3,4	9	x	x	x	x	x	x	x	x	x	x	x
C3-phenanthrenes	3,4	9	x	x	x	x	x	x	x	x	x	x	x
Ethyl phenanthrene	3,4		x	x	x	x	x	x	x	x	x	x	x
4H-cyclopenta(def)phenanthrene	3,4											x	
Fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Acephenanthrylene	4		x	x	x	x	x	x	x	x	x	x	x
Pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[e]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
C1-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
C2-fluoranthenes/pyrenes	4											x	
Ethylfluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
o-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
m-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
p-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[c]phenanthrene	4		x	x	x	x	x	x	x	x	x	x	x
Cyclopenta[cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]anthracene	4		x	x	x	x	x	x	x	x	x	x	x
Triphenylene	4		x	x	x	x	x	x	x	x	x	x	x
Chrysene	4		x	x	x	x	x	x	x	x	x	x	x
C1-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x
C2-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x
1,1'-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
1,2'-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
2,2'-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
Phenylphenanthren.-anthracen	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[b,j]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[k]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[e]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Perylene	4		x	x	x	x	x	x	x	x	x	x	x
C1-benzo[e]pyrenes/perylenes	4		x	x	x	x	x	x	x	x	x	x	x
Indeno[1,2,3-cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]perylene	4		x	x	x	x	x	x	x	x	x	x	x
Dibenzanthracene	4		x	x	x	x	x	x	x	x	x	x	x
11H-benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
11H-benzo[b]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]chrysene	4		x	x	x	x	x	x	x	x	x	x	x
Picene	4		x	x	x	x	x	x	x	x	x	x	x
Dibenzo[def,mno]chrysene	4		x	x	x	x	x	x	x	x	x	x	x
Coronene	4,5		x	x	x	x	x	x	x	x	x	x	x

Table S2–5 continued

Sample	Fraction	Isomers	S2 02/05-1	S2 03/05-1	S2 04/05-1	S2 05/05-1	S2 06/05-1	S2 07/05-1	S2 08/05-1	S2 08/05-2	S2 09/05-1	S2 11/05-1	S2 12/05-1
S-PAC													
Dibenzothiophene	3		x	x	x	x	x	x	x	x	x	x	x
C1-dibenzothiophenes	3	3	x	x	x	x	x	x	x	x	x	x	x
C2-dibenzothiophenes	3	6	x	x	x	x	x	x	x	x	x	*	x
Benzo[b]naphtho[2,1-d]thiophene	1 2		x		x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]thiophene	1 2		x		x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]thiophene	1 2		x		x	x	x	x	x	x	x	x	x
C1-benzonaphthothiophenes			x		x	x	x	x	x	x	x	x	x
O-PAC													
Dibenzofuran	2		x		x	x	x	x	x	x	x	x	x
C1-dibenzofuran	2		x		x	x	x	x	x	x	x	x	x
C2-dibenzofuran	2		x		x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,1-d]furan	2		x		x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]furan	2		x		x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]furan	2		x		x	x	x	x	x	x	x	x	x
C1-benzonaphthofuranes	2		x		x	x	x	x	x	x	x	x	x
C2-benzonaphthofuranes	2		x		x	x	x	x	x	x	x	x	x
Aromatic ketones													
9H-fluoren-one	2 3		x	x	x	x	x	x	x	x			x
9,10-anthraquinon	2 3		x	x	x	x	x	x	x	x			x
7H-benzo[de]anthracen-7-one	2 3		x	x	x	x	x	x	x	x			x
11H-benzo[b]fluoren-11-one	2 3		x	x	x	x	x	x	x	x			x
Personal care products													
Galaxolide	5		(x)	x	(x)	x	x	x	x	x	x	x	x
Tonalide	5		(x)	x	(x)	x	x	x	x	x	x	x	x
α-tocopherol	6		x	x	x	x	x	x	x	x	x	x	x
α-tocopherol acetate	6		x	x	x	x	x	x	x	x	x	x	x

Table S2–6 Results of suspect screening in SPM samples from location R1 (Rhine, Weil; sieved <2 mm)

Sample	Fraction	Isomers	R1 02/05-1	R1 03/05-1	R1 04/05-1	R1 05/05-1	R1 06/05-1	R1 07/05-1	R1 08/05-1	R1 09/05-1	R1 10/05-1	R1 11/05-2	R1 12/05-1
Technical / industrial compounds													
Dimethyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Di-iso-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Di-n-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Bis(2-ethylhexyl) phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Tri-iso-butyl phosphate	5												
Tri-n-butyl phosphate	5												
Tris(2-ethylhexyl) phosphate	5												
Tris(chloropropyl) phosphate	5												
Cresyl-diphenyl phosphate	5												
Methyldiphenylmethane	4	3			x							x	
Methylbisdiphenylmethane	4	12	x	x	x			x	(x)		x	x	(x)
Dioctyldiphenylamine	4		x	x	x	(x)	(x)	x			x	x	x
HCB	1		x	x	x	x	x	x	x	x	x	x	x
PCBs	2,3		x	x	x	x	x	x	x			x	x
Linear alkyl benzenes (LAB)													
C10-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x
C11-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x
C12-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x
C13-LABs	1,2		x	x	x	x	x	x	x	x	x	x	x
Polycyclic aromatic compounds (PAC)													
Naphthalene	2,3		x	x	x	x	x	x	x	x	x	x	x
C1-naphthalenes	2,3	4											
C2-naphthalenes	2,3	6											
C3-naphthalenes	2,3	15											
Biphenyl	2,3												
C1-biphenyl	2,3												
Acenaphthylene	2,3		x	x	x	x	x	x	x	x	x	x	x
Acenaphthene	2,3		x	x	x	x	x	x	x	x	x	x	x
Fluorene	2,3		x	x	x	x	x	x	x	x	x	x	x
C1-fluorenes	2,3												
C2-fluorenes	2,3	5											
Benzo[a]fluorene	2,3												
Benzo[b]fluorene	2,3												
1-phenylnaphthalene	2,3												
2-phenylnaphthalene	2,3												
9-vinylanthracene	3,4												

Table S2–6 continued

Sample	Fraction	Isomers	R1 02/05-1	R1 03/05-1	R1 04/05-1	R1 05/05-1	R1 06/05-1	R1 07/05-1	R1 08/05-1	R1 09/05-1	R1 10/05-1	R1 11/05-2	R1 12/05-1
Phenanthrene	3,4		x	x	x	x	x	x	x	x	x	x	x
Anthracene	3,4		x	x	x	x	x	x	x	x	x	x	x
C1-phenanthrenes	3,4	4	x	x	x	x	x	x	x	x	x	x	x
C2-phenanthrenes	3,4	9	x	x	x	x	x	x	x	x	x	x	x
C3-phenanthrenes	3,4	9	x	x	x	x	x	x	x	x	x	x	x
Ethyl phenanthrene	3,4												
4H-cyclopenta[def]phenanthrene	3,4		x	x	x	x	x	x	x	x	x	x	x
Fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Acephenanthrylene	4		x	x	x	x	x	x	x	x	x	x	x
Pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
C1-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
C2-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
Ethylfluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
o-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
m-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
p-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[c]phenanthrene	4		x	x	x	x	x	x	x	x	x	x	x
Cyclopenta[cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]anthracene	4		x	x	x	x	x	x	x	x	x	x	x
Triphenylene	4		x	x	x	x	x	x	x	x	x	x	x
Chrysene	4		x	x	x	x	x	x	x	x	x	x	x
C1-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x
C2-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x
1,1'-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
1,2'-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
2,2'-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
Phenylphenanthren, -anthracen	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[b,j]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[k]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[e]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Perylene	4		x	x	x	x	x	x	x	x	x	x	x
C1-benzo[e]pyrenes/perylenes	4		x	x	x	x	x	x	x	x	x	x	x
Indeno[1,2,3-cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]perylene	4		x	x	x	x	x	x	x	x	x	x	x
Dibenzoxanthracene	4		x	x	x	x	x	x	x	x	x	x	x
11H-benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
11H-benzo[b]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]chrysene	4		x	x	x	x	x	x	x	x	x	x	x
Picene	4		x	x	x	x	x	x	x	x	x	x	x
Dibenzo[def,mno]chrysene	4		x	x	x	x	x	x	x	x	x	x	x
Coronene	4,5		x	x	x	x	x	x	x	x	x	x	x

Table S2–6 continued

Sample	Fraction	Isomers	R1 02/05-1	R1 03/05-1	R1 04/05-1	R1 05/05-1	R1 06/05-1	R1 07/05-1	R1 08/05-1	R1 09/05-1	R1 10/05-1	R1 11/05-2	R1 12/05-1
S-PAC													
Dibenzothiophene	3												
C1-dibenzothiophenes	3	3											
C2-dibenzothiophenes	3	6											
Benzo[b]naphtho[2,1-d]thiophene	1,2		(x)	(x)	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]thiophene	1,2		(x)	(x)	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]thiophene	1,2		(x)	(x)	x	x	x	x	x	x	x	x	x
C1-benzonaphthothiophenes			(x)	(x)	x	x	x	x	x	x	x	x	x
C-PAC													
Dibenzofuran	2		x	x	x	x	x	x	x	x	x	x	x
C1-dibenzofuran	2												
C2-dibenzofuran	2												
Benzo[b]naphtho[2,1-d]furan	2		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]furan	2		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]furan	2		x	x	x	x	x	x	x	x	x	x	x
C1-benzonaphthofuranes	2		x	x	x	x	x	x	x	x	x	x	x
C2-benzonaphthofuranes	2												
Aromatic ketones													
9-H-fluorene-one	2,3		x									x	
9,10-anthraquinon	2,3		x									x	
7-H-benzo[de]anthracene-7-one	2,3		x				x					x	
11H-benzo[b]fluorene-11-one	2,3		x				x					x	
Synthetic musks													
Galaxolide	5												
Tonalide	5												
α-tocopherol	6		x	x	x	x	x	x	x	x		x	x
α-tocopherol acetate	6		x	x	x	x	x	x	x	x		x	x

Table S2–7 Results of suspect screening in SPM samples from location R2 (Rhine, Iffezheim; sieved <2 mm)

Sample	Fraction	Isomers	R2 02/05-1	R2 03/05-1	R2 04/05-1	R2 05/05-1	R2 06/05-1	R2 07/05-1	R2 08/05-1	R2 08/05-2	R2 09/05-1	R2 10/05-1	R2 11/05-1	R2 12/05-1
Technical / industrial compounds														
Dimethyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x	x
Di-iso-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x	x
Di-n-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x	x
Bis(2-ethylhexyl) phthalate	5		x	x	x	x	x	x	x	x	x	x	x	x
Tri-iso-butylphosphate	5													
Tri-n-butyl phosphate	5													
Tris(2-ethylhexyl) phosphate	5													
Tris(chloropropyl) phosphate	5													
Cresyl-diphenyl phosphate	5													
Methyldiphenylmethane	4	3												
Methylbisdiphenylmethane	4	12	x		x	x		x						
Dioctyldiphenylamine	4													
HCB	1		x	x	x	x	x	x	x	x	x	x	x	x
PCBs	2,3		x	x	x	x	x	x	x	x	x	x	x	x
Linear alkyl benzenens (LAB)														
C10-LABs	1,2		x	x	x	x	x	x						
C11-LABs	1,2		x	x	x	x	x	x						
C12-LABs	1,2		x	x	x	x	x	x						
C13-LABs	1,2		x	x	x	x	x	x						
Polycyclic aromatic compounds (PAC)														
Naphthalene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
C1-naphthalenes	2,3	4												
C2-naphthalenes	2,3	6												
C3-naphthalenes	2,3	15												
Biphenyl	2,3													
C1-biphenyl	2,3													
Acenaphthylene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
Acenaphthene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
Fluorene	2,3		x	x	x	x	x	x	x	x	x	x	x	x
C1-fluorenes	2,3													
C2-fluorenes	2,3	5												
Benzo[a]fluorene	2,3													
Benzo[b]fluorene	2,3													
1-phenylnaphthalene	2,3													
2-phenylnaphthalene	2,3													
9-vinylanthracene	3,4													

Table S2–7 continued

Sample	Fraction	Isomers	R2 02/05-1	R2 03/05-1	R2 04/05-1	R2 05/05-1	R2 06/05-1	R2 07/05-1	R2 08/05-1	R2 08/05-2	R2 09/05-1	R2 10/05-1	R2 11/05-1	R2 12/05-1
Phenanthrene	3,4		x	x	x	x	x	x	x	x	x	x	x	x
Anthracene	3,4		x	x	x	x	x	x	x	x	x	x	x	x
C1-phenanthrenes	3,4	4	x	x	x	x	x	x	x	x	x	x	x	x
C2-phenanthrenes	3,4	9	x	x	x	x	x	x	x	x	x	x	x	x
C3-phenanthrenes	3,4	9	x	x	x	x	x	x	x	x	x	x	x	x
Ethyl phenanthrene	3,4													
4H-cyclopenta[def]phenanthrene	3,4		x	x	x	x	x	x	x	x	x	x	x	x
Fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x	x
Acephenanthrylene	4		x	x	x	x	x	x	x	x	x	x	x	x
Pyrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x	x
C1-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x	x
C2-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x	x
Ethylfluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x	x
o-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x	x
m-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x	x
p-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[c]phenanthrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Cyclopenta[cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[a]anthracene	4		x	x	x	x	x	x	x	x	x	x	x	x
Triphenylene	4		x	x	x	x	x	x	x	x	x	x	x	x
Chrysene	4		x	x	x	x	x	x	x	x	x	x	x	x
C1-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x	x
C2-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x	x
1,1´-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x	x
2,2´-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x	x
Phenylphenanthren. -anthracen	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b,j]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[k]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[e]pyrene	4		x	x	x	x	x	x	x	x	x	x	x	x
HBenzo[a]pyrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Perylene	4		x	x	x	x	x	x	x	x	x	x	x	x
C1-benzo[e]pyrenes/perylenes	4		x	x	x	x	x	x	x	x	x	x	x	x
Indeno[1,2,3-cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]perylene	4		x	x	x	x	x	x	x	x	x	x	x	x
Dibenzoanthracene	4		x	x	x	x	x	x	x	x	x	x	x	x
11H-benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x	x
11H-benzo[b]fluorene	4		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]chrysene	4		x	x	x	x	x	x	x	x	x	x	x	x
Picene	4		x	x	x	x	x	x	x	x	x	x	x	x
Dibenzo[def,mno]chrysene	4		x	x	x	x	x	x	x	x	x	x	x	x
Coronene	4,5		x	x	x	x	x	x	x	x	x	x	x	x

Table S2–7 continued

Sample	Fraction	Isomers	R2 02/05-1	R2 03/05-1	R2 04/05-1	R2 05/05-1	R2 06/05-1	R2 07/05-1	R2 08/05-1	R2 08/05-2	R2 09/05-1	R2 10/05-1	R2 11/05-1	R2 12/05-1
S-PAC														
Dibenzothiophene	3						x	x	x	x	x	x		
C1-dibenzothiophenes	3	3					x	x	x	x	x	x		
C2-dibenzothiophenes	3	6					x	x	x	x	x	x		
Benzo[b]naphtho[2,1-d]thiophene	1,2				x	x	x	x	(x)		(x)	(x)	(x)	
Benzo[b]naphtho[1,2-d]thiophene	1,2				x	x	x	x	(x)		(x)	(x)	(x)	
Benzo[b]naphtho[2,3-d]thiophene	1,2				x	x	x	x	(x)		(x)	(x)	(x)	
C1-benzonaphthothiophenes					x	x	x	x	(x)		(x)	(x)	(x)	
O-PAC														
Dibenzofuran	2		x	x	x	x	x	x	(x)	(x)	(x)	(x)	x	x
C1-dibenzofuran	2								(x)	(x)	(x)	(x)		
C2-dibenzofuran	2								(x)	(x)	(x)	(x)		
Benzo[b]naphtho[2,1-d]furan	2		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]furan	2		x	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]furan	2		x	x	x	x	x	x	x	x	x	x	x	x
C1-benzonaphthofuranes	2		x	x	x	x	x	x	x	x	x	x	x	x
C2-benzonaphthofuranes	2								x	x	x	x		
Aromatic ketones														
9H-fluorene-one	2,3		(x)		(x)	(x)	(x)							
9,10-anthraquinon	2,3		(x)		(x)	(x)	(x)							
7H-benzo[de]anthracene-7-one	2,3		(x)		(x)	(x)	(x)							
11H-benzo[b]fluorene-11-one	2,3		(x)		(x)	(x)	(x)							
Personal care products														
Galaxolide	5													
Tonalide	5													
α-tocopherol	6		x		x	x	x					x		
α-tocopherol acetate	6		x		x	x	x					x		

Table S2–8 Results of suspect screening in SPM samples from location R3 (Rhine, Koblenz; sieved <2 mm)

Sample	Fraction	Isomers	R3 02/05-1	R3 03/05-1	R3 04/05-1	R3 05/05-1	R3 07/05-1	R3 08/05-1	R3 08/05-2	R3 09/05-1	R3 11/05-1	R3 11/05-2	R3 12/05-1
Technical / industrial compounds													
Dimethyl phthalate	5												
Di-iso-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Di-n-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Bis(2-ethylhexyl) phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Tri-iso-butylphospnate	5												
Tri-n-butyl phosphate	5												
Tris(2-ethylhexyl) phosphate	5												
Tris(chloropropyl) phosphate	5												
Cresyl-diphenyl phosphate	5												
Methyldiphenylmethane	4										(x)		
Methylbisdiphenylmethane	4		x		x	x							
Dioctyldiphenylamine	4												
HCB	1		x	x	x	x	x	x	x	x	x	x	x
PCBs	2,3												
Linear alkyl benzenens (LAB)													
C10-LABs	1,2		x	x	x	x							x
C11-LABs	1,2		x	x	x	x							x
C12-LABs	1,2		x	x	x	x							x
C13-LABs	1,2		x	x	x	x							x
Polycyclic aromatic compounds (PAC)													
Naphthalene	2,3		x	x	x	x	x	x	x	x	x	x	x
C1-naphthalenes	2,3	4	x	x	x	x	x	x	x	x	x	x	x
C2-naphthalenes	2,3	6											
C3-naphthalenes	2,3	15											
Biphenyl	2,3												
C1-biphenyl	2,3												
Acenaphthylene	2,3		x	x	x	x	x	x	x	x	x	x	x
Acenaphthene	2,3		x	x	x	x	x	x	x	x	x	x	x
Fluorene	2,3		x	x	x	x	x	x	x	x	x	x	x
C1-fluorenes	2,3												
C2-fluorenes	2,3	5											
Benzo[a]fluorene	2,3												
Benzo[b]fluorene	2,3												
1-phenylnaphthalene	2,3												
2-phenylnaphthalene	2,3												
9-vinylanthracene	3,4												

Table S2–8 continued

Sample	Fraction	Isomers	R3 02/05-1	R3 03/05-1	R3 04/05-1	R3 05/05-1	R3 07/05-1	R3 08/05-1	R3 08/05-2	R3 09/05-1	R3 11/05-1	R3 11/05-2	R3 12/05-1
Phenanthrene	3.4		x	x	x	x	x	x	x	x	x	x	x
Anthracene	3.4		x	x	x	x	x	x	x	x	x	x	x
C1-phenanthrenes	3.4	4	x	x	x	x	x	x	x	x	x	x	x
C2-phenanthrenes	3.4	9	x	x	x	x	x	x	x	x	x	x	x
C3-phenanthrenes	3.4	9	x	x	x	x	x	x	x	x	x	x	x
Ethyl phenanthrene	3.4												
4H-cyclopenta(def)phenanthrene	3.4		x	x	x	x	x	x	x	x	x	x	x
Fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Acephenanthrylene	4												
Pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
C1-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
C2-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
Ethylfluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
o-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
m-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
p-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]fluoranthene	4												
Benzo[c]phenanthrene	4		x	x	x	x	x	x	x	x	x	x	x
Cyclopenta[cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]anthracene	4		x	x	x	x	x	x	x	x	x	x	x
Triphenylene	4		x	x	x	x	x	x	x	x	x	x	x
Chrysene	4		x	x	x	x	x	x	x	x	x	x	x
C1-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x
C2-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x
1,1´-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
1,2´-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
2,2´-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
Phenylphenanthren. -anthracen	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[b,j]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[k]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[e]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Perylene	4		x	x	x	x	x	x	x	x	x	x	x
C1-benzo[e]pyrenes/perylenes	4		x	x	x	x	x	x	x	x	x	x	x
Indeno[1,2,3-cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]perylene	4		x	x	x	x	x	x	x	x	x	x	x
Dibenzoanthracene	4		x	x	x	x	x	x	x	x	x	x	x
11H-benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
11H-benzo[b]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]chrysene	4		x	x	x	x	x	x	x	x	x	x	x
Picene	4												
Dibenzo[def,mno]chrysene	4		x	x	x	x	x	x	x	x	x	x	x
Coronene	4,5		x	x	x	x	x	x	x	x	x	x	x

Table S2–8 continued

Sample	Fraction	Isomers	R3 02/05-1	R3 03/05-1	R3 04/05-1	R3 05/05-1	R3 07/05-1	R3 08/05-1	R3 08/05-2	R3 09/05-1	R3 11/05-1	R3 11/05-2	R3 12/05-1
S-PAC													
Dibenzothiophene	3		x	x	x	x	x	x	x	x	x	x	x
C1-dibenzothiophenes	3	3	x	x	x	x	x	x	x	x	x	x	x
C2-dibenzothiophenes	3	6	x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,1-d]thiophene	1,2		x	x	x	x	x	x	(x)	x	x	x	x
Benzo[b]naphtho[1,2-d]thiophene	1,2		x	x	x	x	x	x	(x)	x	x	x	x
Benzo[b]naphtho[2,3-d]thiophene	1,2		x	x	x	x	x	x	(x)	x	x	x	x
C1-benzonaphthothiophenes			x	x	x	x	x	x	(x)	x	x	x	x
O-PAC													
Dibenzofuran	2						(x)	x	(x)	(x)	(x)	x	(x)
C1-dibenzofuran	2						(x)	x	(x)	(x)	(x)	x	(x)
C2-dibenzofuran	2						(x)	x	(x)	(x)	(x)	x	(x)
Benzo[b]naphtho[2,1-d]furan	2		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]furan	2		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]furan	2		x	x	x	x	x	x	x	x	x	x	x
C1-benzonaphthofurenes	2		x	x	x	x	x	x	x	x	x	x	x
C2-benzonaphthofurenes	2		x	x	x	x	x	x	(x)	x	x	x	x
Aromatic ketones													
9H-fluorene-one	2,3				x				x			x	
9,10-anthraquinon	2,3				x				x			x	
7H-benzo[de]anthracene-7-one	2,3				x				x			x	
11H-benzo[b]fluorene-11-one	2,3				x				x			x	
Personal care products													
Galaxolide	5												
Tonalide	5												
α-tocopherol	6				x				x			x	
α-tocopherol acetate	6				x				x			x	

Table S2–9 Results of suspect screening in SPM samples from location R4 (Rhine, Bimmen; sieved <2 mm)

Sample	Fraction	Isomers	R4 02/05-1	R4 03/05-1	R4 04/05-1	R4 05/05-1	R4 06/05-1	R4 07/05-1	R4 08/05-1	R4 08/05-2	R4 09/05-1	R4 11/05-2	R4 12/05-1
Technical / industrial compounds													
Dimethyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Di-**iso**-butyl phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Di-n-butyl **phthalate**	5		x	x	x	x	x	x	x	x	x	x	x
Bis(2-ethylhexyl) phthalate	5		x	x	x	x	x	x	x	x	x	x	x
Tri-**iso**-**butyl**phosphate	5												
Tri-n-**butyl phosphate**	5												
Tris(2-ethylhexyl) phosphate	5			(x)	x	x	x			x	x		
Tris(chloropropyl) phosonate	5			(x)	x	x	x			x	x		
Cresyl-**diphenyl** phosphate	5												
Methyldiphenylmethane	4	3											
Methylbisdiphenylmethane	4	12	(x)	x	x	x	(x)				(x)		
Dioctyld phenylamine	4		(x)	x	x		(x)	(x)					
HCB	1												
PCBs	2,3												
Linear alkyl benzenes (LAB)													
C10-LABs	1,2		x	x	x	x	x	x	x	x	x		x
C11-LABs	1,2		x	x	x	x	x	x	x	x	x		x
C12-LABs	1,2		x	x	x	x	x	x	x	x	x		x
C13-LABs	1,2		x	x	x	x	x	x	x	x	x		x
Polycyclic aromatic compounds (PAC)													
Naphthalene	2,3		x	x	x	x	x	x	x	x	x	x	x
C1-naphthalenes	2,3	4											
C2-naphthalenes	2,3	6											
C3-naphthalenes	2,3	15											
Biphenyl	2,3												
C1-biphenyl	2,3												
Acenaphthylene	2,3		x	x	x	x	x	x	x	x	x	x	x
Acenaphthene	2,3		x	x	x	x	x	x	x	x	x	x	x
Fluorene	2,3		x	x	x	x	x	x	x	x	x	x	x
C1-fluorenes	2,3												
C2-fluorenes	2,3	5											
Benzo[a]fluorene	2,3												
Benzo[b]fluorene	2,3												
1-phenylnaphthalene	2,3												
2-phenylnaphthalene	2,3												
9-vinylanthracene	3,4												

Table S2–9 continued

Sample	Fraction	Isomers	R4 02/05-1	R4 03/05-1	R4 04/05-1	R4 05/05-1	R4 06/05-1	R4 07/05-1	R4 08/05-1	R4 08/05-2	R4 09/05-1	R4 11/05-2	R4 12/05-1
Phenanthrene	3,4		x	x	x	x	x	x	x	x	x	x	x
Anthracene	3,4		x	x	x	x	x	x	x	x	x	x	x
C1-phenanthrenes	3,4	4	x	x	x	x	x	x	x	x	x	x	x
C2-phenanthrenes	3,4	9	x	x	x	x	x	x	x	x	x	x	x
C3-phenanthrenes	3,4	9	x	x	x	x	x	x	x	x	x	x	x
Ethyl phenanthrene	3,4												
4H-cyclopenta[def]phenanthrene	3,4		x	x	x	x	x	x	x	x	x	x	x
Fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Acephenanthrylene	4		x	x	x	x	x	x	x	x	x	x	x
Pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
C1-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
C2-fluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
Ethylfluoranthenes/pyrenes	4		x	x	x	x	x	x	x	x	x	x	x
o-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
m-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
p-terphenyl	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]fluoranthene	4												
Benzo[c]phenanthrene	4		x	x	x	x	x	x	x	x	x	x	x
Cyclopenta[cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]anthracene	4		x	x	x	x	x	x	x	x	x	x	x
Triphenylene	4		x	x	x	x	x	x	x	x	x	x	x
Chrysene	4		x	x	x	x	x	x	x	x	x	x	x
C1-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x
C2-benzo[a]anthracenes/chrysenes	4		x	x	x	x	x	x	x	x	x	x	x
1,1´-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
1,2´-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
2,2´-binaphthyl	4		x	x	x	x	x	x	x	x	x	x	x
Phenylphenanthren, -anthracen	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[b,j]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[k]fluoranthene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[e]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[a]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Perylene	4		x	x	x	x	x	x	x	x	x	x	x
C1-benzo[e]pyrenes/perylenes	4		x	x	x	x	x	x	x	x	x	x	x
Indeno[1,2,3-cd]pyrene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[ghi]perylene	4		x	x	x	x	x	x	x	x	x	x	x
Dibenzoanthracene	4		x	x	x	x	x	x	x	x	x	x	x
11H-benzo[a]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
11H-benzo[b]fluorene	4		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]chrysene	4		x	x	x	x	x	x	x	x	x	x	x
Picene	4												
Dibenzo[def,mno]chrysene	4		x	x	x	x	x	x	x	x	x	x	x
Coronene	4,5		x	x	x	x	x	x	x	x	x	x	x

Table S2–9 continued

Sample	Fraction	Isomers	R4 02/05-1	R4 03/05-1	R4 04/05-1	R4 05/05-1	R4 06/05-1	R4 07/05-1	R4 08/05-1	R4 08/05-2	R4 09/05-1	R4 11/05-2	R4 12/05-1
S-PAC													
Dibenzothiophene	3			x	x	x	x	x	x	x	x	x	x
C1-dibenzothiophenes	3	3		x	x	x	x	x	x	x	x	x	x
C2-dibenzothiophenes	3	6		x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,1-d]thiophene	1,2		x	x	x		x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]thiophene	1,2		x	x	x		x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]thiophene	1,2		x	x	x		x	x	x	x	x	x	x
C1-benzonaphthothiophenes			x	x	x		x	x	x	x	x	x	x
O-PAC													
Dibenzofuran	2		x	(x)	(x)	(x)	x	x	x	x	x	x	x
C1-dibenzofuran	2		x	(x)	(x)	(x)	x	x	x	x	x	x	x
C2-dibenzofuran	2		x	(x)	(x)	(x)	x	x	x	x	x	x	x
Benzo[b]naphtho[2,1-d]furan	2		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[1,2-d]furan	2		x	x	x	x	x	x	x	x	x	x	x
Benzo[b]naphtho[2,3-d]furan	2		x	x	x	x	x	x	x	x	x	x	x
C1-benzonaphthofurans	2		x	x	x	x	x	x	x	x	x	x	x
C2-benzonaphthofurans	2		x	x	x	x	x	x	x	x	x	x	x
Aromatic ketones													
9H-fluorene-one	2,3			x	x	(x)	x			x	x	x	x
9,10-anthraquinon	2,3			x	x	(x)	x			x	x	x	x
7H-benzo[de]anthracene-7-one	2,3		(x)	x	x	x	x		x	x	x	x	x
11H-benzo[b]fluorene-11-one	2,3		(x)	x	x	x	x		x	x	x	x	x
Personal care products													
Galaxolide	5								x				
Tonalide	5								x				
α-tocopherol	6		x	x	x	x	x		x	x	x	x	x
α-tocopherol acetate	6		x	x	x	x	x		x	x	x	x	x

Script S2–1 Matlab script for running the SOM Toolbox 2.0

```
% Script file: Create_SOM.m

% Requires: SOM Toolbox 2.0 (http://www.cis.hut.fi/somtoolbox/download/)
% The program is licensed under GNU General public license V 3.0
% see: http://www.gnu.org/licenses/gpl.html

% Purpose:
% Program to create a self organizing map (SOM) or Kohonen map with a hit
% histogram for the given input data using the SOM Toolbox 2.0
% The program reads the given input data and creates a U-Matrix. The
% dimension is set automatically from input data array. The U-matrix is
% fitted with a hit histogram. The principal component plot is also
% printed. The SOM is classified using k-means with automatic estimation of
% optimal cluster numbers using
%
% Created:          05.05.2012 T Schulze
% Last Revision:    05.05.2012 T Schulze
%
% This program includes code from SOM Toolbox 2.0

% The input format of the files is (Tab delimited file):
% 5                 %Number of parameter
% #n V1 V2 V3 V4 V5     %Parameters
% 1 2 3 4 5 Name        %Values, last=row name

% Clear variables and screen
clearvars
clc
% Define input variables:

% File name or path to file name (String)
FileName = uigetfile('*.data','Please select a data file');
FileName

% Place holder for missing data (String)
sMissing = 'NaN'
```

```
% Method for normalization ('var', 'range', 'log', 'logistic', 'histD' or
% 'histC') (String)
sNormal = 'logistic'

% U-matrix Plot, defines the data level of the colorbars (n: uses
% normalized data, d: uses denormalized data) (String)
sColorbar = 'n'


% read data file and normalize the data
sD=som_read_data(FileName,sMissing)
sD=som_normalize(sD,sNormal)

% make the SOM with a default hexagonal grid size and Gaussian neighborhood
% function, unattended calculation of the map size
sM=som_make(sD,'hexa','sheet','gaussian','long','name','')

% reads map size and stores it in a varible to use as map delimiter

munits = prod(sM.topol.msize)
munits
% checks if map size is same or below as dimension of input array, else
% dimension of input array is used as map delimiter
if munits > sD.dim
   munits = sD.dim
end

% add labels to SOM data set
sM=som_autolabel(sM,sD,'add1')

% plot the umatrix with all parameters as subplots, create an empty plane
% for the Labels plane;

som_show(sM,'umat','all','comp',1:munits,'empty','Labels','norm', sColorbar)

% plot the labels plane
som_show_add('label',sM,'TextSize',6,'subplot',munits+2)
```

```
% create and plot a marker for the first node
h=zeros(sM.topol.msize); h(1,1) = 1
som_show_add('hit',h(:),'markercolor','r','markersize',0.3,'subplot','all')

% create and plot a hit histogram in U-matrix
h = som_hits(sM,sD)
som_show_add('hit',h,'MarkerColor','magenta','Subplot',1)

% Principal component projection
%    Next, the projection of the data set is investigated. A
%    principle component projection is made for the data, and applied
%    to the map. The colormap is done by spreading a colormap on the
%    projection. Distance matrix information is extracted from the
%    U-matrix, and it is modified by knowledge of zero-hits
%    (interpolative) units. Finally, three visualizations are shown:
%    the color code, with clustering information and the number of
%    hits in each unit, the projection and the labels.

f2=figure;
[Pd,V,me,l] = pcaproj(sD,2); Pm = pcaproj(sM,V,me); % PC-projection
Code = som_colorcode(Pm); % color coding
hits = som_hits(sM,sD);  % hits
U = som_umat(sM); % U-matrix
Dm = U(1:2:size(U,1),1:2:size(U,2)); % distance matrix
Dm = 1-Dm(:)/max(Dm(:)); Dm(find(hits==0)) = 0; % clustering info

subplot(1,3,1)
som_cplane(sM,Code,Dm);
hold on
som_grid(sM,'Label',cellstr(int2str(hits)),...
      'Line','none','Marker','none','Labelcolor','black');
hold off
title('Number of hits')

subplot(1,3,2)
som_grid(sM,'Coord',Pm,'MarkerColor',Code,'Linecolor','k');
hold on, plot(Pd(:,1),Pd(:,2),'k+'), hold off, axis tight, axis equal
```

```
title('PC projection')

subplot(1,3,3)
som_cplane(sM,'none')
hold on
som_grid(sM,'Label',sM.labels,'Labelsize',8,...
        'Line','none','Marker','none','Labelcolor','black');
hold off
title('Labels and clusters')

%    Here, the KMEANS_CLUSTERS function is used to find an initial
%    partitioning. The plot shows the Davies-Boulding clustering
%    index, which is minimized with best clustering.
f3=figure;
subplot(1,3,1)
[c,p,err,ind] = kmeans_clusters(sM,munits); % find the optimal number of clusters

plot(1:length(ind),ind,'x-')
xlabel('Number of clusters')
ylabel('DBI')
title('DBI plot')
[dummy,i] = min(ind)
cl = p{i};

% Here is the clustering info
% calculated previously and the partitioning result:

subplot(1,3,2)
Code = som_colorcode(Pm); % color coding
som_cplane(sM,Code,Dm)
hold on
som_grid(sM,'Label',cellstr(int2str(hits)),...
        'Line','none','Marker','none','Labelcolor','k');
hold off
title('Number of hits')

subplot(1,3,3)
```

```
som_cplane(sM,cl)
hold on
som_grid(sM,'Label',sM.labels,'Labelsize',8,...
        'Line','none','Marker','none','Labelcolor','r');
hold off
title('Labels and clusters')
```

Section 3 Comparison of different exhaustive and one biomimetic extraction techniques for chemical and biological analysis of polycyclic aromatic compounds in river sediments

Table S3–1 Bio-TEQ and Chem-TEQ values of primary extracts obtained with different extraction methods and the respective Bio-TEQ values of the artificial mixtures in pg/g

pg/g	**S1**			**S2**		
Extraction method	$Bio\text{-}TEQ_{extract}$	$Bio\text{-}TEQ_{artifical}$	Chem-TEQ	$Bio\text{-}TEQ_{extract}$	$Bio\text{-}TEQ_{artifical}$	Chem-TEQ
HBCD	213 ± 6	829 ± 76	27	165 ± 24	1250 ± 66	33
USE	4788 ± 160	5569 ± 70	352	11964 ± 474	8161 ± 1784	282
SOX	17059 ± 2916	6816 ± 783	498	17894 ± 5750	6667	412
MDE	7560 ± 871	7312	467	9591 ± 1238	10727 ± 1456	498

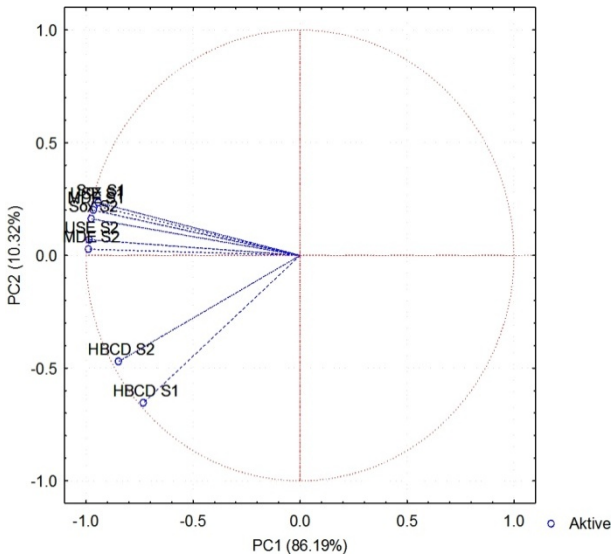

Figure S3-1 Results of principal components analysis (PCA) using the factors extraction method, sediment and results of chemicals analysis of PAHs; PC=principal component

Section 5 Risk assessment of river suspended particulate matter and floodplain soils in the Rhine catchment using chemical analysis and in vitro bioassays

Table S5-1 Recoveries of aqua regia extractable elements in certified reference soil BCR CRM 143 R (mg/kg dw.); the Cu values are not certified and thus indicative

Element	Measured value (n=2)	Certified value	Recovery (%)
Cd	77.4 ± 3.3	72.0 ± 1.7	107.5
Cr	496.2 ± 31.1	426.5 ± 13.4	116.3
Cu	146.9 ± 2.0	(128.0 ± 7.0)	114.7
Ni	317.5 ± 0.9	296.0 ± 3.8	107.3
Pb	188.5 ± 1.2	174.2 ± 5.2	108.2
Zn	1051.8 ± 6.5	1062.9 ± 20.5	99.0

Table S5–2 Sample codes of soil samples from non inundated area, the content of TOC, TIC and trace metals as well as the grain size distribution (type of soil according Arbeitsgemeinschaft Boden 1996) (gray highlighted: core sample)

Sample code	B1	B2	B3	B4	B5	B6	B7
As (mg/kg)	12.4 ± 0.3	5.1 ± 0.2	13 ± 0.9	13.3 ± 0.4	13.5 ± 0.3	13.3 ± 0.1	12.3 ± 0.02
Cd (mg/kg)	0.7 ± 0.2	0.2 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.8 ± 0.01	0.7 ± 0.01	0.7 ± 0.02
Cr (mg/kg)	29.1 ± 1.2	19.2 ± 1.1	31.2 ± 0	31.9 ± 0.8	35.2 ± 0.8	34.8 ± 0.4	27.8 ± 1.2
Cu (mg/kg)	16.7 ± 0.5	3.8 ± 0.6	18.1 ± 0.1	20 ± 0.3	17.6 ± 0.3	16.4 ± 0.1	16.7 ± 0.04
Hg (mg/kg)	0.3 ± 0.01	0.7 ± 0.2	0.5	0.4 ± 0.1	0.4 ± 0.01	0.5 ± 0.3	0.5
Ni (mg/kg)	27.1 ± 1.1	15.5 ± 0.3	31.5 ± 0.9	35.0 ± 3.0	27.9 ± 3.0	37.7 ± 8.6	29.1 ± 6.6
Pb (mg/kg)	29.4 ± 0.3	5.8 ± 0.9	32.6 ± 0.6	32.1 ± 0.3	28.5 ± 0.3	27.6 ± 0.5	28.1 ± 0.3
Zn (mg/kg)	72.9 ± 0.9	16.3 ± 1.3	122.9 ± 0.6	121.2 ± 1.3	71.8 ± 1.3	66.9 ± 0.4	94 ± 0.6

Table S5–3 Sample codes of soil samples from inundated area, the content of TOC, TIC and trace metals as well as the grain size distribution (type of soil according Arbeitsgemeinschaft Boden 1996) (gray highlighted: core sample)

Sample code	BT1	BT2	BT3	BT4	BT5	BT6	BT7
As (mg/kg)	9.7	11.3	10.7	10.0	14.0	9.7	7.7
Cd (mg/kg)	0.6	0.5	0.5	0.4	0.7	0.6	0.5
Cr (mg/kg)	53.3	50.0	50.0	43.4	49.9	53.3	53.3
Cu (mg/kg)	51.3	49.4	50.0	43.4	53.9	54.7	50.0
Hg (mg/kg)	0.8	0.4	0.4	0.3	0.4	0.4	0.7
Ni (mg/kg)	40.0	33.4	35.0	30.0	39.9	38.3	31.6
Pb (mg/kg)	45.0	38.4	36.7	25.0	41.6	41.7	46.6
Zn (mg/kg)	153.2	150.1	143.3	126.8	159.7	153.3	116.6

Table S5–4 Sample codes and sampling periods of the SPM samples collected at Iffezheim barrage as well as the content of TOC, TIC and trace metals

Sample code	S1	S2	S3	S4	S5	S6
Start date	01.02.2001	14.02.2001	06.03.2001	07.03.2001	12.03.2001	13.03.2001
End date	14.02.2001	06.03.2001	07.03.2001	13.03.2001	13.03.2001	(1–5 p.m.)
As (mg/kg)	18.6 ± 0.1	12.54 ± 1.0	13.0 ± 0.9	12.1 ± 0.8	9.6 ± 0.1	9.1 ± 0.8
Cd (mg/kg)	1.0 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.5 ± 0.1
Cr (mg/kg)	50.5 ± 4.6	43.9 ± 0.3	31.9 ± 0.3	31.3 ± 0.2	26.1 ± 0.4	25.7 ± 0.7
Cu (mg/kg)	40.3 ± 0.9	30.8 ± 4.0	26.5 ± 0.5	22.7 ± 0.6	19.1 ±0.9	26.7 ± 12.0
Hg (mg/kg)	0.8 ± 0.2	0.6 ± 0.3	0.5	0.5 ± 0.3	0.7 ± 0.1	0.5 ± 0.1
Ni (mg/kg)	37.6 ± 3.1	30.7 ± 0.2	29.4 ± 0.1	29.1 ± 1.0	30.3 ± 6.1	29.2 ± 3.5
Pb (mg/kg)	31.7 ± 2.3	26.2 ± 1.3	23.9 ± 3.8	20.5 ± 0.7	17.0 ± 0.6	15.0 ± 0.3
Zn (mg/kg)	137.6 ± 1.3	107.3 ± 2.6	102.4 ± 1.9	94.0 ± 0.1	78.8 ± 1.2	74.1 ± 3.3

Table S5–5 Results of analysis of polychlorinated dibenzofuranes (PCDF) and dibenzodioxines (PCDD) in selected samples in inundated (BT) and infrequently inundated (B) top soil layers as well as in SPM samples (S) (gray highlighted: core samples); data given in pg/g dry weight (dw) and Chem-TEQ (in pg/g) according to Clemons et al. (1997)

Congener	**B1**		**B2**	**BT1**		**BT7**		**S2**	
	dw (pg/g)	TEQ (pg/g)	dw (pg/g)	dw (pg/g)	TEQ (pg/g)	dw (pg/g)	TEQ (pg/g)	dw (pg/g)	TEQ (pg/g)
2,3,7,8-TCDF	5.8	1.1	<0.2	4.9	0.9	5.7	1.1	6.5	1.2
1,,2,3,7,8-PeCDF	2.1	0.4	<0.1	1.3	0.3	2.7	0.5	5	1
2,3,4,7,8-PeCDF	3.5	6.7	<0.1	4.1	7.8	4.6	8.7	<1.3	
1,,2,3,4,7,8-HxCDF	3.4	3.7	<0.1	7.6	8.4	6.1	6.7	5.4	5.9
1,,2,3,6,7,8-HxCDF	1.8		<0.1	3.2		1.9		2.2	
1,,2,3,7,9,9-HxCDF	<0.4		<0.1	<0.6		<0.6		<0.6	
2,3,4,6,7,8-HxCDF	2		<0.1	2.2		2.3		3.3	
1,2,3,4,6,7,8-HpPCDF	16		<0.8	27.8		32		25.9	
1,2,3,4,7,9,9-HpPCDF	<1.8		<0.8	<3.8		<5.6		<4.2	
OCDF	17.3		<29.7	211		315		684	
2,3,7,8-TCDD	<0.4		<0.1	<0.8		<2.8		<2.1	
1,2,3,7,8-PeCDD	<0.3		<0.1	<2.2		0.6	1.56	2.2	5.7
1,2,3,4,7,8-HxCDD	<0.4		<0.1	<0.2		<0.6		<1.1	
1,2,3,6,7,8-HxCDD	0.4	0.1	<0.2	<0.4		4.2	0.84	<1.3	
1,2,3,7,9,9-HxCDD	0.5		<0.2	<0.3		2.7		<1.2	
1,2,3,4,6,7,8-HpCDD	13.2	2.6	<0.8	74.7	14.9	108	21.6	59.1	11.8
OCDD	52.3		<4.0	1680		1690		2510	
∑PCDD/F	118.3	14.63	n.n.	2016.8	32.3	2175.8	41.1	3303.5	25.7

Table S5–6 EC_{50}-values or LOEC-values of all acetone extracted samples (mg soil per ml test solution). n.b. = not quantifiable, n.a. = not tested; limits 95% confidence limits are shown in brackets; the results from the drilling samples are highlighted in gray

Sample	Landside soils (B)		Floodplain soils (BT)		Suspended particulate matter (SPM)	
Number	Bacteria	Fish eggs	Bacteria	Fish eggs	Bacteria	Fish eggs
1	271.5 [201.2;282.3][a]	114 [101.6;127.8]	70.45 [-]	7.2 [5.4;9.5]	85.7 [82.2;89.1]	17.7[c]
2	100 [LOEC][a]	128.7 [116.4;142.3]	87.4 [78.9 – 96.8]	21.4 [12.8;35.8]	241.8 [200.0;292.8]	16.0 [15.9;16.1]
3	25 [LOEC]	128.7 [111.9;148]	25 [LOEC]	50 [-]	25.7 [;31.1]	16.0 [15.9;16.1]
4	203.2 [154.4;267.3][a]	90.1 [88.8;91.5]	132.1 [97.5 – 152.9]	n.a.	14.8 [;291.2]	19.1 [18.9;19.4]
5	33.1 [;156.3][a]	76.3 [70.4;82.7]	n.a.	n.a.	72.5 [60.9;88.8]	21.4 [17.5;26.0]
6	200 [LOEC][a,b]	106.6 [58.2;155.0]	n.a.	n.a.	n.a.	[d]
7	85.8 [37.3;99.5][a]	110.4 [91.9;132.5]	16.3 [-273.9]	n.a.	-	-

[a] positive dehydrogenase activity

[b] high negative effects (positive dehydrogenase activitiy)

[c] perfect fit no confidence intervall calculated

[d] interupted concentration-response relationship

Table S5–7 EC_{50}-values or LOEC-values of all native samples (mg soil per ml test solution). n.b. = not quantifiable, n.a. = not tested; limits 95% confidence limits are shown in brackets; the results from the drilling samples are highlighted in gray

Sample	Landside soils (B)		Floodplain soils (BT)		Suspended particulate matter (SPM)	
Number	Bacteria	Fish eggs	Bacteria	Fish eggs	Bacteria	Fish eggs
1	311.7 [261.9;358.7]	32.0 [30.1;34.0]	25 [LOEC]	n.q.	377.6 [228.9;424.5]	344 [278;425.6]
2	1235 [913.9;1726]	not toxic	25 [LOEC]	43.4 [-]	226.5 [137.1;250]	n.q.
3	128.6 [;133.8]	45.8 [40.2;52.2]	41.7 [17.2;47.5]		125 [LOEC][a]	98.5 [91.6;106.0]
4	250 [LOEC]	20.7 [15.1;24.5]	81.8 [71.7;93.3]	66.1 [52.7;102.3]	321.2 [289.3;356.5][a]	112.8 [100.5;184]
5	238.7 [226.8;251.3]	28.9 [25.5;32.4]	n.a.	n.a.	552.9 [486;629][a]	n.q.
6	25 [LOEC]	75.7 [66.1;86.7]	n.a.	n.a.	n.a.	n.a.
7	118.6 [;127.4]	61.9 [56.3;66.2]	99.0 [83.9;118.1]	n.q.	-	-

[a] positive dehydrogenase activity

[b] high negative effects (positive dehydrogenase activitiy)

[c] perfect fit no confidence intervall calculated

[d] interupted concentration-response relationship

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Declaration of originality

I hereby certify, as the author of this thesis, and as one of the main authors of the publications arising, that I was the person involved in fieldwork, organization, implementation, analysis, evaluation, and manuscript preparation.

I declare that this thesis and the work reported herein is to the best of my knowledge original, except as acknowledged in the text, and that the work was not submitted previously to any other institution. Any technical help by third persons is declared in the preface and introduction.

Berlin, 02.01.2013

Tobias Schulze