Improving exposure assessment of complex hydrocarbon mixtures in the aquatic environment

Een bijdrage aan het verbeteren van de blootstellingskarakterisering van complexe koolwaterstofmengsels in het aquatische milieu.
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 6 december 2011 des middags te 12.45 uur

door

Barry Muijs

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Promotoren:	Prof.dr. W. Seinen
	Prof.dr. M. van den Berg
Co-promotor:	Dr.ir. M. T. O. Jonker

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Het leven is te kort om ideeën in de ijskast te stoppen, dus werd het tijd
dat ik mijn plan ging verwezenlijken

Sue en Victoria Riches
Poolvrouwen
Kosmos -Z&K Uitgevers BV, Utrecht, 2002

On the cover:

Flooded water meadows of the River IJssel near Deventer, The Netherlands, November 2002. Picture taken by the author.

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Chapter 1

Introduction

Complex organic mixtures in the environment

Our extensive use of professional and consumer products has brought vast amounts of hydrophobic organic compounds (HOCs) into the aquatic environment, resulting in concentrations far above acceptable levels. The majority of these chemicals is persistent, bioaccumulative, and toxic, and therefore has posed a threat to organisms for a long time (*1*). Considering that these HOCs are released from various sources or emitted as mixtures, organisms are exposed to numerous HOCs simultaneously. Polycyclic aromatic hydrocarbons (PAHs) and petroleum hydrocarbons (oil) are examples of such mixtures and are ubiquitous in the aquatic environment. PAHs are organic compounds that consist of two or more fused benzene rings. Including their alkylated homologues, hundreds of PAH congeners are possible. Most PAHs originate from the incomplete combustion of organic material (*2*), although some may be synthesised by microbiological activity as well. Forest fires are typical natural sources, but PAHs largely find their origin in coal and oil (*3*). The majority of PAHs in the environment is therefore anthropogenic and emitted to the atmosphere by traffic and industries. Because PAHs are able to be transported over long distances once airborne (*4*), these compounds are even found in remote Arctic and Antarctic areas where nearby sources are absent (*5*). Atmospheric deposition of airborne PAHs is seen as the major source of PAHs in soils. Due to the transportation of polluted soil particles by water, wind, and cattle, PAHs eventually settle into sediments, because ditches, rivers, and canals are the lowest parts in the landscape. Run-off water from adjacent

roads (*6*), oil-spills, and discharges from ships are important sources as well. Because PAHs, including their metabolites, are biologically active, toxic (*7,8*), and even mutagenic and carcinogenic; they are contaminants of concern in many countries.

Unlike PAHs that are individual recognizable and detectable compounds, petroleum hydrocarbons are complex mixtures that consist of hundreds to thousands of different hydrophobic compounds, including straight-chain, branched, and cycloalkanes, monoaromatics and polyaromatics, and aromatic compounds that contain sulphur, nitrogen, and oxygen in their ring structure (*9*). Oil has a biogenic origin and its formulation starts when dead aquatic micro-organisms (phytoplankton and zooplankton) are deposited to the ocean floor and buried under new layers of sediment. Due to elevated temperature and pressure, the organic-rich material is converted into a brown-black viscous liquid. Subsequently, functional groups that are typical for biogenic organic compounds, such as alcohols, acids, and esters disappear, resulting in a complex hydrophobic mixture of hydrocarbons known as oil. Eventually, the liquid is converted into methane or graphite. However, the majority of the liquids and gasses is transported to the earth's surface by natural forces, but are often trapped in reservoirs underneath dense rock formations deep in the earth's crust (*10,11*). Only where such dense rock formations are absent oil will finally reach the earth's surface.

Oil and oil products have been used by mankind since 6000-7000 BC (*12*), although some have reported that bitumen was already used by the Middle Palaeolithic people in 40.000 BC (*13*). Tar and asphalts from nearby wells where oil reached the earth's surface were used to reinforce the walls and towers of the ancient city of Babylon, as a water-proofing agent, in jewels, and for mummification. The use of petroleum products (bitumen) was also reported for the construction of Noah's arc (*12*). The further consumption of oil during those days was limited to lighting and medicinal applications, and only small quantities were used; however, consumption changed radically after the introduction of the distillation process in 1849 and the invention of the combustion engine in 1876 (*14*). Today, oil is actively being pumped out of reservoirs and distilled into various products, each having its own physical-chemical properties and usage (table 1). A modern world without oil is nearly unimaginable due to its numerous applications in transportation, energy supply, and plastics, among others.

Our almost unlimited use of oil, however, has its downside. The amount of oil in reservoirs is limited and easily-exploitable oil seems soon to be finished. Some believe that we already combusted more than 50% of the world's total oil capacity (*15,16*). Although not all reservoirs have been discovered yet, it seems that the remaining reserves are hard to access because remaining oil fields are distant or hardly exploitable as the locations are covered with ice or snow for the major part of the year. Oil will thus become more expensive in the near future; yet our consumption is still growing (*17*). Furthermore, the combustion of fossil fuels results in increasing concentrations of carbon dioxide in the earth's atmosphere (*18*), for which the climactic and environmental consequences are still unknown. The recent oil disaster in the Gulf of Mexico has shown the consequences of emission of vast amounts of oil to the aquatic environment. Unfortunately, this accident was not unique, but merely the

Table 1. Major oil fractions produced in a refinery and their physical-chemical properties and applications (after reference (*10*)).

name	boiling point (°C)	boiling point range[1]	main application
petrol	0-120	C_5-C_{10}	car fuel
paraffin	121-190	C_{11}-C_{13}	lamp oil, jet fuel
diesel fuel	191-260	C_{14}-C_{18}	(heavy) car fuel,
heavy gas oil	261-360	C_{19}-C_{25}	shipping fuel
lubricating oil	361-530	C_{26}-C_{40}	lubricating
residuum	>530	>C_{40}	road constructions

[1] Although oil is a mixture of both aliphatic and aromatic compounds each having their own physical-chemical characteristics, characterisation of oil and oil fractions is commonly based on the boiling points of linear alkanes.

most recent in a series of infamous oil spills resulting in well-documented destruction of wildlife, polluted beaches and shores, and consequences for those who are dependent on the sea for their food-supply or income. The real threat of oil pollution, however, is not what gets reported in the news; it lies underneath the water's surface, where large amounts of oils are dispersed or dissolved (*19-21*). Due to their low water solubility and strong hydrophobic character (*22*), dispersed and dissolved oils eventually accumulate into organisms and sediments (*23-25*), threatening the aquatic environment for many years and possibly decades (*26*).

Risk assessment of organic contaminants

In order to prevent unacceptable human and environmental exposure to chemicals, risk assessment is of vital importance. It plays a key role in the authorisation and regulation of new and existing chemicals, and rationalises any remediation practice of soil and sediment pollution. As the basic principle of risk assessment is to compare exposure with effect concentrations, environmental risks are expected when predicted environmental concentrations (PECs) exceed the predicted no-effect concentrations (PNECs). PECs are the concentrations in water and sediments, soils and groundwater, sewage treatment plants, and predators modelled from the expected release during manufacturing, formulation, and service life, and the compound's physical-chemical properties (*1*). PNECs are based on standard toxicity tests on organisms representing three trophic levels: ideally an algae, an invertebrate, and a fish. Here, the PNEC is defined as the lowest value multiplied by an assessment factor that counts for organisms not involved in testing and uncertainties, or the value at which 5% of the species tested is affected (*1*). Risk assessment of mixtures, however, requires a different approach, because in this case effect assessment is not always as simple as summarizing the toxicological responses for each individual compound. Mixtures might for instance be synergistic or antagonistic, in which the total toxicological response is respectively higher or lower than that of the individual compounds (*1*). Moreover, taking into account that the composition of mixtures may change in time, PNECs obtained for mixtures are not necessarily representative for field exposure. Considering that these chemicals are characterised by a non-specific mode of action, effect concentrations are

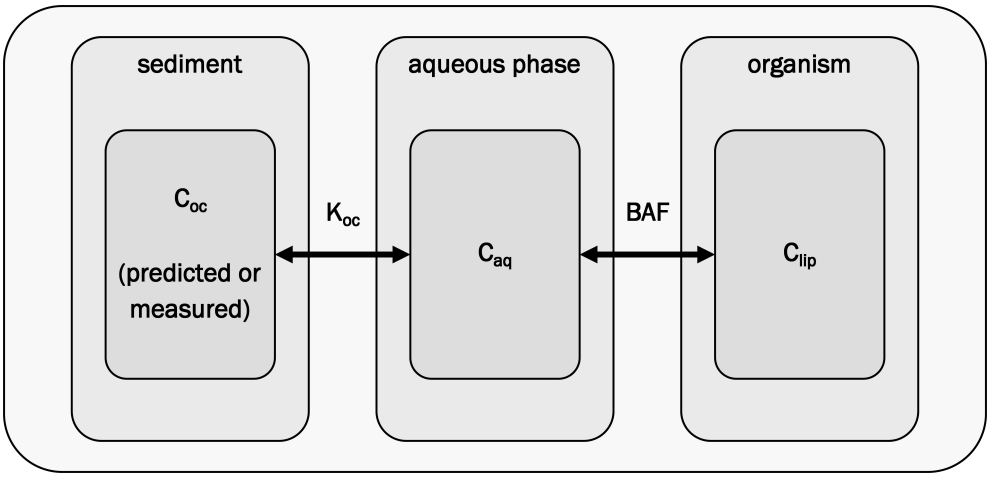

Figure 1. The equilibrium partition theory. C_{oc}: measured or predicted concentration of an HOC in the sediment's organic carbon phase; C_{aq} and C_{lip}: modelled concentrations in the aqueous phase and organism's lipid phase, respectively; K_{oc}: organic carbon-water partition coefficient; BAF: bioaccumulation factor

available for mixtures consisting exclusively of nonpolar HOCs, which was for instance demonstrated for petroleum hydrocarbons (*27*). This non-specific mode of action causes HOCs accumulated in the cells' phospholipid membranes to disturb the membrane functionality, resulting in a disturbed water balance in cells and the eventual death of the organisms involved. Death occurs once the amount of accumulated molecules in cell membranes exceeds the threshold of about 50-200 mmol/kg lipid, a threshold referred to as the critical body residue (CBR) or lethal body burden (LBB) (*28*). Unacceptable risks are thus expected when internal concentrations exceed this threshold, but this approach requires adequate assessment of bioaccumulation, the net result of contaminant uptake in organisms from their intermediate environment, including food[1].

Bioaccumulation is currently assessed by the generally accepted assumption that only chemicals dissolved in (pore) water are available for uptake in organisms (bioavailability). Bioaccumulation is therefore more closely related to concentrations in the aqueous phase than the total amounts of HOCs present in soils and sediments (*29-31*). Exposure concentrations, i.e. the concentrations in the aqueous phase, and bioaccumulation are estimated according to the equilibrium partition theory (EPT) from the amount of HOCs measured or predicted in sediments (*32,33*), which assumes that HOCs at steady-state are distributed over the sediments' organic phase, the aqueous phase, and the organisms' lipids according to their partition coefficients (figure 1). Considering that these partition coefficients are positively related with the octanol-water partition coefficient (K_{ow}), the distribution of HOCs over the three compartments

[1] Whereas the term bioaccumulation is used for the net accumulation of contaminants via all routes of exposure, bioconcentration is exclusively reserved for the accumulation of contaminants in hydrophobic tissues directly from the aqueous phase (*1*).

is driven by hydrophobicity. EPT is based on two types of partition coefficients, namely the organic carbon-water partition coefficients (K_{oc}) and the bioaccumulation factor (BAF). The first describes the distribution of HOCs between the sediment's organic phase and the aqueous phase, and the second refers to the accumulation of HOCs in the animal lipid phase from the aqueous phase or food (figure 1). Considering K_{oc} and BAF can differ by as little as one order of magnitude, bioaccumulation is often directly estimated from solvent-extracted, organic carbon-normalized concentrations in sediments by applying a biota to sediment accumulation factor (BSAF) of 1-2 (*34*).

Shortcomings of bioaccumulation assessment

Although EPT-based exposure assessment is simple, labour extensive, and generally accepted by policy makers, its application may result in an overestimation of bioaccumulation as solid phase, chemical, and biotic characteristics that determine bioavailability are not included (*8,35,36*). Exposure concentrations are assumed to be completely controlled by sorption to organic material in the solid phase (*32*), but they actually depend on numerous factors, including the contact time between contaminant and organic phase (aging) (*36-38*), the quality of the organic matter (*39*), and the presence of additional sorption phases such as black carbon (*40-42*), soot (*43*), and even oil and coal particles (*40,44,45*). Therefore, values for K_{oc} can vary enormously amongst soils and sediments (*8,30,46*), making these parameters not only chemical- but also site-specific. The generally accepted BSAF of 1-2 (*34*) is therefore not valid for many the organic chemicals, i.e., mostly PAHs (*30,42,47,48*). As K_{oc}s and BAFs are not always available, they are often estimated from a relationship with hydrophobicity (*1,35,49*). Where K_{oc} increases proportionally with hydrophobicity, BAFs remain constant or even decrease beyond log K_{ow} of 5.5-6 (*35,50*), a phenomenon which has been related to size-exclusion (*51*). This so-called hydrophobicity cut-off is however most likely caused by experimental artefacts and bioaccumulation thus proportionally increases with hydrophobicity as well (*52*). Risks assessed by models incorporating a hydrophobicity cut-off are therefore not realistic because they ignore bioaccumulation of more hydrophobic compounds. Moreover, BAFs are organism-specific and lipid-specific (*53-55*), and BAFs obtained with standard tests (*56*) may thus not be representative for the assessment of bioaccumulation in all organisms involved.

The aforementioned uncertainties may result in outcomes that are not representative for actual risks as observed *in-situ*. Moreover, partition coefficients are based on laboratory experiments for which the exposure conditions are stable and standardized. Bioaccumulation is, however, influenced by factors such as temperature, salinity and animal size (*35*). Therefore, bioaccumulation in field-exposed organisms may be different from that estimated using EPT-based models. For instance, it was demonstrated that temperature influences partitioning of HOCs to sediments (*57*) and thereby exposure concentrations, as well as bioaccumulation in lipids (*54*). K_{oc} is often determined at room temperature, while BAFs are determined at a temperature which is optimal for the organism under investigation (*56*). BAFs obtained in laboratory testing might therefore not be representative for the field, because exposure conditions *in-situ* are highly variable. Therefore, EPT-based risk assessment requires correction factors compensating for this. As a result, current exposure assessment based on EPT might

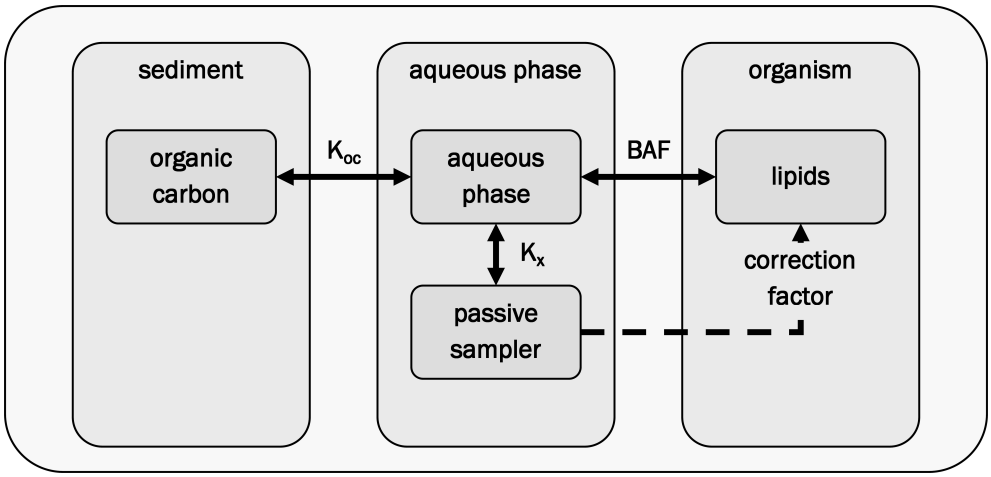

Figure 2. Principle of passive samplers. K_{oc}: organic carbon-water partition coefficient, BAF: bioaccumulation factor, K_x: partition coefficient between sampler and water.

not be adequate, because it is not able to predict exposure concentrations properly in all cases and is not necessarily representative for bioaccumulation *in-situ*. New analytical tools to measure exposure concentrations or the bioavailable fraction and new insights into how to assess exposure and effects of mixtures may, however, be able to accurately estimate exposure, as will be discussed below.

New developments in exposure assessment

Because only contaminants that are bioavailable will accumulate in organisms, bioavailability plays an important role in exposure assessment. Direct measurements of exposure concentrations or the bioavailable fraction (i.e., the fraction of the solid-bound concentration being available for uptake by organisms) will therefore result in a more realistic approach (*58*) than assessing exposure concentration modelling using EPT. Although direct analyses of the concentrations in the aqueous phase would result in reliable exposure concentrations, such analyses are not always feasible as analytical detection limits require large sample sizes. The best results are still obtained from bioaccumulation studies, though such studies are not preferable given that they are laborious and require animal testing. New developments in analytical chemistry have, however, provided methods that are able to measure exposure concentrations directly or to determine the amount of HOCs in the bioavailable fraction. Here, exposure concentrations are extracted by adding an extra hydrophobic phase (passive sampler) to water, sediment, or soil samples after which contaminants will be redistributed over the different phases according to their partition coefficients (figure 2). Once the system has equilibrated (i.e. after the redistribution is complete), the sampler is removed, cleaned, and the absorbed contaminants are subsequently extracted with a suitable solvent and analyzed. The concentrations in the aqueous exposure phase are then calculated from the concentration of HOCs accumulated in the sampler using partition

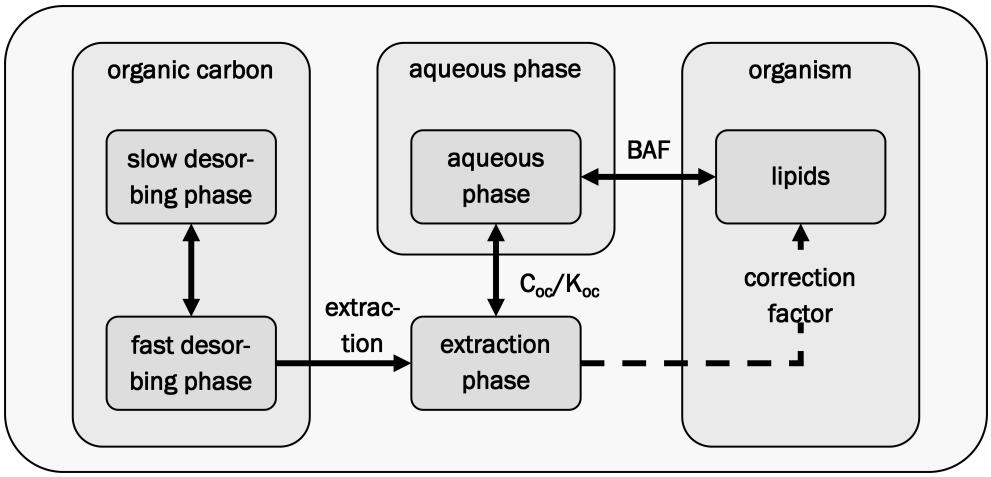

Figure 3. Principle of extractions of the fast desorption fraction. K_{oc}: organic carbon-water partition coefficient, BAF: bioaccumulation factor, C_{oc}: organic carbon-normalized concentration in the fast desorbing phase.

coefficients (*59*). Finally, bioaccumulation is estimated from the concentrations in the aqueous phase by using BAFs (figure 2). When partition coefficients are lacking, the concentration in the sampler might even be directly compared with bioaccumulation assuming that the sampler is as a surrogate organism, although a correction factor might often be needed to correct for differences in uptake ratios (*60*). Semi permeable membrane devices (SPMD) (*61*), solid phase micro-extraction (SPME) (*59*), and polyoxymethylene-solid phase extractions (POM-SPE) (*62*) are well-known examples of such passive sampling techniques.

HOCs accumulated in the sediment's organic carbon are distributed over three fractions from which they will desorb into the aqueous phase rapidly, slowly, or very slowly (*63*). The distribution of HOCs over these three phases strongly influences bioavailability (*64,65*), because only chemicals in the rapidly and a negligible amount of HOCs sequestered in the slowly desorbing fraction are released to the pore water (*66,67*). As such, bioavailability is related to the concentrations in the fast desorbing phase (*63*) (figure 3), which can be extracted with several chemical-analytical methods, including mild solvent extractions (*68*), supercritical fluid extractions (*69*), persulphate oxidation (*70*), and extractions with Tenax (*71*) or cyclodextrine (*72*). Subsequent bioaccumulation assessment is applied by equilibrium partitioning assumptions or directly by assuming that the amount of HOCs extracted (*30*) can be taken up completely (figure 3).

Many studies have demonstrated that the methods described above are valuable alternatives for equilibrium partitioning based risk assessment, because they significantly improve risk assessment of organic chemicals. For instance, bioaccumulation of PAHs in earthworms exposed to contaminated soils was overestimated when assessed from total, solvent-extracted concentrations as K_{oc}-

values varied widely amongst the soils. However, predicted body burdens based on the determination of exposure concentrations using SPME closely resembled observed bioaccumulation (*8*). Similar results were obtained in a study in which several chemical-analytical methods for the assessment of bioaccumulation of PAHs in aquatic worms were compared (*30*). Furthermore, promising results were reported using supercritical fluid extraction (*73*), 6-h Tenax extractions (*74-76*), solid phase microextraction (*31,60,77*), semipermeable membrane devices (*38*), and extractions with polyoxymethlylene (POM) (*47*) and cyclodextrin (*78*). Based on these findings, one might expect the chemical-analytical methods to be beneficial for the exposure assessment of complex mixtures as well, but this has not yet been tested.

Improving exposure assessment of complex mixtures

Risk assessment of petroleum hydrocarbons was significantly improved by introducing the hydrocarbon block approach (*79*). Here, oil extracts are analysed and the resulting response from a gas chromatograph, usually the signal between the retention times of decane (C_{10}) and tetracontane (C_{40}) and referred to as total petroleum hydrocarbons (TPH)[2], is divided in blocks with similar physical-chemical and toxicological characteristics. Internal exposure concentrations of each individual block are subsequently modelled using equilibrium partitioning and (estimated) partition coefficients of a representative marker compound for the specific hydrocarbon block (*80*). Crucial for this approach is the definition of hydrocarbon blocks. The simplest approach is to divide the response obtained by gas chromatography into blocks determined by the retention time of straight-chain alkanes. However, this approach will not result in adequate risk assessment given the fact that hydrophobicity and thus hydrophobicity-dependent partition coefficients are poorly related with retention time (*22*). Better results are obtained when extracts from soils or sediments are fractionated into aliphatic and aromatic compounds prior to GC-analysis (*80*) or when sophisticated analytical equipment such as two-dimensional gas chromatography (*9,81*) is used. The hydrocarbon block approach was incorporated in a model that estimates concentrations in cell membranes in organisms exposed to oil-polluted sediments, which can subsequently be compared with effects based on critical body residues (*80*). Another model was able to predict TPH concentrations in pore- and groundwater based on the concentrations measured in the solid phase using exhaustive solvent extractions (*82*).

Although the above–mentioned models might be good starting points to improve exposure assessment of complex mixtures, additional and sophisticated analyses are required. Moreover, the models rely strongly on partitioning to the organic carbon phase and therefore ignore the effects of additional sorption phases and organic matter composition on the exposure concentration, although sorption to solid and liquid oil particles was incorporated in the model presented by Verbruggen *et al*. (*80*). Assessment of internal exposure concentrations by the application of the aforementioned chemical-analytical methods and subsequent comparison with critical

[2] The response on a gas chromatograph depends on many factors such as extraction technique and applied clean-up method. Therefore, the definition of total petroleum hydrocarbons depends on the analytical method applied.

body residues might be therefore an alternative. This approach was successful to assess toxicity of PAHs in earthworms exposed to PAH-polluted soils (*8*). Similar studies have shown that SPME fibres were adequate tools to assess toxicity of petroleum hydrocarbons to daphnids and fish exposed to the water accommodated petroleum hydrocarbons (*27,83*). However, both fibres and organisms were exposed to dissolved oil and not directly to oil-polluted sediments. When both the organic and aqueous phase become saturated with petroleum hydrocarbons, oil is present as a separate phase (*45,80*). This phase may foul both passive samplers and organisms, which may have consequences for the assessment of exposure concentrations. Previous work already suggested fouling, as petroleum hydrocarbon concentrations in polyacrylate-coated SPME fibres exposed to oil-contaminated sediments were not related with initial TPH concentrations above 1000 mg/kg (*84*). However, below this concentration the fibres adequately predicted lethality of midge larvae (*84*), suggesting that chemical-analytical methods are applicable to assess bioaccumulation from oil-polluted sediments. However, little research on this topic has been conducted to date; consequently, none of these methods can yet be concluded to adequately assess exposure in organisms exposed to numerous HOCs simultaneously.

Objectives and outline

The aim of this thesis is to contribute to the improvement of risk assessment, and exposure assessment in particular, of complex hydrocarbon mixtures in sediments. Starting point is the use of chemical-analytical methods that have been demonstrated successful for individual contaminants, but not yet fully developed for both application in the field, and for complex mixtures such as petroleum hydrocarbons. Although the SPME method is applicable to assess bioaccumulation of PAHs in aquatic worms, their outcomes are not necessarily representative for bioaccumulation as it occurs in the field, because parameters necessary to assess exposure concentrations and bioaccumulation are laboratory-based and influenced by environmental conditions such as temperature and salinity. Therefore, the effect of temperature on partition of PAHs to SPME fibres and bioaccumulation in aquatic worms was studied (Chapter 2). For complex mixtures such as petroleum hydrocarbons, the SPME method is also insufficiently investigated and developed, and therefore it currently is not clear whether it can be used to assess internal exposure concentrations. Moreover, no reliable information is available on bioaccumulation of petroleum hydrocarbons as only a few studies have been performed addressing this issue. Additionally, analyses of petroleum hydrocarbon concentrations in both sediments and biota may often have been biased by the presence of non petroleum-related compounds. Therefore, in this thesis a new method was developed that is able to adequately remove these interfering substances from oil extracts from sediments and biota (Chapter 3). To gain insight in bioaccumulation of complex mixtures, bioaccumulation of petroleum hydrocarbons was intensively studied in Chapter 4. Because current approaches to assess bioaccumulation of complex petroleum mixtures in aquatic organisms are inadequate, a selection of available chemical-analytical methods was applied on oil-polluted sediments and it was tested to which extent they could properly assess actual bioaccumulation (Chapter 5). In Chapter 6, the potential of SPME for PAHs and the best

method resulting from Chapter 5 for petroleum hydrocarbons to predict actual exposure concentrations in worms exposed in the field was finally tested. The last chapter therefore aimed to answer the question whether the research described in the current thesis indeed resulted in an improvement of exposure assessment of complex mixtures in the real world.

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Chapter 2

Temperature-dependent bioaccumulation of Polycyclic Aromatic Hydrocarbons

Barry Muijs, Michiel T.O. Jonker

Institute for Risk Assessment Sciences, Utrecht University

Abstract

Bioaccumulation factors (BAFs) play a key role in risk assessment of chemicals in sediments and soils. For hydrophobic organic chemicals (HOCs), BAFs are however difficult to determine and values are mostly obtained by modelling. Apart from a lack of reliable data, the applicability of lab-derived values in the field situation is unknown, as exposure conditions (e.g., temperature, pH, salinity, test species, number of chemicals) are standardized in the lab, whereas they may vary in the field. In this study, the effect of temperature on the bioaccumulation of a series of moderate to very hydrophobic PAHs in aquatic worms was studied by using polydimethylsiloxane (PDMS)-coated solid phase microextraction (SPME) fibres. The results indicated that bioaccumulation of non-metabolisable HOCs is an exothermic, enthalpy-driven process, thus decreasing with increasing temperature. As such, biotic concentrations may be several times higher in winter than in summertime, which could have ecotoxicological consequences. A two-parameter linear free energy relationship was derived with which PAH bioaccumulation can be predicted from temperature and the chemicals' hydrophobicities. Comparing the determined (thermodynamics of) PAH partitioning into organisms and PDMS indicated that the latter phase cannot be used as a surrogate phase for animal lipids. Still, SPME provides an appropriate analytical tool for the measurement of aqueous concentrations, from which bioaccumulation can subsequently be estimated by using BAFs.

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Introduction

For thousands of chemical compounds, risk assessment has to be performed in order to determine their human and environmental safety. Risk assessment largely relies on scenarios with models, containing input parameters that characterize the compounds' physicochemical properties, fate, and effects. The parameters describing uptake into organisms are the bioconcentration factor (BCF) in case the uptake of contaminants is via the water phase only, or the bioaccumulation factor (BAF) if organisms accumulate contaminants via both the water phase and an additional exposure matrix (soil or sediment) or food (*1*). These factors describe the equilibrium distribution of organic chemicals between organism and water and are both given by the ratio of the concentration in biota (C_{org}) and the aqueous concentration (C_w) (*2*). BCF and BAF values are mostly estimated from relationships with the octanol-water partition coefficient ($logK_{ow}$) (*3,4*), as values are only available for a limited number of chemicals. In particular for very hydrophobic organic chemicals (HOCs) having a $logK_{ow}$ > 5.5-6, reliable BCFs and BAFs are often lacking, because they are very difficult to determine as measurements are liable to several experimental artefacts (*5*). Apart from a lack of data and a questionable reliability for specific chemicals due to experimental difficulties, very little is known about the extrapolation possibilities of lab-derived BCFs and BAFs to the field situation, i.e., the situation to which risk assessment should ultimately apply. During laboratory studies, species, body size and weight, the number of test chemicals, exposure temperature, salinity, and pH are usually standardized. However, in the field, conditions will deviate from those normally applied in the lab and *in situ* uptake of chemicals may therefore differ from expectations (*3*).

Amongst the factors mentioned above, temperature may be considered the most important and relevant one for non-ionic HOCs (e.g., dioxins, polychlorinated biphenyls, polycyclic aromatic hydrocarbons (PAHs), flame retardants, chlorobenzenes, petroleum hydrocarbons). These chemicals remain unaffected by pH and lipid normalization presumably (largely) removes differences regarding organisms (*2,6*). Although salinity does influence partitioning of HOCs into organic phases (*2,7*), salinity changes in actual practice may rather result in reduced animal survival rates. In contrast, temperature has been observed to affect bioaccumulation as well (see below), but it changes seasonally over a wide range without a determined effect on survival of local species. Bioaccumulation of HOCs that are not being metabolized can be expected to decrease with increasing temperature. After all, storage lipids and membrane phospholipids are the major partitioning sites for these chemicals and in order to maintain optimal membrane functioning and accessibility to the energy pool, the lipid phases are kept fluid over a wide temperature range (*8,9*). Partitioning to these fluid lipids is enthalpy-driven (*10*), which consequently should result in a negative temperature effect. BCF values for HOCs have indeed been observed to correlate inversely with temperature (*10,11*), although positive (*12-14*) and no correlations have been reported as well (*15,16*). The positive or no effects observed can however probably be explained by shortcomings in the experimental setups applied (discussed later on). All in all, there is little detailed knowledge on the effects of temperature on bioaccumulation. Relationships between temperature and uptake are lacking for the vast majority of

organisms and chemicals, which obviously hampers extrapolation of lab-derived BCF and BAF values to field situations.

Urged by this knowledge gap, the present study was designed to establish the effect of temperature on the bioaccumulation of 13 moderate to very hydrophobic polycyclic aromatic hydrocarbons (PAHs; $4.5 < \log K_{ow} < 7$) into aquatic worms (*Lumbriculus variegatus*). Worms were chosen as test organisms, because they are abundant in sediments and soils, are a major food source for fish, mammals, and birds, and bioaccumulation into these organisms is already included in risk assessment models (*1*). Moreover, worms have not previously been used as test organism to study the effects of temperature on bioaccumulation. PAH BAF values for *L. variegatus* were determined at different temperatures between 5 and 24°C, following a recently described method (*5*). The method pays special attention to avoiding experimental artefacts that may bias true uptake of very hydrophobic chemicals and involves a four phase system in which PAHs are equilibrated among worms, water, cellulose, and solid phase micro-extraction (SPME) fibres. The cellulose acts as substrate and PAH source for the worms, and aqueous PAH concentrations are determined with SPME. The temperature-dependent SPME fibre-water partition coefficients required in this study were determined first, and their existence additionally allowed a thermodynamics-based judgment of the suggested biomimetic potential of the SPME technique (*17*). The full experimental setup is illustrated in appendix I, figure S1.

Experimental section

Chemicals, fibres, and glassware

Solvents used (hexane, acetone, methanol, and acetonitrile) were obtained from Lab-Scan (Dublin, Ireland) and were of Pestiscan or HPLC grade. Testing chemicals (phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*e*]pyrene (BeP), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), benzo[*g,h,i*]perylene (BghiP), dibenz[*a,h*]anthracene (DahA), and indeno[1,2,3-*c,d*]pyrene (InP); all > 98%) were from Aldrich (Steinheim, Germany). Other chemicals used were 2-methylchrysene (99.2%; BCR, Geel, Belgium), aluminium oxide (Super I; ICN Biomedicals, Eschwege, Germany), calcium chloride and sodium azide (extra pure; Merck, Darmstadt, Germany), and penicillin and streptomycin (Gibco BRL, Breda, the Netherlands). Polydimethylsiloxane (PDMS)-coated, disposable SPME fibre (glass fibre core diameter 110 μm, PDMS coating thickness 28.5 μm) was obtained from Poly Micro Industries (Phoenix, AZ, USA). The fibre was cut into pieces of 5 cm length and washed two times with acetonitrile and two times with Millipore water prior to use. Photodegradation of PAHs was prevented during all experiments by using amber-coloured glassware or glassware covered with aluminium foil. All glassware was thoroughly rinsed with Pestiscan grade acetone before use.

Organisms

Aquatic worms (*Lumbriculus variegatus*) were cultured in our laboratory and were acclimatized for six weeks to three different temperatures (5, 12, and 24°C) in 10 L aquaria filled with pulverized cellulose and tap water. The water was aerated and filtered continuously with air-driven carbon filters, and partly changed every two weeks. During acclimatization, worms were fed with fish flake food once a week.

PDMS-water partition coefficients

Glass bottles were almost completely filled (about 110 mL) with a solution of calcium chloride (0.01 M) and sodium azide (50 mg/L) in Millipore water. After the addition of 2 fibres of 5 cm each, the solution was spiked with 50 µL of a solution containing 13 PAHs at a concentration of 0.7 mg/L in acetone. The bottles were closed with glass stoppers and put on 1-dimensional shakers, placed in three climate-controlled rooms (4, 12, and 20°C), or in a water bath shaker (30°C). Each treatment was replicated five times and actual temperatures were monitored regularly in dummy flasks also placed on the shakers.

After six weeks, PAH concentrations in the water phase were determined by transferring about 100 mL of test solution to a 100 mL cylinder, containing 4 mL of hexane. The volume of the test solution was determined by using a balance and a temperature-dependent density correction. The cylinders were closed with glass stoppers and intensively shaken for 2h at 120 rpm. Subsequently, the hexane layer from each cylinder was carefully transferred to a calibrated pointed flask. The water phase was then extracted two times more with 3 mL of hexane. The hexane phases were pooled, gently evaporated to 0.5 mL, and solvent-exchanged to 0.45 mL of acetonitrile. Fifty µL of internal standard solution (10 mg of 2-methylchrysene / L acetonitril) was then added and after vortexing, the extract was transferred to a 2 mL autosampler vial and stored at -18°C until analysis. All final concentrations were corrected for PAH recoveries, which were determined to be 92-100%, depending on the PAH. Fibres were also collected, wiped with a clean tissue wetted with Millipore water, measured to the nearest 0.5 mm, cut, and transferred to autosampler vials filled with 180 µL of acetonitrile in 250 µL inserts. Twenty µL of internal standard solution was added to each vial and closed vials were stored at -18°C until analyses. PAHs were analyzed by HPLC as described previously (*5*).

Bioaccumulation factors

Bioaccumulation factors were determined at different temperatures according to the method described in (*5*). For each temperature, seven systems were prepared. Erlenmeyers flasks (500 mL) were filled with 3.5 g of pulverized and hexane-extracted cellulose and 400 mL of tap water, and subsequently spiked with 30 µL of a PAH solution containing 13 PAHs in acetone at a concentration of about 300 mg/L each. The Erlenmeyers flasks were closed, divided over two temperature-controlled rooms (5 and 12 °C) and a water bath shaker (24°C), and shaken thoroughly (160 rpm) for two days to establish PAH equilibration between water and cellulose. Then, three 5 cm long

fibres and 3.6 g of wet weight worms acclimatized to the respective temperature were added. The worms had been gut-purged overnight in clean tap water of the same temperature. Also penicillin (100 Units/L) and streptomycin (100 µg/L) were added to prevent bacterial growth. The systems were then gently shaken (100 rpm) for four (24 °C) to six weeks (5 and 12°C) to allow equilibration (four weeks is sufficient for reaching equilibrium conditions at 20 °C (5), but longer times may be necessary for lower temperatures, due to slower kinetics). Temperature was monitored frequently and actual temperatures are used throughout this paper. Despite the successful application of the experimental setup before (5), worm mortality was observed in about half of the systems after 1-2 weeks, possibly induced by the formation of toxic degradation products of decaying worms. To reduce chances on mortality in the other systems, the headspace of these Erlenmeyer flasks was purged with oxygen (5). Consequently, survival was not affected in these systems and here worms were healthy. Only these systems were therefore used for bioaccumulation measurements.

Upon finishing the exposure, fibres were collected and treated as described above. Worms were separated from the cellulose and gut-purged for eight hours in 250 mL Erlermeyers flasks, filled with tap water of the respective exposure temperature. The flasks were placed on a 2-dimensional shaker (100 rpm) and water was changed three times in order to prevent re-ingestion of faeces. Finally, the worms were collected, frozen, and freeze-dried. Part of the freeze-dried material (80 mg) was extracted with 120 mL of acetone:hexane (1:3 v/v) in pre-extracted Soxhlet devices. The extracts were evaporated to 3 mL on a modified Kuderna-Danish apparatus and subsequently to 1 mL under nitrogen. Extracts were cleaned-up through aluminium oxide columns (4g, deactivated with 10% of Millipore water) by elution with 30 mL of hexane. The purified extracts were evaporated to about 0.5 mL and solvent-exchanged to 3.6 mL of acetonitrile. Finally, 400 µL of internal standard solution was added and after vortexing, the extracts were transferred to autosampler vials for PAH analysis with HPLC.

Lipid content of the worms was determined in triplicate as follows: 100 mg of freeze-dried material was weighed into 10 mL tubes and extracted with 4 mL of acetone:hexane (1:3 v/v) by vortexing (1 min at 2200 rpm) and sonification (5 min), followed by centrifugation (5 min at 1500 rpm). After collecting the solvent phase, the remaining pellet was extracted another two times and the pooled extract was evaporated to dryness under nitrogen. Lipid contents were subsequently determined gravimetrically.

Data analysis

Bioaccumulation factors were calculated by dividing PAH concentrations in worms (C_{org}, mg/kg lipid) and those in the aqueous phase. The latter concentrations were calculated from PAH concentrations in the fibres (C_f, mg/L) and temperature-corrected fibre-water partition coefficients (K_f):

$$BAF = C_{org} \frac{10^{aT+b}}{C_f} \quad (1)$$

where T is the temperature (°C), and *a* and *b* are the slope and the intercept, respectively, of the relationship between K_f and temperature (see Supporting Information for a derivation of equation. 1).

Sorption enthalpies (ΔH) and entropies (ΔS) were calculated from measured K_f and BAF values, according to Kwon *et al*. (*18*). First, partition coefficients were converted to mole fractions using:

$$\log K^{m}_{sorbent} = \log K_{sorbent} + \log\left(\frac{V_{water}}{V_{sorbent}}\right) \quad (2)$$

in which $K_{sorbent}$ represents K_f or BAF, V_{water} is the molar density of water (55.5 mol/L) and $V_{sorbent}$ is the molar volume of the sorption phase (lipids or PDMS). The molar density of the artificial membrane lipid 1-palmytoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC, 1.32 mol/kg) (*18*) was used for lipids and for PDMS, 12.81 mol/kg was applied. The latter value was derived from the density (0.95 cm^{-3}) and molar weight (74.15) of a single dimethylsiloxane unit. Although molar densities depend on lipid composition, the degree of polymerization, and temperature, sensitivity analysis indicated that these parameters had no or little effect on the final sorption entropy. Subsequently, sorption enthalpies and entropies of solute partitioning into PDMS or lipids were calculated using the van 't Hoff relationship:

$$\log K^{m}_{sorbent} = -\frac{\Delta H}{2.303R}\frac{1}{T} + \frac{\Delta S}{2.303R} \quad (3)$$

where R is the gas constant (8.314 10^{-3} kJ/mol/K) and T the temperature (K). The parameters were fit using Graphpad Prism (v3.00, Graphpad Software, Inc.). Finally, Gibbs free energies (ΔG) were calculated at 293K:

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

Results and discussion

Temperature-dependent partitioning of PAHs into PDMS.

SPME is widely applied for the quantification of organic compounds in environmental matrixes (*19*), the determination of freely dissolved (bioavailable) concentrations in soils and sediments (*20*), and the estimation of bioaccumulation (*5,21*). All these applications rely on proper fibre-water partition coefficients (K_f), which generally however are only available for one exposure temperature. The partition coefficients measured at 24°C in the present study (appendix I, table S1) were comparable to values published before (*20,21*). As expected (*7*), partitioning of PAHs into the fibre coating decreased with increasing temperature (appendix I, table S1), yielding negative values for the Gibbs free energies (appendix I, table S2) and thus indicating an exothermic process. The sorption process appears to be dominated by an enthalpy change. The relatively large negative ΔH values suggest a low amount of energy needed for creating a cavity in the PDMS phase or favourable interactions between PAHs and PDMS, once the sorbates have entered the sorbent (*2,6*). The entropy term

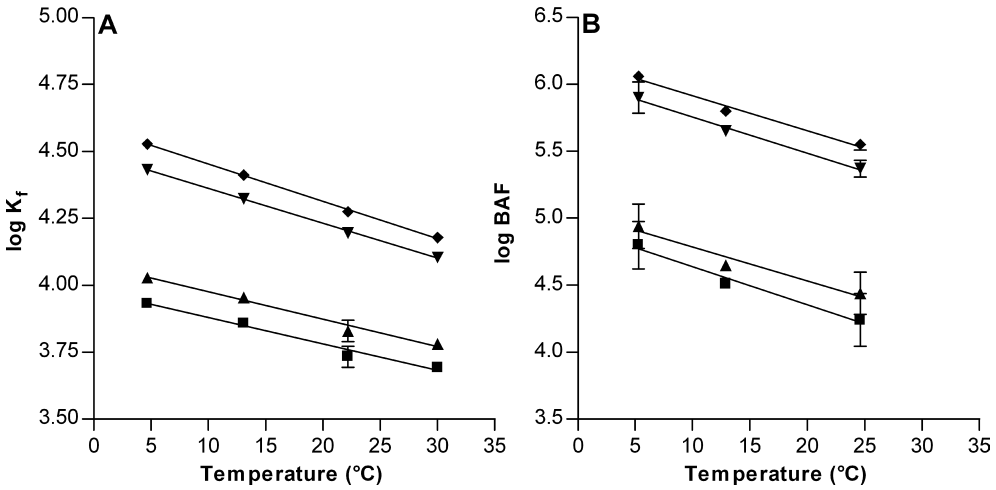

Figure 1: Temperature-dependent uptake of phenanthrene (■), anthracene (▲), fluoranthene (▼), and pyrene (♦) into 30 µm PDMS-coated SPME fibres (A) and *Lumbriculus variegatus* (B). Values are averages ± standard deviations. The lines are linear regression curves, for which the parameters (slopes, intercepts, r^2s, and p-values) are presented in appendix II, table S1 (SPME fibres) and appendix II, table S3 (*L. variegatus*).

being >0 would be explained by a gain in freedom of molecular movement of the hydrophobic chemicals in PDMS as compared to that in water. For a few representative PAHs, partitioning as a function of temperature is illustrated in figure 1A. This figure shows that a temperature change of 25°C resulted in a small, but significant ($p<0.016$) decrease in the partition coefficients of 0.24 log units for Phe to 0.55 log units for BeP. The relationship between partitioning and temperature for BghiP, DahA, and InP was less straightforward, because partition coefficients at 5°C were similar to or even lower than those at 12°C. Due to their relatively low aqueous solubilities at 5°C, a fraction of these PAHs may have been present as crystals, resulting in an overestimation of aqueous concentrations. Alternatively, non-equilibrium conditions due to slow equilibration kinetics at this low temperature might be an explanation. The 5°C data for these PAHs were therefore considered biased, and were omitted from the data analysis. A consistent data set then resulted with similar slopes of the log K_f-temperature relationship for all PAHs, except DahA. Also, enthalpies in appendix I, table S2 display a consistent pattern, with values being similar for PAHs with the same number of rings, and showing an increase of about -6 kJ/mol per ring added. For the PAHs tested in this study (except DahA), partitioning into 30µm PDMS-coated fibres at any temperature between 5 and 30°C can consequently be predicted, using a linear relationship ($r^2>0.846$) for which the parameters are given in appendix I, table S1. These predictions were used to derive bioaccumulation factors at different temperatures using SPME, as will be discussed below.

To our knowledge, only one study has been published in which the relationship between uptake of hydrophobic compounds into PDMS and temperature was

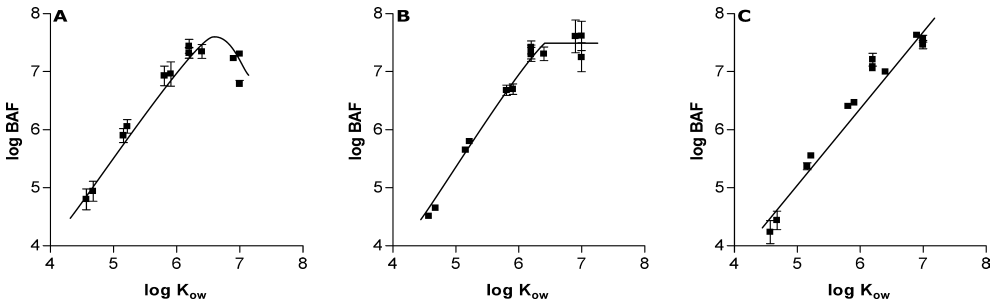

Figure 2. Relationships between bioaccumulation factor (logBAF) and octanol-water partition coefficient (logK_{ow}) for PAHs at 5 (A), 12 (B), and 24°C (C). Values are averages ± standard deviations. The lines serve to guide the eye.

described. This study demonstrated that the partitioning of (alkylated) mono-aromatics (BTEX) into PDMS also increased with decreasing temperature (*22*). Sorption enthalpies calculated from the data in ref. (*22*) were combined with those derived for the PAHs in the present study and plotted against the chemicals' octanol-water partition coefficients (logK_{ow}). As shown in appendix I, figure S2, sorption enthalpies increase (become more negative) linearly with increasing hydrophobicity (r^2=0.941, p<0.001), suggesting that partitioning of mono- and poly-aromatics into PDMS is a thermodynamic process predominantly driven by sorbate hydrophobicity. Replacing ΔH in the well-known relationship between sorption and temperature (*2,7*) by this logK_{ow} relationship, the following equation, in which partitioning of (alkylated) mono-aromatics and PAHs into PDMS is estimated from logK_{ow} (20°C data adopted from (*5*)) and exposure temperature, can be derived:

$$K = K_0 e^{-\frac{-5.134 \log K_{ow} + 4.563}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right)} \quad (5)$$

In which K_0 is the partition coefficient measured at T_0, R is the universal gas constant (8.314 10^{-3} kJ/mol/K), and T is the actual temperature in °K. Obviously, this relationship is valid only for the chemicals and temperature range (5-30°C) it was derived for.

Temperature dependence of bioaccumulation.

Bioaccumulation factors of PAHs in *L. variegatus* measured at 5, 12, and 24°C are presented in appendix I, table S3. The majority of values determined at 24°C appear to be not significantly different (t-test) from values reported in ref (*5*), which were measured at 20°C, or differences were within 0.1 log unit (except for Phe and DahA). The present results being similar to previous determinations at comparable temperature indicates a good reproducibility of the experimental approach introduced in (*5*). In figure 2, BAF values determined at all three temperatures are plotted against the chemicals' hydrophobicities. It should be mentioned that K_{ow} values will depend on the temperature as well, but as values are not available for the three testing

temperatures, fixed values (adopted from ref (*5*)) were used to construct the graph. figure 2 shows a linear log-log relationship for BAF values measured at 24°C. For the other two lower temperatures, however, a clear levelling/cut-off for PAHs having a logK_{ow} ≥ 6.5 is observed, with the effect being most pronounced for the lowest temperature. In other words, bioaccumulation of high-molecular-weight PAHs seems to be reduced at lower temperatures. Analogous to the 'classical' BCF hydrophobicity cut-off at logK_{ow} of 5.5-6, this phenomenon might either be explained by a true mechanism or by experimental artefacts (*5*). In the first case, temperature should have an effect on the lipid composition of worms and the resulting differences in lipid composition should specifically affect partition behaviour of high-molecular-weight PAHs (i.e., size-exclude them from lipids occurring at lower temperatures). Support for the first part of this hypothesis comes from observations that the relative amount of unsaturated fatty acids in both membrane and storage lipids increases with decreasing temperature (*8,9,23*). The biological purpose of this process, which has been observed to be completed within 4-7 weeks (*9*), is to have a constant lipid fluidity to maintain optimal membrane functioning (*8*) and accessibility of energy reserves (*9*). The effect of temperature on lipid composition however varies among species (*23*) and even among populations (*9*). If and to which extent the lipid composition of the population of *L. variegatus* used changed during the present experiments is therefore not clear. Investigating the effects in detail was however outside the scope of this study. Although it has been demonstrated that partitioning of organic compounds into unsaturated artificial membrane lipids (thermodynamically) differs from that into saturated lipids (*18*), it is also unclear if the worms' lipid composition (can have) changed such that bioaccumulation of only the most hydrophobic PAHs was reduced to cause the observed levelling/cut-off. Probably, however, this is not the case, because of two reasons. First, sorption of a wide range of organic compounds was influenced by lipid composition (*18*). In our study, only BAFs for the most hydrophobic PAHs were lower at 5° than at 12°C. If temperature really has a significant effect on both lipid composition and partitioning, lower BAF values could be expected for all PAHs. Second, recent unpublished data indicate that the presumed compositional change has no effect on partitioning of PCBs to dead worms (*pre mortum* acclimatized to different temperatures). Therefore, the levelling/cut-off observed in figure 2 is most likely caused by an experimental artefact, i.e., non-equilibrium conditions (*5*) at 5° and 12°C. Kinetics are slower at lower temperatures and the exposure time applied (four to six weeks) presumably was too short to achieve full equilibrium conditions at these temperatures, with the degree of non-equilibrium increasing with decreasing temperature (figure 2). Longer exposure times were not feasible, however, because worms could not be maintained longer than six to eight weeks in these systems. The non-equilibrium hypothesis is supported by the results from an additional experiment, in which bioconcentration factors were measured at 5°C after 33, 40, and 54 days. With the exception of Phe, Ant, Flu, and Pyr, BAF values increased gradually with increasing exposure time (appendix I, figure S3). A hydrophobicity cut-off was only observed for BghiP, DahA, and InP, and at 5°C also for BaP. For all other PAHs, full equilibration can therefore be assumed after 40 or 54 days. Note that non-equilibrium conditions do not necessarily fully explain the exact shapes of the logK_{ow}-logBAF curves measured at 5 and 12°C (levelling/cut-off), as an underestimation of K_{ow} values at

these lower temperatures will contribute to some extent as well (assuming that partitioning of different PAHs into octanol is affected differently by temperature, as also observed for PDMS – see above). In fact, such an effect might explain the increase in curve slope with decreasing temperature, as observed in figure 2. In our opinion, other explanations for the levelling/cut-off at the lower temperatures (i.e., metabolization, third phase effects/bioavailability, elimination into faeces) are not plausible, leaving non-equilibrium conditions as the main explanatory factor. Throughout this paper, all values for Phe, Ant, Flu, and Pyr at 5 °C are based on the average of the 40 and 54 days exposure (maximum number of replicates), whereas for all other PAHs, data from the 54 days exposure were used. Due to the presumed non-equilibrium conditions, the 5 and 12°C BAF values for BghiP, DahA, and InP, as well as the 5°C value for BaP should be used with caution.

Similar to the relationship between temperature and K_f discussed above, BAF values decreased with increasing temperature for most of the PAHs (see appendix I, table S3; obvious exceptions are BghiP, DahA, and InP). For a selection of PAHs, the temperature dependence is illustrated in figure 1B. The differences in BAFs determined at 5 and 24°C were small (0.35 logunits for BeP to 0.56 logunits for Phe), but significant, as the slopes of the temperature-logBAF curves significantly differed from zero ($p<0.0064$). Similar to PDMS, sorption enthalpies were negative and the entropy terms ($T\Delta S$) were relatively small (appendix I, table S2), indicating that partitioning of PAHs into worm lipids is an enthalpy-driven process as well. As for PDMS, apparently only little energy is required for creating a sorption cavity in the lipid phases, or favourable interactions between chemicals and lipids occur as soon as the chemicals have partitioned into the lipids (*10*). Due to relatively large standard errors and presumed non-equilibrium conditions for some PAHs, no clear relationship between sorption enthalpy and hydrophobicity could be observed. Still, PAH uptake in *L. variegatus* at temperatures between 5 and 24°C is predictable by simple, PAH-specific linear models, of which the slopes and intercepts are given in appendix I, table S3. Again, it should be stressed that one should be cautious with using the values for the more hydrophobic compounds.

The inverse relationship between bioaccumulation and temperature observed in the present study is according to expectations, as outlined in the Introduction. Although the extent may depend on the chemical and the organism studied, a negative trend between temperature and uptake has previously been observed for chlorobenzenes, PAHs, and Bisphenol A in fish lipids, worms, and frog tadpoles (*10,11*). On the other hand, a positive trend was observed for chlorobenzene BCFs in fish (*13*) and Bisphenol A uptake in salmon embryos (*14*). The exposure time in ref (*13*) however only measured 48 h, a period most probably too short to reach equilibrium. When measuring at non-equilibrium, a positive trend with temperature is obvious, as kinetics at higher temperature are faster, thus resulting in increased uptake as compared to that at lower temperatures. An indication for non-equilibrium conditions in (*13*) is the observation that the (marginal) change in BCF with temperature increased with chlorobenzene hydrophobicity, even though one would expect the change to be similar for different compounds within the same class (c.f., the data for the first six PAHs in appendix I, table S3). Also the BCF values in ref (*14*) were determined under non-

equilibrium conditions, as acknowledged by the authors. Some researchers have reported the absence of a clear effect of temperature (*15,16*). This seems to be due to insufficient data resolution, but non-equilibrium conditions may explain part of these observations as well, as can be deduced from the exposure times applied, relative to the chemicals' hydrophobicities and/or the organism size (*15*). Based on the above, we believe that bioaccumulation of non-metabolisable HOCs in multicellular aquatic organisms is an enthalpy-driven process, accompanied by an inverse relation between temperature and accumulation. For unicellular organisms (algae, bacteria), presumably the same applies, although Koelmans and Jimenez (*12*) concluded otherwise. Trichlorobenzene/algae BCFs measured after 48 h in (*12*) did however negatively correlate with temperature, whereas no trend was observed for tetrachlorobenzene, and a predominantly positive relation was found for penta- and hexachlorobenzene. These results may suggest non-equilibrium measurements as well.

For risk assessment purposes, BCF and BAF values are mostly estimated from relationships with $\log K_{ow}$, without considering any effects of environmental conditions. From the present study, it is however clear that temperature does influence the uptake of PAHs in aquatic worms and actual bioaccumulation may therefore be different from model estimates. Even though temperature effects are relatively small, one can calculate that bioaccumulation of PAHs in *L. variegatus* in wintertime is four times higher than during summer. A factor of four may be within the confidence intervals of the models used, but still may have ecotoxicological consequences.

Based on the present experiments, the following equation, which estimates PAH bioaccumulation as a function of both octanol-water partition coefficient at 20°C and temperature in °C (r^2=0.961, p<0.001) can be derived:

$$\log BAF = 1.47 \log K_{ow} - 0.0278T - 1.59 \quad (6)$$

Note that K_{ow} values are fixed values, i.e., the temperature-dependence for this partition coefficient is not included. Obviously, equation 3 is only valid for bioaccumulation of PAHs in *L. variegatus* between 5 and 24°C.

PDMS as a biomimetic tool.

It has been suggested that PDMS might serve as a surrogate for biomembranes and thus could be used to mimic bioaccumulation of hydrophobic organic contaminants in animal lipids, because chemical concentrations absorbed in PDMS were observed proportional to concentrations in animal lipids (*17,24*). Such a relationship was shown for several organic chemicals, including chlorinated compounds (*17,24,25*) and TNT (*26*). Being a good surrogate in our opinion would however imply that PDMS should act as an artificial lipid phase and that it should be possible to straightforwardly substitute uptake studies with animal lipids by uptake studies using PDMS. At least for the PAHs and organisms tested in the present study, such a substitution does not seem applicable, because of three reasons: (i) The present study demonstrates partition coefficients for PDMS being lower than for biota lipids (cf. appendix I, tables S1 and S3), which is in accordance with studies published before (*17,24,25*). The ratio between concentrations in lipids and PDMS however appears to depend on the

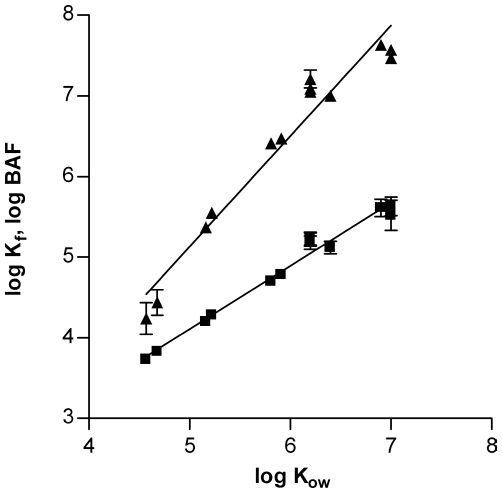

Figure 3. Hydrophobicity-dependent partitioning of PAHs into 30 µm PDMS-coated SPME fibres (■) and *Lumbriculus variegatus* (▲). Values are average ± standard deviations. Lines represent the following linear regression curves (± standard errors): log K_f = 0.785 (± 0.0365) logK_{ow} + 0.182 (± 0.219); r^2=0.977; p<0.001, and logBAF = 1.37 (± 0.0963) logK_{ow} - 1.72 (± 0.577); r^2=0.949; p<0.0001.

(combination of) chemical and organisms studied. Therefore, a 1:1 relationship between accumulation in PDMS and lipids does not exist. Using PDMS as a surrogate for lipids would thus require calibration experiments for every single combination of chemical (group)/organism in order to derive case-specific correction factors; (ii) Although one fixed correction factor per group of compounds would be feasible (*17*), figure 3 clearly demonstrates that for PAHs such a correction factor, or the difference between partitioning into PDMS and lipids, significantly increases with hydrophobicity. This indeed would imply the need for chemical-specific correction factors, which is in contrast to previous studies that reported the ratio between concentrations of chlorinated compounds in organisms and in PDMS not to be influenced by hydrophobicity (*17,25*). Interestingly, figure 3 also shows that the relationship between PAH partitioning into PDMS and octanol has a slope of less than unity, whereas the slope of the relationship between partitioning into lipids and octanol is >1; (iii) PAH partitioning into PDMS and lipids differs in terms of thermodynamics: although both processes are enthalpy-driven, enthalpies for PDMS are less negative than for lipids (appendix I, table S2). Also, the values become more negative with hydrophobicity, whereas the opposite seems to be the case for lipids. The first observation, which applies to at least the 3 and 4-ring PAHs, implies that with a certain decrease in temperature, the increase in PAH uptake in PDMS is less than the uptake increase in lipids. One explanation for this observation might be a change of lipid composition with temperature (*8,23*), resulting in a change in sorption characteristics, whereas the composition of PDMS will be chemically stable, at least over a temperature range of 25°. Alternatively, it may indicate that less energy is required to create a sorption cavity within the lipid phase as compared to the PDMS phase, or that favourable (π-

electron) interactions between PAHs and lipids take place, whereas they do not between PAHs and PDMS. In conclusion, the present results demonstrate that PDMS cannot be used as a surrogate for animal lipids, at least not for PAHs. Rather, PDMS should be considered an analytical tool, which can be applied for the estimation of bioaccumulation, as done in e.g. ref (*21*). This tool involves a two-step calculation approach in which the freely dissolved concentration in (pore) water is calculated first from the concentration accumulated in PDMS by using the K_f, followed by the estimation of concentrations in organism lipid by applying bioaccumulation factors. For PAHs, the required fibre-water partition coefficients and bioaccumulation factors are reported in this paper, as well as their relationships with temperature, allowing (in situ) bioaccumulation to be estimated at any temperature within the range investigated here.

Acknowledgments

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Chapter 3

Evaluation of clean-up agents for total petroleum hydrocarbon analysis in biota and sediments

Barry Muijs, Michiel T.O. Jonker

Institute for Risk Assessment Sciences, Utrecht University

Abstract

Petroleum hydrocarbons (oil) are common environmental contaminants. For risk assessment purposes, their concentrations in environmental matrixes, such as biota and soils/sediments are frequently determined by solvent extraction and subsequent analysis with gas chromatography (GC) equipped with flame ionization detection (FID) or mass spectrometry (MS). Because the total GC detector response is labelled as total petroleum hydrocarbon (TPH) concentration and matrix compounds (lipids, organic matter) will contribute to this response, proper extract clean-up is crucial. Still, the choice for a specific clean-up material during open column chromatography often seems arbitrary, since no comparative study on clean-up agents for TPH analysis is available. Here, such a study is described and it is demonstrated that none of the commonly used agents fulfils the requirements of complete matrix compound removal and TPH recovery. A novel column filled with (top-down) 1 g of 33% w/w 1 M NaOH-impregnated and 2.2 g of 7% w/w H_2SO_4-impregnated silica gel is recommended for cleaning-up biota extracts, as it fully removes extracted lipids and yields acceptable TPH recoveries of around 90%, based on a certified oil reference standard. For sediment extracts, most columns tested resulted in a negligibly low contribution of matrix compounds to the overall detector response, but 5% deactivated Florisil or 10% deactivated aluminium oxide are preferable, because these materials yield the highest (~95%) TPH recoveries.

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Introduction

Petroleum hydrocarbons are ubiquitous contaminants in terrestrial and aquatic environments. Their environmental occurrence is driven by multiple sources, including accidental and deliberate spills from ships, offshore oil production, automobile waste, but also natural seeps. As a result, petroleum hydrocarbons (generally colloquially referred to as "oil") are found in soils, sediments, water, and biota (*1-8*). Crude oils as well as most refined petroleum products are complex mixtures of thousands of mostly nonpolar organic compounds, such as aliphatic and aromatic hydrocarbons (*9*). Because oil, or at least specific compounds in oil, are proven to be toxic to biota (*2,6,10*), risk assessment of oil-contaminated environmental matrixes is needful. To this end, oil concentrations have to be quantified in matrixes such as sediments and biota. Oil concentrations are usually determined by extraction of the matrix with a suitable solvent and subsequent analysis by gas chromatography (GC) with flame ionization detection (FID) or sometimes mass spectrometry (MS). In general, quantification of oil is based on the total peak area between fixed retention times of marker compounds, resulting in a so-called 'total petroleum hydrocarbon (TPH)' concentration. Unfortunately, solvent extraction also yields non-petroleum hydrocarbons, such as soil or sediment organic matter (i.e. degradation products of plants and animals), or lipids in case organisms are under investigation. If not properly removed, these 'interfering matrix compounds' (IMC) will cause an overestimation of TPH concentrations, as they will contribute to the overall detector response by FID or MS. Therefore, appropriate sample clean-up is of great importance when determining TPH concentrations in environmental matrices.

Open column chromatography is frequently used for the purification of extracts from soils, sediments, and biota, prior to TPH analysis. Although standard procedures are available for soils and sediments, no consensus exists on techniques and agents to be applied. For instance, open column chromatography using Florisil is recommended by the ISO procedures (*11*), while Dutch protocols state that extracts should be shaken with this agent (*12*). Standard procedures for the purification of TPH extracts from biota are lacking, which results in the application of a wide variety of agents. Aluminium oxide, Florisil, or silica gel are often used, either alone or in combination, and having different levels of deactivation (*5-7,13-16*). Sometimes, potassium hydroxide is applied prior to column chromatography in order to hydrolyse lipids to free fatty acids and alcohols (*7,13,15*). The choice for a clean-up material in TPH analyses often seems to be based on studies performed on single organic compounds or well-defined mixtures, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). For these chemicals, the performance of different clean-up agents has been well-documented (*17,18*), in contrast to oil, for which there are no comparative studies on the clean-up efficiency of different materials available in the literature. The suitability of an agent for specifically-detected chemicals does however not guarantee satisfactory results for non-specifically-detected TPHs. Still, the wrong choice of clean-up (methodology and) material may lead to highly inaccurate TPH concentrations, not only because of the incomplete removal of IMC, but also due to TPH recovery, which might vary significantly among different materials and methods.

In the present study, the performance of a series of clean-up agents for TPH analysis was compared. Different agents for the purification of extracts from sediments and biota obtained by means of Soxhlet extraction with hexane/acetone were tested in open column chromatography experiments. The agents were evaluated in terms of both clean-up efficiency and TPH recovery, with TPH being defined as the total FID response between the retention times of decane (C_{10}) and tetracontane (C_{40}), thus including both aliphatics and aromatics. The ultimate goal of the work was to identify the most optimal clean-up material to be applied in TPH analysis of sediments, soils, and organisms. Clean-up agents tested were the commonly applied chromatography materials silica gel, aluminium oxide, and Florisil. These materials were tested as pure material, in combination with each other, and/or treated with different percentages of water, acid, or base. Following an initial screening of 15 of these different materials, a stepwise approach was undertaken in which promising agents were selected, modified, and subsequently evaluated in the next step, until the most appropriate agent/combination of agents remained. This material was then validated through testing different oils, different oil concentrations, and extracts from different organisms.

Experimental section

Chemicals and glassware

All solvents (acetone, *n*-hexane, and *n*-heptane) were purchased from Labscan (Dublin, Ireland) and were of Pestican grade. Silica gel (63-200 µm; for column chromatography), Florisil (150-250 µm; for column chromatography), and sodium hydroxide (NaOH; ISO analysis) were from Merck (Darmstadt, Germany). Aluminium oxide (50-200 µm; Super I) was from MP Biomedicals (Eschwege, Germany) and sulphuric acid (H_2SO_4; 95-98%) was from Baker (Deventer, The Netherlands). Tetracontane (C_{40}; >95%) was purchased from Fluka (Steinheim, Germany). Other alkanes (decane (C_{10}), undecane (C_{11}), hexadecane (C_{16}), docosane (C_{22}), and octacosane (C_{28}); all >98 %) were from Sigma Aldrich (Steinheim, Germany) or Fluka. A certified standard oil (RIVM-LOC-001) was purchased from NMi (Nederlands Meetinstituut, Delft, The Netherlands). This oil is a mixture of one mass part of Kuwait gas oil without volatile compounds, and one mass part of Agip Base Oil HVI60. The Dutch Standardization Institute (NEN) recommends this mixture as a standard oil for the quantification of TPH concentrations in environmental samples (12). 'Bilge oil' was sampled from a bilge water depot in a yacht basin (Wageningen, The Netherlands); Distillate Marine grade A (DMA), a nautic fuel oil was provided by Gulf Oil (Nigtevecht, The Netherlands); and an engine oil (Visco2000, BP) was purchased from a local gas station.

Extractions and clean-up experiments were performed in brown-coloured glassware to prevent photo degradation. Glassware was carefully cleaned and rinsed with Pestiscan grade acetone before use. Only glass pipettes and PTFE-free syringes were used for transferring solutions to prevent any loss of hydrophobic compounds by sorption to plastics.

Sediment and biota extracts

Dutch freshwater sediments were sampled from Oostvaardersplassen (OVP) and from a local rural ditch (LB). A Dutch estuarine sediment was sampled from location Oesterput (OP). Organic carbon contents were 3.25 ± 0.08 % for OVP sediment, 4.09 ± 0.126 % for LB sediment, and 1.20 ± 0.08 % for OP sediment, and TPH and PAH concentrations in all sediments were around or below GC-FID detection limits. All sediment samples were passed through a 1 mm sieve, freeze-dried, and ground in a mortar.

Aquatic worms (*Lumbriculus variegatus*) were cultured in 45 L aquaria, using cellulose as substrate. The water was continuously refreshed and aerated, and worms were fed once a week with flake fish food. Worms were scooped out of the aquaria, separated from the substrate, and allowed to clear their guts overnight in running tap water. Juvenile fish (*Poecilia reticulata*) of about 8 weeks old (2 cm) were from our culture, for which it was necessary to reduce the number of fish in order to maintain a healthy population. Fish were placed in aerated clean water for two days to empty their guts, after which they were killed in liquid nitrogen. Living mussels (*Mytilus edulis*) were obtained from a local seafood store and were allowed to clear their guts in artificial seawater (during 20 h). All organisms were frozen, freeze-dried, and ground in a mortar. Lipid contents were determined gravimetrically after extraction with hexane:acetone (3:1) and measured 15.0 ± 0.0140 % of dry weight (dw) for worms, 25.8 ± 0.0507 % for fish, and 8.95 ± 0.0446 % for mussels.

Lipids were extracted from about 7.5 g dw of worms, 2 g dw of mussels, or 1 g dw of fish. Solvent-extractable organic matter (SEOM) was extracted from 20 g of LB sediment and 40 g of OP or OVP sediments. Extractions were performed on freeze-dried samples with 120 mL (mussels and fish) or 250 mL (all other samples) of a 1:3 (v/v) mixture of acetone and hexane in a pre-extracted Soxhlet apparatus for 16 hours. Extracts were concentrated to about 10 mL on a modified Kuderna-Danish apparatus. Subsequently, hexane was added until each mL of the extract was an equivalent of 250 mg dw worms and mussels, 100 mg dw fish, or 800 – 1000 mg dw sediment. These values were assumed to reflect common sample amounts for TPH analysis. Accompanying lipid concentrations in the extracts were about 38 mg/mL for worms, 26 mg/mL for fish, and 22 mg/mL for mussels; and SEOM concentrations were about 1.4 mg/mL for OVP sediment, 3.1 mg/mL for LB sediment, and 1.8 mg/mL for OP sediment. Ten mL test tubes were filled with 1.00 mL of lipid or SEOM solution, 1.00 mL of oil (1000 mg/L), or with both. The combined solutions mimicked a TPH concentration of 4000 mg/kg dw in organisms and 1000 – 1250 mg/kg dw in sediments. Masses of the added oil solution were recorded on an analytical balance to rule out pipette inaccuracies. Tubes were closed and stored at -18°C until clean-up. Subsamples of the extracts were evaporated to dryness and the lipid and SEOM concentrations were determined gravimetrically. Lipid reference samples were made by solvent-exchanging the original lipid solutions to heptane, diluting them ten times, and adding 200 µL of internal standard solution (tetracontane at 300 mg/L in heptane) to 1 mL of the diluted solution. Oil reference samples were made by adding 200 µL of internal standard solution to 1 mL of the standard oil solution.

Table 1. Column details and evaluation results of seven clean-up agents tested for lipid clean-up efficiency and TPH recovery. Values are average ± SD (*n*=4).

Clean-up agent[1]	Eluent (mL)	Colour after purification	TPH recovery (%)	Lipid clean-up efficiency (%)
SiO_2 (2 g, 0 % deact.[2])	30	yellow	83.8 ± 0.90	58.5 ± 1.06
Al_2O_3 (4 g, 10% deact.[2]	30	yellow	94.4 ± 3.69	93.2 ± 0.66
Al_2O_3 (4 g, 10% deact.) + SiO_2 (2 g, 0 % deact.[2])	55	clear	89.1 ± 2.35	98.4 ± 0.61
Florisil (2 g, 5% deact.[2])	30	white/yellow	93.3 ± 1.01	89.1 ± 1.62
SiO_2 (1.7 g, 44% H_2SO_4) + SiO_2 (1 g, 33% 1M NaOH) (44% ABS) [3]	30	clear	74.7 ± 3.77	99.4 ± 0.14
SiO_2 (1 g, 33% 1M NaOH) + SiO_2 (1.7 g, 28% H_2SO_4) (28% BAS) [3]	30	clear	83.8 ± 1.16	99.8 ± 0.11
SiO_2 (1 g, 33% 1M NaOH) + SiO_2 (2.2 g, 7% H_2SO_4) (7% BAS) [3]	30	clear	89.0 ± 1.78	101 ± 0.30

[1] The agent mentioned first is on top. SiO_2: silica gel, Al_2O_3: aluminum oxide.

[2] Deactivation with Millipore water. Values are % (w/w).

[3] Treatment with 95-98% sulfuric acid (H_2SO_4) or 1 M sodium hydroxide (NaOH). Percentages mentioned are on a weight basis.

Open column chromatography

Prior to use, all clean-up agents were heated overnight (16h) at 180°C. Aluminium oxide and Florisil were then deactivated with Millipore water, and, if applicable, silica gel was treated with different amounts of pure sulphuric acid or 33% (w/w) of 1 M sodium hydroxide. Treated agents were shaken thoroughly until no lumps remained.

Glass columns (height 12 cm, internal diameter 1.0 cm) were plugged with quartz wool, filled with the desired amount of agent, and pre-eluted with 25 mL of hexane. When both acidified and basic silica gel were used, the agents were separated by a thin layer of activated, untreated silica gel (0.1 g). The content of each test tube (see above) was then carefully transferred to the accompanying column and the test tubes were rinsed 4 times with 1 mL of hexane by thoroughly vortexing. Once the 4 x 1 mL of hexane had disappeared under the agent's surface, the columns were eluted with a predefined volume of hexane. Final eluates were concentrated to approximately 3 mL on a modified Kuderna-Danish apparatus and then to 0.5 mL under a gentle stream of nitrogen. Resulting extracts were solvent-exchanged to 0.8 mL of heptane, and 200 µL of internal standard solution was added. After vortexing, the extracts were transferred to autosampler vials and stored at 4°C until analysis. All agents were tested in triplicate or quadruplicate, except during the initial screening experiment and for those cases in which comparison and selection was based on FID response only (see below). Here, tests were done singular or in duplicate, respectively. For each agent, the background signal (blank) was determined at least in triplicate. Column details (treatment, amount of agent(s), and eluent volume) are presented in table 1 and appendix II, tables S2-S5.

To determine possible TPH losses during Kuderna-Danish and nitrogen concentration steps, one mL of standard oil solution was dissolved in 30 mL of hexane and treated as eluate, as described above. Only for the hydrocarbon block C_{10}-C_{16} (see section 2.4), significant TPH losses were observed. All final C_{10}-C_{16} concentrations in samples were therefore adjusted for this fraction's recovery (89.1±1.87%; *n*=4).

As outlined in the introduction and appendix II, figure S1, the present study was started with an initial screening in which silica gel, aluminium oxide, and Florisil were compared, either as pure material, in combination, or treated with water, acid, or base (appendix II, table S1). Based on this screening, six (out of the 15 tested) potential agents were selected for further study. The selection was based on both clean-up efficiency and TPH recovery, but frequent use by other laboratories was also included as a criterion. The materials selected were (see appendix II, figure S1) aluminium oxide (deactivated with 10% of water), Florisil (deactivated with 5% of water), silica gel (100%, activated), a combination of (top-down) aluminium oxide (deactivated with 10% of water) and silica gel (100%, activated), a combination of silica gel impregnated with (top-down) 44% (w/w) sulphuric acid and 33% (w/w) 1 M sodium hydroxide (hereafter referred to as 44% ABS – Acid Base treated Silica gel), and an upside-down and modified version of the last column: 33% (w/w) 1 M NaOH-impregnated silica gel on top and 28% (w/w) H_2SO_4-impregnated silica gel at the bottom (hereafter referred to as 28% BAS – Base Acid treated Silica gel). Details of these columns are presented in table 1. The evaluation of all agents was performed with lipids extracted from worms and SEOM extracted from OP, OVP, and LB sediments, and the certified standard oil. The finally selected column was ultimately tested as well with lipids extracted from fish and mussels, three additional oils, and five standard oil-contaminated worm samples containing oil concentrations ranging from 100 to 10 000 mg/kg dw.

GC-analyses

TPH concentrations were measured on a Carlo Erba (Milan, Italy) 8065 gas chromatograph, equipped with a Carlo Erba autosampler, detector, and amplifier. One µL aliquots of the samples were injected on-column on a 2 m pre-column (J&W Scientific, Folsom, CA, USA), coated with deactivated silica. Subsequent separation was accomplished on a 12 m 0.25 mm i.d. DB5.625 column (J&W Scientific). Hydrocarbons were detected with a FID-80 flame ionization detector, operating at 325°C, and the signal was amplified with an EL980 amplifier before recording. Helium was used as carrier gas at 85 kPa. Note that the choice for FID instead of MS is not crucial within the present setup; extracts analyzed either way require clean-up. FID was however preferred for this study, because it is recommended for TPH analysis and it has a lower liability to detector contamination. The oven temperature was programmed as follows: 7 min at 50°C, increasing to 300°C with 10°C/min, and held on 300°C for 12 min. Chromatograms were recorded with Chromcard software (version 2.3.3, Thermo Electron Corporation, Milan, Italy). The area of the tetracontane peak was determined with Chromcard, but the TPH 'hump' area was calculated with Excel (version 2003, Microsoft Corporation), because of the limited capability of Chromcard to integrate similar humps. Accurately integrating the TPH hump often appears problematic, because the detector response usually increases during each run as a result of column

bleeding. We corrected for this by injecting Pestican grade heptane at least ten times during each sequence. In Excel, the areas of both samples and heptane blanks were then calculated against a horizontal baseline, which was based on the average signal value just before the solvent peak. Subsequently, the average value of the heptane blanks was subtracted from each TPH chromatogram. TPH quantification was performed on the basis of a five-point calibration curve, made from the standard oil. Decane and tetracontane had been added as marker and/or internal standard. Each concentration level of the calibration curve was injected at least 4 times during a sequence, resulting in a goodness of fit (r^2) of >0.99 and a low standard error for slope values (<0.1%). Because of the absence of appropriate noise, the detection limit of the GC (ca. 40 mg/L) was defined as 2 times the standard deviation of the area of the heptane blanks, and was well below the lowest calibration standard of 100 mg/L. All samples, including calibration standards, were normalized to the internal standard. To interpret chromatograms and to distinguish TPH recoveries and clean-up efficiencies of different boiling point fractions, chromatograms were divided into four hydrocarbon blocks (C_{10-16}, C_{16-22}, C_{22-28}, and C_{28-40}), based on the highly stable (<0.1%) retention times of C_{10}, C_{16}, C_{22}, C_{28}, and C_{40}.

Results and discussion

The importance of sample clean-up

Unpurified worm extracts resulted in GC-FID chromatograms with a clear hump from C_{15} to far beyond C_{40} with several individual peaks (see figure 1). Based on retention times, none of the individual peaks could be identified as *n*-alkanes, illustrating the non-petrogenic origin of the response. Because of the solvents used, extracted lipids will likely consist of non-polar triacylglycerols and other simple lipids, whereas polar phospholipids might be absent (*19*). In contrast to lipids, sediment organic matter is more complex and consists of a mixture of numerous organic compounds, including straight chain and branched alkanes, fatty acids, and aromatics (*20*). Only a part of the organic matrix present in soils and sediments is solvent-extractable. Therefore, SEOM concentrations in unpurified extracts were always lower than the organic matter contents of the sediments. All unpurified sediment extracts produced a GC-FID response that consisted of noise around the baseline, a clear hump from C_{18} to C_{40}, and several sharp peaks in the range of C_{20}-C_{34} (see figure 1). The majority of these peaks were identified as odd-numbered straight chain alkanes, which might be an indication of biogenic sources, such as plant waxes (*21,22*) or algae (*6,23,24*).

In order to quantitatively illustrate the importance of clean-up, the chromatographic areas produced by IMC extracted from worms and sediments were interpolated in the TPH calibration curve and the accompanying artificial 'TPH concentrations' in the samples were calculated. These measured 33 100 ± 1310 mg/kg dw for worms, 240 ± 90.0 mg/kg dw for OVP sediment, and 924 ± 60.0 mg/kg dw for LB sediment. Due to the low organic content of OP sediment, 'TPH concentrations' for unpurified extracts of this sediment were below the detection limit (<40 mg/kg dw). The high FID responses

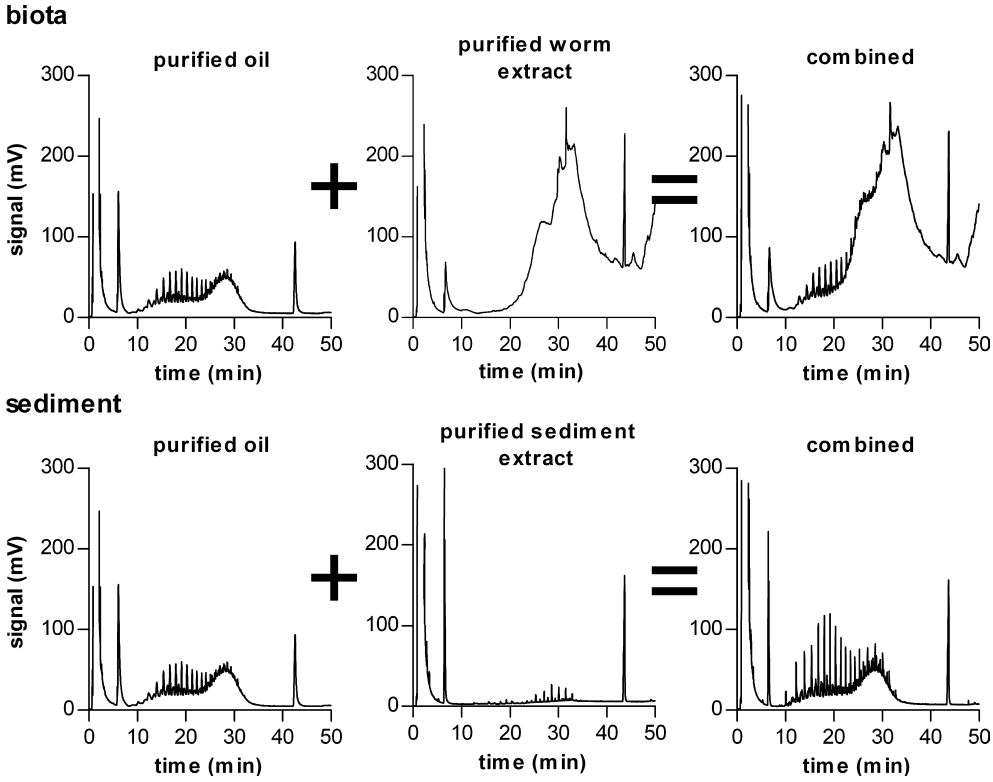

Figure 1. GC-FID chromatograms demonstrating the effect of incomplete clean-up of worm and sediment extracts through activated silica gel.

caused by IMC in both unpurified worm and sediment extracts unambiguously stress the necessity of clean-up prior to TPH analysis.

This necessity also became visible when analyzing oil solutions that were mixed with worm extracts and subsequently purified through various materials. The upper part of figure 1 demonstrates that the resulting contribution of lipids to the overall FID response can be very large. Under the conditions applied (i.e. mimicking a TPH concentration in worms of 4000 mg/kg dw), lipids for instance contributed for 78% to the response in case of clean-up through silica gel (figure 1). Florisil reduced this contribution to 45%, aluminium oxide to 38 %, and the combination of aluminium oxide and silica gel to 14 % (figure 2). Lipids did not significantly increase the total FID response in case silica gel was impregnated with sulphuric acid (figure 2). It should be noted however that TPH concentrations in organisms sampled from oil-contaminated soils, sediments, and water have often been observed lower (e.g. 86-3975 mg/kg dw (*6,7,13*)) than the concentration tested in this study. In such cases, the contribution of IMC will be higher. In sediment extracts however, the contribution of IMC to the overall FID response was negligible, as shown in the lower part of figure 1. The extracts (1000-1250 mg oil/kg dw) resulted in some additional peaks, mainly from odd-numbered

alkanes, but did not significantly increase the overall response. TPH concentrations in contaminated soils and sediments are usually higher than the testing concentration applied here. For instance, oil concentrations of >8000 mg/kg dw have been reported (*8,25,26*), which implies an even lower contribution of IMC to the total FID response.

From the above, it can be concluded that the necessity of clean-up generally depends on the TPH concentration in the sample under investigation and the relative contribution of IMC to the overall detector response. Purification of biota extracts however appears unavoidable and the choice of clean-up material is crucial. For sediment extracts, clean-up is required, but the type of the clean-up material used is less essential.

Purification of biotic extracts

As mentioned in section 2.3 and depicted in appendix II, figure S1, the initial screening study resulted in the selection of six agents to be evaluated further for lipid removal efficiency. Out of these six materials, application of silica gel, aluminium oxide, Florisil, and the combination of aluminium oxide with silica gel did not result in lipid-free extracts. Clean-up efficiencies ranged from 58.5 % for silica gel to 98.4 % for the combined aluminium oxide/silica gel column (table 1) and neither of the extracts eluted through these agents resulted in a flat baseline (figure 2A-D). The low clean-up of silica gel is probably explained by the fact that this material retains polar compounds (e.g., phospholipids), whereas non-polar compounds (e.g., triacylglycerols) that likely dominate the composition of the worm extracts and oil will be eluted with non-polar solvents (*19,27*). Although the observed chromatographic pattern (figure 2A) may also result from column overload, this artefact is not plausible here, because the lipid amount tested was below the recommended load of 30 mg/g silica gel (*19*). Note that silica gel's clean-up efficiency cannot be improved by deactivation of the material or by decreasing the amount of eluent volume (*19*).

The mechanism behind clean-up by Florisil and aluminium oxide is not only based on the retention of polar compounds by polar interactions, but probably also on specific Lewis acid/base interactions with the electrophilic carbonyl groups in lipid molecules (*28*). The basic character of aluminium oxide and Florisil (pH 9-10) might play an important role, because clean-up efficiencies of neutral aluminium oxide were reported to be lower than those of basic aluminium oxide (*18,29*). Autoxidation of double bonds and/or hydrolysis of lipids has also been suggested as clean-up mechanism for aluminium oxide (*19*). Such additional interactions may explain the higher clean-up efficiencies of these materials compared to that of silica gel, but extracts still contained considerable amounts of lipids (figure 2B-C, table 1).

Less IMC were observed in extracts cleaned-up through the combined aluminium oxide-silica gel column, for which the eluent volume was increased to 55 mL, because of positive effects on TPH recovery (table 1). Still, an elevated GC-FID response was clearly visible (figure 2D). A similar pattern has been observed after the purification of mussel extracts (*6,12*), even in case the extracts were saponified with potassium hydroxide prior to open column chromatography (*12*). Although it was suggested that the interfering signal may have been caused by the presence of algae in the mussels'

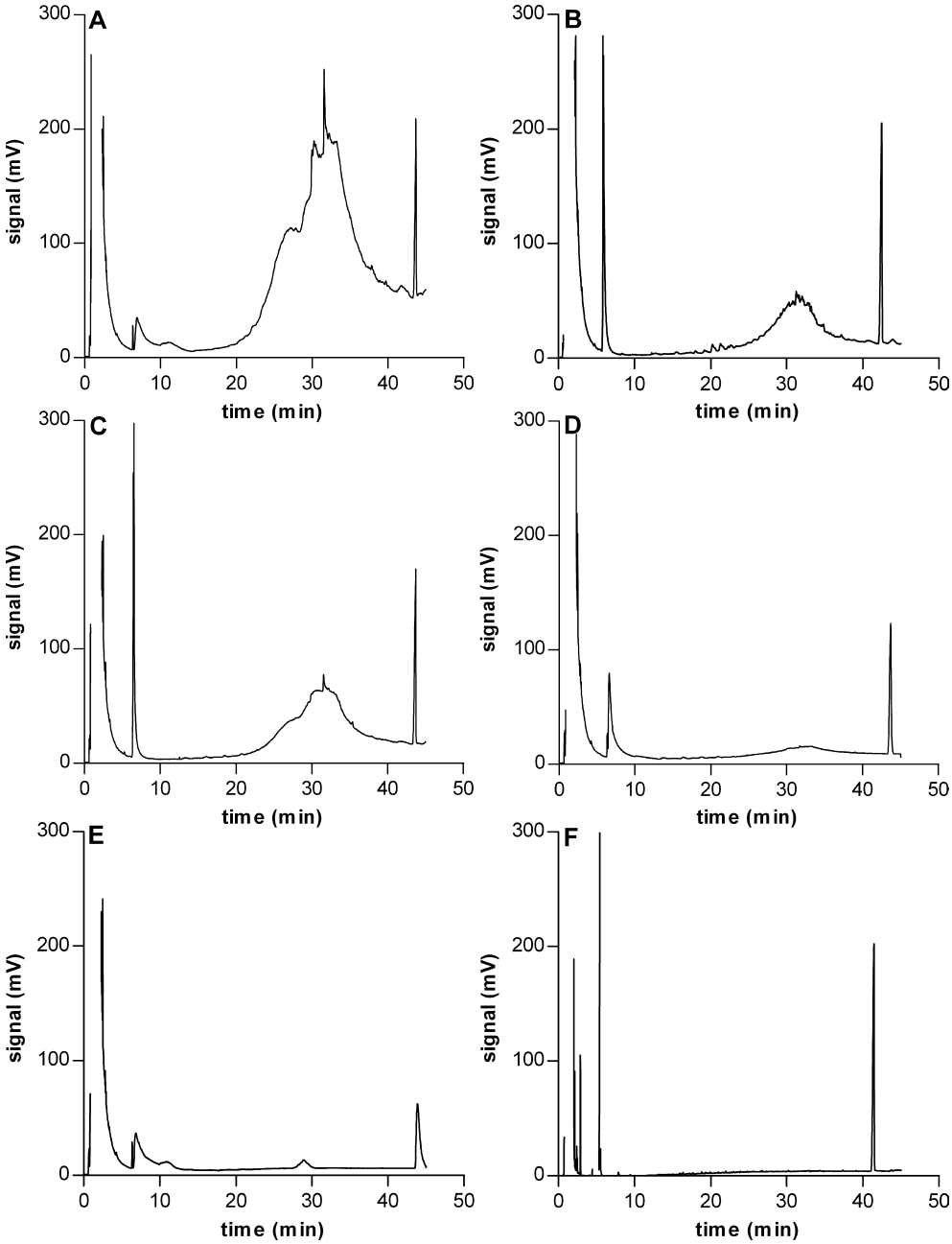

Figure 2. GC-FID chromatograms of worm extracts cleaned-up through silica gel (A), aluminium oxide (B), Florisil (C), aluminium oxide/silica gel (D), H_2SO_4 (top, 44% w/w)/NaOH (33% w/w)-impregnated silica gel (E), and NaOH (top, 33% w/w)/H_2SO_4 (28% w/w)-impregnated silica gel (F).

guts (animals were not gut-purged prior to analysis) (*6*), the present results indicate that aluminium oxide-silica gel columns are not able to remove all solvent-extractable biotic material.

The performance of the above-discussed columns (except silica gel) might be improved by increasing the activity of the agents through adding less water, or by decreasing the eluent volume. This was tested for Florisil and aluminium oxide. Clean-up efficiency of both agents indeed consequently increased to about 95% with increasing deactivation (appendix II, table S2), whereas reducing eluent volumes had no effect (appendix II, table S3). In spite of the relatively high clean-up efficiency, however, accompanying chromatograms were still noisy. Therefore, Florisil and aluminium oxide were finally concluded inappropriate for cleaning-up biotic extracts for TPH analysis. Silica gel and the combined aluminium oxide-silica gel column were concluded unsuitable for the same reason, but also because of their low TPH recoveries, which will be discussed in the TPH recoveries section.

In contrast to the agents discussed above, clean-up efficiencies for multilayered columns filled with silica gel impregnated with (sulphuric) acid and silica gel impregnated with a base (sodium hydroxide) were towards 100% (table 1). Multilayered columns filled with (top-down) acid and basic silica gel were introduced for the analysis of dioxins in environmental samples, including biota and human milk (*30,31*). In such columns, lipids are oxidized in the acidic part and neutralized in the basic part. The latter step prevents charring during the subsequent solvent evaporation steps (*31*). These columns are recommended by the US-EPA for the analysis of dioxins in solid waste samples (*32*), but also have been used for cleaning-up extracts from biota for the analysis of PCBs (*33*) and halogenated benzenes (*34,35*). In some cases, only acidified silica gel is used without the addition of a second, base-treated layer (*17,18,36,37*). In the present study, application of the 44% ABS column resulted in almost completely lipid-free extracts. Despite a high clean-up efficiency of >99% for this column (table 1), a small chromatographic hump was however still visible (figure 2E). Because it was expected that caustic digestion (saponification) will occur when lipids are treated with a base first, the column was also tested upside-down (i.e., base on top). Treatment with an acid in the second stage is then expected to result in full hydrolysis of lipids, yielding alcohols and free fatty acids. At the same time, the percentage of acid was reduced to 28%, because of an expected positive effect on TPH recovery, which was relatively low for the 44% ABS column (see section 3.4). Remarkably, the resulting unconventional 28% BAS column produced completely lipid-free chromatograms (figure 2F). Therefore, this column was subjected to further evaluation.

In this next evaluation stage, first of all the percentage of sulphuric acid was further reduced to 14 and 7%, because of an expected beneficial effect on TPH recovery (discussed in section 3.4). The reduction however did not significantly affect the clean-up efficiency (appendix II, table S4). Unfortunately, the capacity of the column containing 7% of sulphuric acid appeared to be limited to 250 mg dw worms, which corresponds to ca. 50 mg of hexane:acetone-extractable lipids. Larger sample amounts gave breakthrough effects; the entire acidic part of the column coloured black and the

eluates were deep yellow. Increasing the amount of acidic silica gel from 1.7 to 2.2 g however increased the column capacity to 75 mg of hexane:acetone-extractable lipids, without affecting TPH recovery (appendix II, table S5). Varying the amount of basic silica gel had no significant effect on both clean-up efficiency and TPH recovery (appendix II, table S5). In order to test this column's applicability for lipids extracted from other organisms, also fish and mussel hexane/acetone extracts were cleaned-up. As demonstrated by appendix II, figure S2, all eluates were fully free of IMC. A column containing (top-down) 1 g of 33% w/w 1 M sodium hydroxide-impregnated silica gel and 2.2 g of 7% w/w sulphuric acid-impregnated silica gel (7% BAS) therefore seems most optimal for the purification of biota extracts for TPH analysis (table 1).

In contrast to the agents discussed before (Florisil, aluminium oxide, silica gel), the mechanism of lipid removal by sulphuric acid-impregnated silica gel is based on oxidation of lipids (*31,37*), rather than on retention. This is visually illustrated by the formation of black 'burning residues' upon elution of lipids through acid-treated columns. Still, when worm extracts were purified through acidic silica gel only, the chromatograms showed several small peaks, an observation reported before (*17,18,38*). Along with the results from the BAS column evaluation experiment (discussed above), this indicates that the addition of basic silica gel, as well as the order of the two agents, is essential for obtaining lipid-free extracts. The exact reason behind this phenomenon is not completely clear. Caustic digestion, as was suggested above, is however unlikely, because an attempt to purify worm extracts through basic silica gel only resulted in clean-up efficiencies and chromatograms being comparable to those observed for unmodified silica gel (appendix II, table S1). The addition of 1 M sodium hydroxide results in deactivation of the silica gel and therefore a decrease in retention of nonpolar compounds (*39*), rather than hydrolysis of lipids. Moreover, appendix II, table S5 demonstrates that the clean-up efficiency was not improved when the amount of basic silica gel was increased. Possibly, the basic part of the column retains acidic compounds, which cause interferences when they are oxidized with sulphuric acid first. Whatever the exact explanation for the order requirement may be, basic silica gel on top reduced the percentage of sulphuric acid needed for full lipid removal, which advantageously prevents clogging of the column by burning residues in the acidic part.

Purification of sediment extracts

In addition to functional groups, as present in lipids, SEOM may also contain molecules without functional groups, like straight chain alkanes (*20,40*), derived from plant waxes (*21,22*) or algae (*6,23,24*). Such compounds are also present in oil (*9*) and full removal of IMC from sediment and soil extracts may therefore be problematic. However, distinguishing between petrogenic and biogenic sources may be possible, because odd-numbered alkanes prevail in extracts with biogenic origin, while both even- and odd-numbered alkanes are present in oils (*23*). Although this phenomenon allows classification of a chromatogram as either biogenic or petrogenic, subtracting a 'non-petroleum' background from a TPH signal is unfeasible.

For the sediment extracts in the present study, the clean-up agent comparison was based on GC-FID chromatograms rather than on clean-up efficiencies. Reason was that SEOM concentrations tested were low (0.18 ± 0.0068% of dw in OVP sediment and 0.22 ± 0.0084% of dw in OP sediment) and extracts after purification only caused FID responses around the detection limit, which impeded reliable calculations of clean-up efficiencies. Also, specific biogenic compounds present in the extracts could not be removed by the clean-up procedure. Consequently, clean-up efficiencies could never reach 100%. Therefore, minimal noise between SEOM peaks was taken as main criterion of judgment. For LB sediment, however, the SEOM concentration was higher (0.31 ± 0.0044 % dw) and this sediment's extracts therefore did allow reliable calculations of the clean-up efficiency.

Initially, only the performance of silica gel (activated), aluminium oxide (10 % deactivated), Florisil (5% deactivated), and a combination of silica gel and aluminium oxide was evaluated with extracts from OVP and OP sediment (see table 1 for column details). Because of the presumed low occurrence of animal lipids, it was not expected that acidified and basic silica gel would be additionally beneficial and therefore, these agents were excluded from this evaluation. The clean-up procedure with the above four agents resulted in colourless extracts and little noise between the odd (biogenic) alkane peaks (figure 3A-D), although the heights of these peaks were similar to those in the unpurified sediment extracts. The performances of the four agents were all comparable. In a second series of experiments, it was tried to reduce the FID response by using 7% BAS columns, the preferable column for lipid removal. These tests were done with extracts from OVP, OP, and LB sediments. For comparison, LB extracts were also purified through 5% deactivated Florisil, resulting in a clean-up efficiency of 92.2 ± 2.89% and a FID response similar to that obtained for OP and OVP sediment extracts. Purification of the sediment extracts through 7% BAS columns removed the noise between the odd-numbered alkane peaks (appendix II, figure S3), probably due to the removal of animal lipids. Quantitatively, however, purification through 7% BAS columns was not favourable as compared to Florisil, because the materials' clean-up efficiencies for LB sediment extracts were not significantly different (94.1 ± 2.23 vs. 92.2 ± 2.89%).

In summary, the contribution of SEOM to the overall detector response was low in the present setup and was mainly caused by the presence of biogenic alkanes. No significant differences between the different clean-up agents were observed. Most probably, the choice for a clean-up agent for sediments with relatively low (<4 %) organic carbon contents is therefore not crucial, although Florisil, which is used by many laboratories working according to ISO 16703 (*11*), or aluminium oxide are preferable due to their highest TPH recoveries (to be discussed in section 3.4).

TPH recoveries

TPH recovery upon purification through silica gel and a combination of aluminium oxide and silica gel was relatively low (table 1), which might be caused by the retention of aromatic compounds by silica gel upon elution with hexane (*9,41*). TPH recoveries of aluminium oxide and Florisil were 93-94 % (table 1). Both eluent volume and degree of

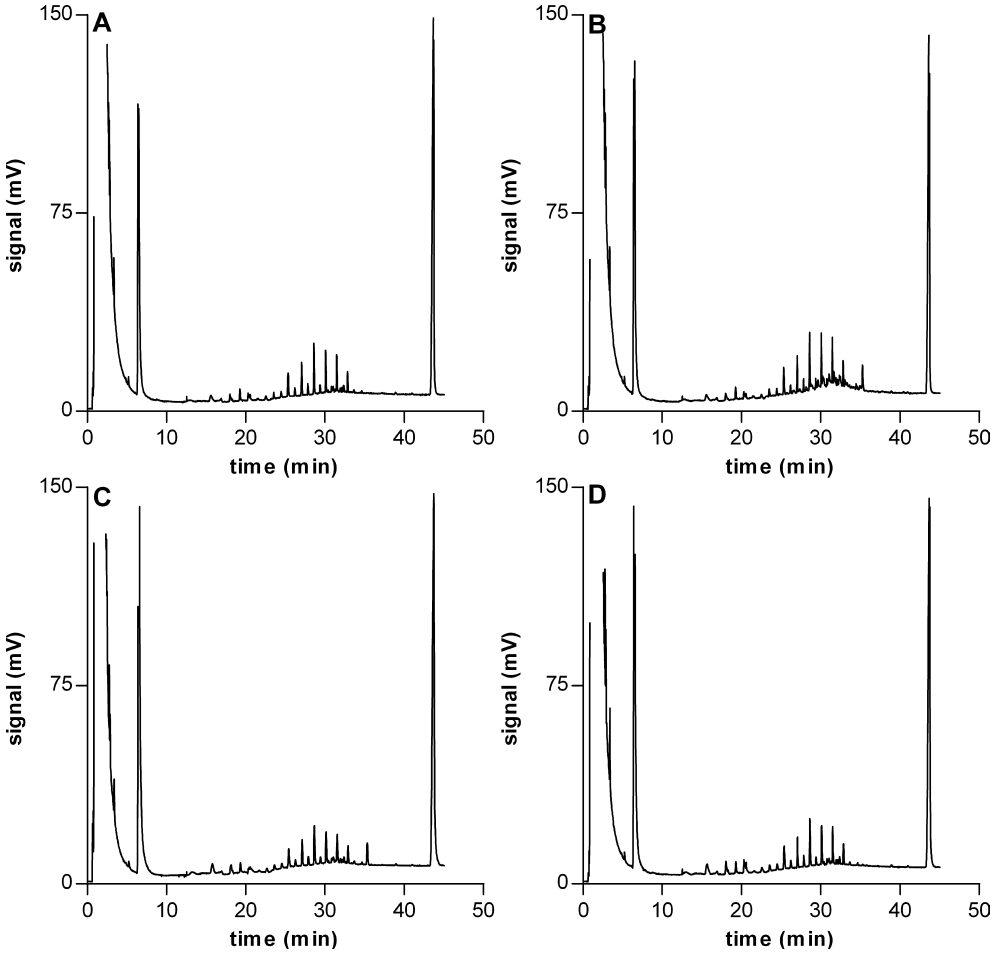

Figure 3. GC-FID chromatograms of sediment (OVP) extracts cleaned-up through silica gel (A), aluminium oxide (B), Florisil (C), and aluminium oxide/silica gel (D).

deactivation appeared to have no significant effect on the TPH recovery of these materials, with the exception of activated (i.e., 0% deactivated) Florisil, for which recoveries were around 80% (appendix II, tables S2-3). Similar to silica gel, Florisil in its pure form apparently is too active and deactivation is needed to reduce the interaction with specific (probably aromatic) compounds. TPH recovery for the 44% ABS column was the lowest of all columns tested (table 1). This could be the result of loss of unsaturated hydrocarbons and aromatics, which can react with sulphuric acid (*28*). For instance, at room temperature, PAHs react with sulphuric acid to polycyclic aromatic sulphonic acids (*42,43*), which will be retained in the lower, basic part of the column. Also, the acidic part of the 44% ABS column turned slightly grey upon oil elution, which might be an indication of 'burning residues' of easily oxidisable hydrocarbons. Exchanging the acidic and basic parts (28% BAS column) resulted in a significantly

higher TPH recovery (table 1). This increase in recovery might however be due to lowering the acid percentage rather than to the change of material order. Indeed, when the percentage of sulphuric acid was further reduced to 7% (7% BAS column), TPH recovery increased to 90% (appendix II, table S4), without affecting the clean-up efficiency (discussed above). Therefore, the 7% BAS column, being the preferable column for lipid removal, seems also acceptable in terms of TPH recovery (table 1). Although 5% deactivated Florisil, and 5 or 10% deactivated aluminium oxide have higher TPH recoveries, it should be emphasized that they can only be used in case of low concentrations of IMC in extracts (i.e. extracts of organic-poor sediments). In order to investigate the recovery performance of the 7% BAS column over a wide concentration range, the column was loaded with worm extracts, contaminated with oil concentrations of 100 to 10 000 mg/kg dw. As shown in appendix II, figure S4, the TPH recovery was not affected by the concentration, which implies that the recommended column can straightforwardly be used to detect varying concentrations in environmental matrixes.

Finally, it should be mentioned however that TPH recoveries might depend on the composition of the oil investigated. Lower TPH recoveries may for instance be expected for oils containing high concentrations of unsaturated hydrocarbons or PAHs. Also, lubricating oils often contain different amounts and types of (non-petrogenic) additives that may behave differently from the bulk oil when cleaned-up. To test this hypothesis, the recoveries of three additional different oils (a nautic gasoil, a car lubricant, and a waste 'bilge oil') upon elution through the 7% BAS column were determined. Results showed that recoveries indeed somewhat differed and ranged from 75% for the nautic gasoil to 90% for the car lubricant (appendix II, table S6). The relatively low recovery of the nautic fuel oil is probably explained by this oil's extremely high aromatic content of 47% (*44*), which is about twice as high as the percentage generally reported for oils (*41,44*). As mentioned above, specific aromatic compounds may be vulnerable to the sulphuric acid. Because it is expected that the majority of the oil contamination in the aquatic environment concerns mixtures of fuels and lubricants (as represented by the bilge and standard RIVM oil), and the recovery of such oils is about 85-90% on the 7% BAS column, this column should be considered appropriate for a wide range of applications.

Conclusions

The ideal agent for the purification of extracts by open column chromatography prior to TPH analyses should be able to yield both maximum TPH recovery and clean-up efficiency. Because of unavoidable reactions between specific petroleum hydrocarbons and clean-up material on the one hand, and the similarities between IMC and target analytes on the other, an agent completely fulfilling both purification requirements most probably does not exist. Based on the present study, a column filled with (top-down) 1 g of silica gel impregnated with 33% (w/w) 1 M sodium hydroxide and 2.2 g of silica gel impregnated with 7% (w/w) sulphuric acid (7% BAS column) is however recommended for cleaning-up biota extracts. For such extracts, clean-up is indispensable and application of the novel 7% BAS column resulted in completely lipid-free extracts as well as an expected, acceptable TPH recovery of about 90% for

environmentally occurring oils. Because of the common presence of lipid-like substances in sediments, the 7% BAS column is appropriate for sediment extracts as well. In most cases (i.e., at least for sediments having organic carbon contents < 4%), the choice for a clean-up material for sediment extracts is however less essential, as the contribution of unretained SEOM to the overall detector response is similarly low for all materials. Hence, agent selection can be based on TPH recovery only and Florisil (5% deactivated; 2 g) and aluminium oxide (10% deactivated; 4 g), both having somewhat higher TPH recoveries as compared to the 7% BAS column, will generally be preferable.

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Chapter 4

A closer look at bioaccumulation of petroleum hydrocarbon mixtures in aquatic worms

Barry Muijs, Michiel T.O. Jonker

Institute for Risk Assessment Sciences, Utrecht University

Abstract

Petroleum hydrocarbons (oils) are ubiquitously present in the aquatic environment and adequate risk assessment is therefore essential. Bioaccumulation plays a key role in risk assessment, but the current knowledge on bioaccumulation of oils is limited. Therefore, this process was studied in detail, using the aquatic worm *Lumbriculus variegatus* and 14 field-contaminated sediments. The main focus during the study was on uptake kinetics, the relationship between oil boiling point fraction and uptake, and effects of sediment characteristics. Uptake kinetics became slower with increasing boiling point fraction, but 70-90 % of the equilibrium situation was reached within the standard exposure duration of 28 d. Worms accumulated sedimentary petroleum hydrocarbons in the range of C_{10}-C_{34}, a range much wider than expected. Biota-to-sediment accumulation factors (BSAFs) for separate boiling point fractions were constant and around the proposed value of 1-2 up to C_{22}, but gradually decreased beyond this point. The decrease was probably caused by a combination of non-equilibrium conditions and enhanced sorption of higher boiling point fractions to sediments; the latter possibly due to the presence of strongly-sorbing separate oil phases or e.g. black carbon. A negative relationship was observed between BSAF and oil concentration in sediment, which was explained by the presence of separate oil phases at high oil concentrations. These strongly-sorbing phases may limit their own availability (in particular when being highly weathered) and may also be avoided by worms. The observed phenomena have obvious implications for bioaccumulation assessment of oils and suggest that the current risk assessment procedure for oils in sediments may lead to erroneous results.

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Introduction

Natural seepage as well as numerous accidental and deliberate oil spills have led to the ubiquitous presence of petroleum hydrocarbons (oils) in the aquatic environment. Petroleum hydrocarbons affect aquatic life not only via smothering caused by direct exposure to liquid oil phases, but also through bioaccumulation (*1-5*) and subsequent toxicity (*2,6*) when exposed to water soluble fractions or fractions present in sediments. Proper and reliable risk assessment of oil-contaminated aquatic systems is therefore of vital importance.

Within risk assessment, bioaccumulation plays a key role, because only the compounds that are taken up will reach the ultimate biological target site, e.g., membrane lipids in case of narcotic petroleum hydrocarbons (*7,8*). Bioaccumulation studies on oil are however scarce and mainly focused on the assessment of environmental impact after oil disasters (*1,5*) or the effects of anthropogenic sources on aquatic life (*2-4*). The aforementioned studies do have indicated that petroleum hydrocarbons are potentially bioaccumulative, but several (mechanistic) questions remain. For instance, the kinetics of bioaccumulation are yet unknown, the relationship between uptake and specific oil fractions is unclear, and information on any effects of exposure characteristics (e.g., oil concentration) is lacking.

One reason for the lack of data on oil bioaccumulation may be the fact that the process is very complicated. Petroleum hydrocarbons are mixtures of thousands of mainly hydrophobic compounds with a complex environmental behaviour (*9,10*). Most probably because of this, bioaccumulation assessment after oil spills usually focuses on polycyclic aromatic hydrocarbons (PAHs) (*11,12*), which are easily detectable and are generally considered the most toxic petrogenic compounds. However, a similar approach neglects toxic effects of other compounds presents in the mixture, such as branched indanes and tetralins (*13,14*). Assuming that oil mainly has a non-specific mode of action (*7,8*), each individual accumulated compound will contribute to the adverse effects caused by exposure to oil pollution. As such, it would be necessary to include all petrogenic compounds in the risk assessment of oils. Unfortunately, analyzing the thousands of individual compounds is not feasible. In response, the so-called 'hydrocarbon block' approach has been proposed, in which the total petroleum hydrocarbon (TPH) response between the retention times of defined alkanes as derived from gas chromatography, is divided into blocks containing chemicals with similar characteristics and physical-chemical properties (*15*). Although such an approach may require additional analyses or sophisticated analytical equipment like two-dimensional gas chromatography, the hydrocarbon block method definitely improves risk assessment of oils.

The objective of the present study was to gain insight into some mechanistic aspects of bioaccumulation of petroleum hydrocarbons by using the hydrocarbon block approach and thereby to produce basic knowledge for the risk assessment of the complex mixture. To this end, bioaccumulation of oil in the aquatic worm *Lumbriculus variegatus* was determined after exposure to a series of field-contaminated sediments with a known history of oil pollution. Both uptake kinetics and 'steady state'

concentrations were determined and the results were interpreted in terms of uptake rate constants and biota-to-sediment accumulation factors (BSAFs), which were related to both oil and sediment characteristics (i.e., hydrocarbon block number, and organic carbon and TPH concentration, respectively). TPH was defined as the response between the retention times of decane (C_{10}) and tetracontane (C_{40}) on a gas chromatograph with a flame ionisation detector (FID) after Soxhlet extraction and purification through validated clean-up agents (Chapter 2) The present paper is part of a study that aims at developing and verifying methods to improve risk assessment of petroleum hydrocarbons in sediments. Subsequent papers will deal with methods to predict bioaccumulation in the lab as well as the field.

Experimental section

Chemicals

Solvents (*n*-hexane, *n*-heptane, and acetone; all Pestiscan grade) were obtained from Lab-Scan (Dublin, Ireland). Silica gel (63-200 µm), hydrochloric acid (37%, analytical grade), sodium hydroxide (analytical grade), and anhydrous sodium sulphate (analytical grade) were from Merck (Darmstadt, Germany). Aluminium oxide (50-200 µm; Super I) was obtained from MP Biomedicals (Eschwege, Germany) and was deactivated with 10% (w/w) of Millipore water. Sulphuric acid (95-98%) was obtained from Baker (Deventer, the Netherlands) and tetracontane (C_{40}; >95%) was purchased from Fluka (Steinheim, Germany). Other even-numbered *n*-alkanes between C_{10} and C_{36}, as well as C_{11} (all >98 %) were purchased from Sigma Aldrich (Steinheim, Germany) or Fluka. Silver cups (12.5 × 5 mm) were from Van Loenen (Zaandam, The Netherlands) and atropine from Sigma (99%). Silver cups were Soxhlet-extracted with acetone:hexane (1:3 v/v) for 4 h. Sodium sulphate was heated at 600°C for 3 h in a stove and cooled in a desiccator. A certified standard oil (RIVM-LOC-001) was purchased from NMi (Nederlands Meetinstituut, Delft, The Netherlands).

Sediments and worms

Oil-contaminated sediments were sampled from several locations across the Netherlands. ROSL, SCSL, and WOGR sediments were provided by Wageningen University and VK16 was provided by AquaSense (Colijnsplaat, the Netherlands). All sediments were sieved (1 mm), homogenized, and stored at 4°C. Dry weights were determined gravimetrically on dried (105°C) sediment samples. Sediment characteristics, likely oil sources, and TPH concentrations are presented in table 1. Worms (*Lumbriculus variegatus*) were cultured in a 45 L aquarium filled with pulverized cellulose and copper free water (24°C). The water was continuously aerated and refreshed, and worms were fed once a week with dried fish food. Prior to bioaccumulation experiments, worms were gut-purged overnight in running tap water.

Bioaccumulation experiments

One-L bottles were filled with approximately 0.2 L of sediment and 0.7 L of tap water. The bottles were placed in a climate-controlled room (20°C, 12/12 h day/night) and

after the sediments had settled (24 h), 5 g (wet weight) of worms was added. The overlying water was gently aerated with activated carbon-purified air. Uptake kinetics were determined for HIJG and KVHB sediment, by measuring TPH bioaccumulation after 3, 5, 8, 11, 14, 19, 22, 27, 35, and 49 d of exposure. Twenty eight-day bioaccumulation was subsequently determined for all sediments. During exposure, approximately 75% of the overlying water was refreshed every two weeks. Upon finishing the exposures, worms were removed from the sediments and gut-purged for 6 h in 100 ml Erlenmeyer flasks containing tap water. By placing the flasks on a rotary shaker (90 rpm, 20°C) and refreshing the water three times, re-ingestion of faeces was minimized. Visual observations indicated that no sediments particles were present in the worms' digestive tracts after 6 h of gut-purging, while significant loss of less hydrophobic compounds is not expected upon this duration (*16*). Worms were subsequently blotted dry with a tissue, frozen (-18°C), and freeze-dried. All sediments were tested in triplicate or quadruplicate.

Extraction of worms and sediments

Freeze-dried worms (250 mg) and sodium sulphate-dried sediments (1 g of wet weight) were placed in glass fibre thimbles and extracted with 120 ml of acetone:hexane (1:3 v/v) for 16 h in a pre-extracted Soxhlet apparatus. Extracts were evaporated to approximately 3 ml on a modified Kuderna-Danish apparatus and subsequently to approximately 1.5 ml under nitrogen. Worm extracts were then purified through multi-layered columns, containing 1.0 g of 33% sodium hydroxide (1M)-impregnated silica gel and 2.2 g of 7% sulphuric acid-impregnated silica gel (Chapter 2). Sediment extracts were cleaned-up through columns filled with 4 g of deactivated aluminium oxide (Chapter 2). After elution with 30 ml of *n*-hexane, the extracts were collected in calibrated pointed flasks, evaporated to 0.5 ml using Kuderna-Danish and nitrogen, successively, and solvent-exchanged to 0.8 ml of *n*-heptane. Finally, internal standard (200 µl of 300 mg/L tetracontane in heptane) was added and the extracts were transferred to autosampler vials. All samples were stored at room temperature until analysis to prevent precipitation of tetracontane. Blanks and recoveries were determined by extracting the extraction mixture only or a pre-weighed volume of the certified standard oil, respectively. Recoveries were between 85 and 100 % for both the sediment and worm clean-up procedure.

GC analysis

TPH concentrations were measured on a Carlo Erba 8065 gas chromatograph system (Milan, Italy), consisting of an autosampler (AS800), a Flame Ionisation Detector (FID-80), and an amplifier (EL980). Samples were on-column injected on a 5 m pre-column (J&W Scientific, Folsom, CA), coated with deactivated silica, and then separated on a 15 m 0.25 mm i.d. VF-5ms column (Varian, Lake Forest, CA). Injection volumes were 1 µl for the sediment extracts and 3 µl for the worm extracts. The oven was programmed as follows: 5 min at 80°C, increasing with 15°C/min to 300°C, and steady at 300°C for 17 min. Responses were recorded with Chromcard software (version 2.3.3, Thermo Electron Corporation, Milan, Italy). The tetracontane peak was

integrated with Chromcard, but the TPH 'hump' was integrated with Excel (version 2003, Microsoft Corporation) (Chapter 2). TPH quantification was performed on the basis of a five-point calibration curve, made from RIVM oil. All samples, including calibration standards, were normalized to the internal standard. Boiling point fractions (hydrocarbon blocks) were based on the stable retention times of 16 *n*-alkanes between C_{10} and C_{40}.

Lipid and organic carbon analyses

The lipid content of all worm samples was determined by weighing 100 mg of freeze-dried material into 10 ml tubes. The samples were then extracted with 4 ml of acetone:hexane (1:3 v/v) by vortexing (1 min at 2200 rpm) and sonification (5 min), followed by centrifugation (5 min at 1500 rpm). After collecting the solvent phase, the remaining pellets were extracted another two times and the pooled extracts were evaporated to dryness under nitrogen. Lipid contents were then determined gravimetrically. Organic carbon and nitrogen were determined by weighing 5 to 20 mg of freeze-dried sediment into silver cups, after which carbonates were removed by adding hydrochloric acid (2M). The samples were analyzed on an elemental analyzer (Carlo Erba EA1110), equipped with an activated copper column held at 1070°C. Carbon and nitrogen were quantified using atropine as a calibration standard.

Results and discussion

TPH uptake kinetics

TPH concentrations in *L. variegatus* reached an apparent equilibrium after approximately 50 d of exposure (figure 1A). Fitting a first order one compartment model to the uptake profiles of different boiling point fractions indicated that kinetics became slower with increasing boiling point (figure 1C-D). For the HIJG sediment, an uptake rate constant (± standard error) of 0.065 ± 0.0098 d^{-1} was obtained for the C_{16}-C_{22} fraction, which was clearly higher than for the C_{22}-C_{28} and C_{28}-C_{34} fractions (0.033 ± 0.0094 and 0.031 ± 0.0099 d^{-1}, respectively). Uptake rate constants for the KVHB sediment were lower, but demonstrated a similar relationship with boiling point, as has also been reported in the literature for PAHs and polychlorinated alkanes (*17-19*). Note however that for this sediment the concentrations of the fraction C_{28}-C_{34} did not exceed the detection limit. The obtained uptake rate constants suggest that the overall bioaccumulated (C_{11}-C_{34}) fraction will equilibrate within 43 d in case of the HIJG sediment and within 74 d for the KVHB sediment. Equilibration of petroleum hydrocarbon fractions beyond C_{34} may take >90 d, but the contribution of these fractions to the overall TPH uptake expectedly is negligible. For the fraction C_{11}-C_{16}, a deviating kinetic profile was observed; the concentration of this fraction reached a maximum after 14 d, but then decreased again (figure 1B). Although the concentrations of this fraction were close to the detection limit, the observed profile might reflect a true phenomenon, because a similar pattern has also been observed during kinetic PAH bioaccumulation experiments with worms exposed to spiked soils (*17,20*). In these cases, the effect was most pronounced for low-molecular-weight

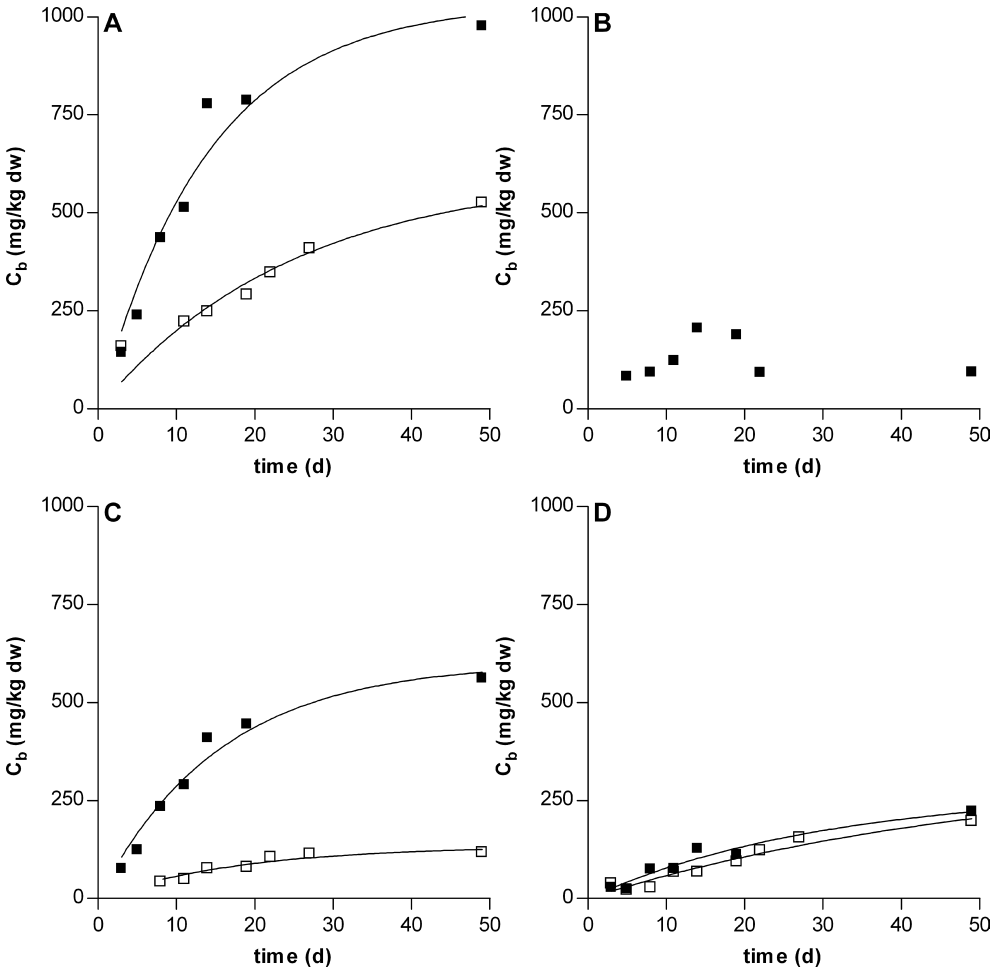

Figure 1. Uptake kinetics of Total Petroleum Hydrocarbons (TPH) in *Lumbriculus variegatus* from HIJG (closed symbols) and KVHB (open symbols) sediment for the boiling point fractions (A) C_{11}-C_{34}, (B) C_{11}-C_{16}, (C) C_{16}-C_{22}, and (D) C_{22}-C_{28}.

PAHs, which agrees with the present observation that only the lowest boiling point fraction displayed the deviating behaviour. For PAHs, the effect was hypothetically explained by a decreased concentration of compounds available for uptake due to biodegradation, evaporation, or weathering (*17*). In the present study, these processes may also apply, because typical half-lives for weathering and degradation of the lowest boiling point fraction are in the order of days, in contrast to half-lives for fractions >C_{20}, which are years to decades (*10*). Degradation of petroleum hydrocarbons usually starts after a lag-phase of a couple of days, while accumulation will start almost immediately. This may result in an initial uptake of compounds, followed by elimination as soon as the available concentration in the interstitial water starts to decrease. Biodegradation may even be stimulated through worm activity (bioturbation) (*21*) by increasing the

oxygen flux into the sediment. For PAHs it has also been suggested that the observed peak in the uptake kinetics could be caused by decreasing lipid contents, as normal kinetics were observed in case concentrations were lipid-normalized (*20*). Any decline in lipid contents can however not explain the peak in figure 1B, since peaks were absent for all other fractions.

During the subsequent 'static' bioaccumulation experiments, worms were exposed for 28 d. Although the estimated uptake rate constants suggest that concentrations in worms will have reached 70-90 (not 100) % of equilibrium upon this exposure duration, exposure for 28 d is in conformity with US-EPA guidelines (*22*). Moreover, prolonged exposure times may result in biased data due to progressive biodegradation, increased mortality as a consequence of chronic toxicity, and decreasing lipid contents.

Lipid contents

During the 28 d bioaccumulation experiments, worms showed normal behaviour and substrate avoidance was not observed, although complete burial took one to two days for the clayey sediment VK16. Recovered worms all appeared healthy and no signs of toxicity (i.e. protusions) were observed. The smallest number of worms was recovered from the most heavily polluted sediment LERI, whereas population growth was observed for the peaty sediments JHOZ and JHWE, which apparently provided optimal substrates for *L. variegatus*. After 28 d of exposure, lipid contents of the worms varied between 10.6 and 13.3 % of dry weight. Except for the lowest value (10.6%; for the S130 sediment), these percentages are slightly higher than those for worms from the culture (11.4 %). This implies that the sediments provided sufficient food and the worms did not need their energy reserves. The observation also strengthens the hypothesis that the above-discussed peak in the uptake kinetics for C_{10}-C_{16} (figure 1B) was not caused by decreasing lipid contents as suggested previously (*20*). The differences in lipid contents of worms exposed to different sediments were significant (ANOVA, p=0.0001) and presumably are related to variations in substrate composition and nutritional status. However, a significant relationship with organic carbon or nitrogen contents was not observed (p=0.12 and 0.057, respectively). Although no relationship between TPH concentrations in organisms and lipid contents has been described in the literature, all TPH concentrations in worms were lipid-normalized, because lipids are the major partitioning site for the hydrophobic petroleum hydrocarbons. Moreover, standard deviations decreased upon the normalization and the calculation of BSAFs (see below) requires lipid-normalized data. It should be noted that differences between sediments were not affected by the normalization.

TPH bioaccumulation profiles

Chromatograms of worm extracts displayed a characteristic 'hump' from C_{14} to C_{28} with a maximum around C_{20} (appendix III, table S1). The hump represents the so-called unresolved complex mixture (UCM), which is comprised of thousands of unidentified petroleum hydrocarbons. Based on the retention times of alkanes in a standard solution (see Experimental section), several sharp peaks on top of the hump could however be indentified as straight-chain alkanes (appendix III, figure S1). In particular

the presence of these compounds is a confirmation of the petrogenic origin of the bioaccumulated mixture (*23*). Although solvent extractions always result in the presence of matrix constituents (organic matter, lipids) in the extracts, interfering effects of such compounds on the present chromatograms were not expected as we applied a clean-up approach specifically validated for this purpose (Chapter 2). The absence of the typical 'lipid hump' around C_{28} (Chapter 2) supports this view. A detailed characterization of the accumulated hydrocarbons was outside of the scope of the present study, but the mixture would be presumed to consist predominantly of the relatively more water soluble and less hydrophobic compounds, such as short-chain alkanes and aromatics (*8*). Surprisingly, however, alkanes with a chain length up to C_{34} were also indentified in worms, while these were not expected based on calculations with a model used to estimate TPH concentrations in membranes, assuming equilibrium partitioning of aliphatic and aromatic compounds between sediment, pore water, and membranes (*8*). This observation may suggest an additional uptake route for these very hydrophobic compounds, that is, through ingestion of organic matter to which the chemicals are sorbed (*24*). Still, only a fraction of the hydrocarbon mixture present in the sediments was accumulated by the worms. Extracts from sediments typically resulted in chromatograms with a hump around C_{28} and responses up to or even beyond C_{40} (appendix III, figure S1), while chromatograms of worm extracts clearly showed a prevalence of the lower boiling point fractions (appendix III, figure S1). This difference between extracts from worms and sediments indicates the absence of any bias in TPH concentrations in worms caused by e.g. sediment particles in the digestion system due to insufficient gut-purging.

Biota-to-Sediment Accumulation Factors

According to the equilibrium partitioning theory (EPT), BSAF values for hydrophobic organic compounds should be 1-2 under equilibrium conditions, based on the assumption that sorption to sediment organic carbon equals partitioning to biota lipids (*25*). BSAFs determined for separate hydrocarbon blocks in the present study however varied between 0.01 and 2.3 (figure 2, and appendix III table S1), thereby representing a similar range as observed previously for individual PAHs and polychlorinated biphenyls (PCBs) (*26-28*). Also, a relatively large variation in BSAFs between sediments was observed, which seemed to be related to differences in oil weathering status. In particular for the peaty sediments JHWE and JHOZ, for which frequent input of fresh oil is most likely because of operational gasoline pumps adjacent to the sample locations, BSAFs were high. For the other sediments characterized by relatively high BSAFs, oil films were observed during sampling and sample treatment. Operational sources and visual observations may be an indication of the presence of fresh, non-weathered oil, having a relatively high bioavailability and corresponding BSAF values. Despite the variation in BSAF values between sediments, two interesting phenomena can be observed from figure 2 and appendix III, table S1. First, BSAFs seemed to negatively correlate with the TPH concentration in sediment. BSAFs for the sediments HIJG, LERI, S160, SCSL, and WOGR, having the highest TPH concentrations, were the lowest for each hydrocarbon block. Yet, differences in BSAFs between these sediments were small. Second, for individual sediments BSAF values for separate hydrocarbon blocks

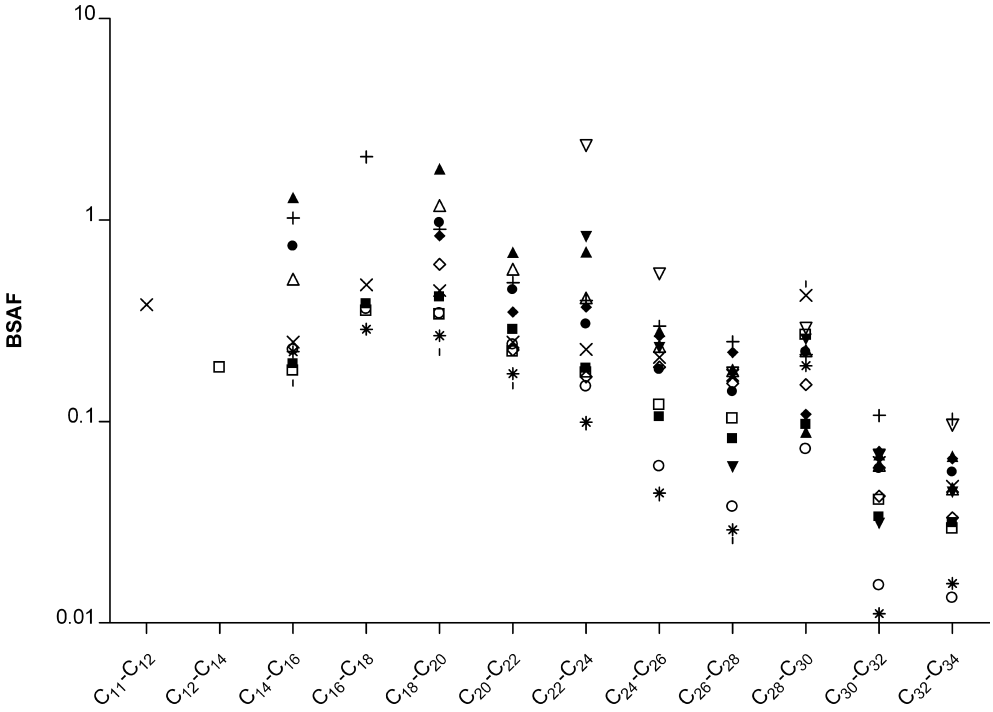

Figure 2. Lipid and organic carbon-normalized Biota-to-Sediment Accumulation Factors (BSAFs) for 12 different petroleum hydrocarbon fractions in *Lumbriculus variegatus*. Different markers represent different sediments: HIJG (■), JHOZ (▲), JHWE (▼), KVGS (◆), KVHB (●), LERI (□), S120 (△), S130 (▽), S150 (◇), S160 (○), SCSL (×), UTNB (+), VK16 (✳), and WOGR (|). Only BSAF values based on concentrations in sediments and biota above detection limits are included.

appeared to be relatively constant up to C_{22}, indicating that worms proportionally accumulated these fractions from the sediments. However, beyond C_{22} BSAFs decreased for all sediments (figure 2, and appendix III, table S1), an observation which has also been reported for PAHs (*26,27*) and PCBs (*28*). Possible explanations for these two phenomena will be discussed below.

Critical Separate Phase Concentration Controls TPH Bioaccumulation

One hypothetical explanation for the above-described correlation between BSAFs and TPH concentrations in sediments (figure 2, and appendix III, table S1) may be that the presence of oil somehow limits the availability of the mixture itself, as will be explained below. TPH concentrations in *L. variegatus* exposed to HIJG, LERI, S160, SCSL, and WOGR sediment only varied between 3222-4817 mg/kg lipids, thereby being not significantly different (ANOVA, p=0.154), whereas TPH concentrations in the accompanying sediments differed significantly between 1900 and 4100 mg/kg dw (appendix III, table S1). In other words, bioaccumulation of petroleum hydrocarbons

seems to level-off above a certain TPH concentration in sediment. However, concentrations alone do not fully explain such a levelling-off, as BSAFs for the peaty sediments JHWE and JHOZ, having THP concentrations in the same range as the five sediments mentioned above, were relatively high. We therefore hypothesize that the phenomenon is caused by the presence of non-aqueous phase liquid (NAPLs) in the five sediments. Analogous to individual hydrophobic organic chemicals, the complex hydrocarbon mixture oil will partition between the pore water and the sediment organic carbon, when occurring in sediments (*29*). However, when the amount of oil present saturates the pore water and the organic carbon matrix, oil will be present as a NAPL, i.e., a film or droplets (*29*). The TPH concentration above which oil will form a separate NAPL has been referred to as the Critical Separate Phase Concentration (CSPC) (*29*). This concentration has been reported to be in the range of 1000-4000 mg/kg dw (*29-31*), but obviously should be normalized to the sediment organic carbon content (*29*). Normalized CSPC values correspond to 10-30% of the organic carbon mass (*29-31*). For the five aforementioned sediments, the presence of NAPLs is likely since the TPH concentrations exceeded the 30% limit on an organic carbon mass basis. Assuming that in these sediments the TPH concentration in pore water therefore was at the aqueous solubility level and that TPH concentrations in worm lipids at such high concentrations are mainly determined by the aqueous concentration according to the EPT, the levelling-off of the TPH concentrations in worms as well as the accompanying reduced BSAFs would be explained. Additionally, it should be noted that NAPLs may act as an additional sorption phase (*29,30*), lowering the bioavailability of (specific) petroleum hydrocarbons. Worms may also be selective feeders, capable of avoiding oil droplets or films; behaviour that was previously suggested for worms exposed to activated carbon-enriched sediments (*32*). When NAPL concentrations become very high, avoidance may however not be possible anymore, resulting in smothering and mortality of the organisms. Finally, the occurrence of NAPLs in the peaty sediments JHWE and JHOZ is not plausible, because for these sediments the TPH concentrations only comprised 5% of the organic carbon mass. This accordingly might explain the relatively high BSAF values observed for these sediments.

TPH bioaccumulation cut-off: artefact or true mechanism?

As discussed above, BSAF values for separate hydrocarbon blocks were approximately constant up to C_{22} for all sediments, but decreased gradually beyond this point (figure 2, and appendix III, table S1). In other words, bioaccumulation of compounds with a high boiling point seems to be reduced and the cut-off value for the offset of the process is located around C_{22}. A similar phenomenon has previously been observed for individual PAHs and PCBs, for which an inverse relationship between BSAF and hydrophobicity was reported (*26-28*). In the concerning studies, both experimental artefacts (i.e., non-equilibrium conditions) and true mechanisms (i.e., size-exclusion of large chemicals from biological membranes or enhanced sorption of the compounds to sediments) were discussed as possible causes. At least for PAHs, non-equilibrium conditions and size-exclusion were conclusively ruled out, leaving increased sorption of the more hydrophobic compounds to sediments as the only plausible explanation (*27,33*). For the present study focusing on oil, a non-equilibrium

condition artefact may however be applicable, considering the extreme hydrophobicity of the higher boiling point fractions (*34*) and their accompanying slow kinetics. As discussed above, high boiling point fractions may need over 90 d to reach equilibrium between sediment and worms, whereas worms were exposed for 28 d only. Regarding actual mechanisms underlying the BSAF decrease with boiling point fraction, size-exclusion seems unlikely as well, since compounds up to C_{34} were detected in worm extracts (see above), some of which were indentified as alkanes. This observation suggests that alkanes with a molecular size as large as that of C_{34} can actually be taken up in biota. Any reduced uptake of such large compounds in this respect should then be considered the result of slow uptake kinetics (*35*) (i.e., an experimental artefact), which is supported by the fact that these compounds were only detected in trace amounts. One might argue that the presence of these compounds may also be related to oil phases present in or on the worms. However, this does not agree with the chromatographic profiles of worm extracts being clearly different from those of sediment extracts (see above). Reduced bioavailability of higher boiling point fractions due to enhanced sorption to sediments, as suggested to occur for PAHs, does seem a plausible explanation for the BSAF decrease with boiling point fraction. In case oil is present at concentrations above the CSPC, the mixture will form a separate phase, which has been demonstrated to be an additional and strong sorption matrix for hydrophobic compounds (*29,30*). The results of a modelling study indeed indicated that the majority of the high boiling point fractions will be present in these NAPLs, which consequently will cause the fractions to be unavailable for uptake in biota (*8*). Moreover, as mentioned above, *L. variegatus* might even avoid NAPLs, thereby further limiting bioaccumulation of the very hydrophobic fractions. Still, NAPLs are likely present in only five out of the 14 sediments tested, whereas decreasing BSAFs were observed for all sediments. Possibly, the sediments may have contained other strong sorption phases, such as soot originating from e.g. (shipping) traffic, which are also common in sediments (*36*). On the other hand, since non-equilibrium conditions are plausible as well, the reduced BSAF phenomenon may be caused by a combination of factors, rather then by a single one.

Implications for TPH risk assessment

Within the current risk assessment protocols, environmental risks of petroleum hydrocarbons are assessed on the basis of total concentrations in the solid (soil, sediment) phase. The observation of BSAF values in field sediments being less than one (see above), already indicates that this way of assessing will lead to an overestimation of risks. Figure 3A indeed demonstrates that organic carbon-normalized TPH concentrations in sediments are approximately a factor of five to ten higher than TPH concentrations measured in worms. It should be noted though that the organic-carbon normalization does lead to an improvement, as non-normalized concentrations do not show any relationship with concentrations in worms (See figure 1B). Still, the relationship between organic carbon-normalized TPH concentrations in sediments and concentrations in worms is poor (r^2=0.59), which is explicable since only at low concentrations oil will be associated with organic carbon. At high concentrations oil will form separate phases and concentrations will thus be independent of the organic

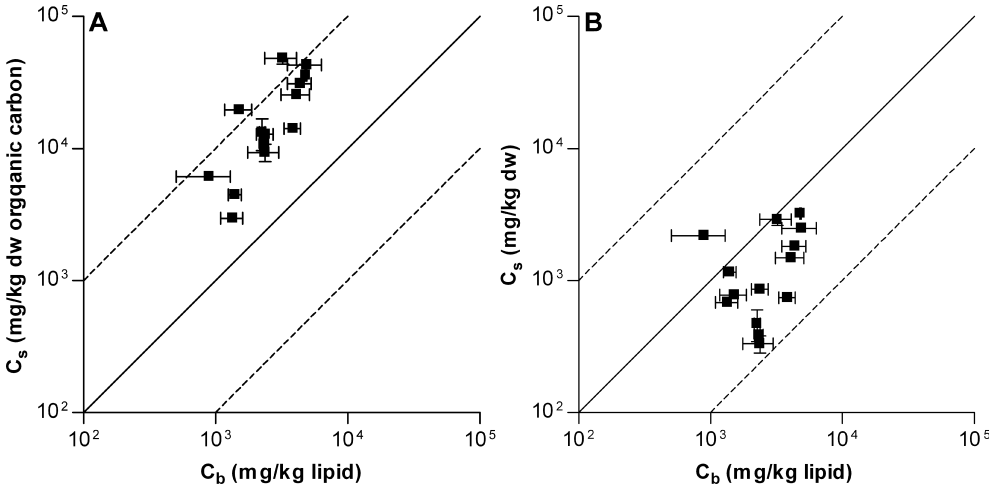

Figure 3. Relationships between lipid-normalized Total Petroleum Hydrocarbon (TPH) (C_{11}-C_{34}) concentrations in *Lumbriculus variegatus* (C_b) and (A) organic carbon-normalized TPH concentrations in sediments, and (B) TPH concentrations in sediments. The solid lines represent the 1:1 relationships, whereas the dotted lines delimit the one order of magnitude deviation interval. Values are averages ± standard deviations.

carbon content. This behaviour is actually reflected by the data in figure 3A, which show the strongest overprediction for the sediments with the highest organic carbon-normalized concentrations. This corresponds with the lowest BSAF values being observed for the most heavily oil-polluted sediments (figure 2). In summary, the current risk assessment procedure for TPH present in sediments and soils may lead to erroneous results in many cases. In subsequent papers, tools to improve TPH risk assessment will be discussed.

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Chapter 5

Assessing the bioavailability of complex petroleum hydrocarbon mixtures in sediments

Barry Muijs, Michiel T.O. Jonker

Institute for Risk Assessment Sciences, Utrecht University

Abstract

Several experimental methods have been developed to assess the bioavailability of individual organic compounds. So far none of them has however been applied to complex mixtures, such as oil (petroleum hydrocarbons), which is an ubiquitous pollutant. In the present study, we tested the potential of five of these experimental methods and that of a model approach to predict bioaccumulation of oil in the aquatic worm *Lumbriculus variegatus* exposed to 14 field-contaminated sediments. Actual and predicted bioaccumulation were compared in terms of both total bioaccumulative petroleum hydrocarbon concentrations and the relative distribution pattern of separate boiling point fractions (hydrocarbon blocks). None of the experimental methods was able to directly assess bioaccumulation in *L. variegatus* and correction factors were needed to match predicted and actual concentrations. These factors appeared concentration-dependent for solid phase-micro extraction (SPME) and extractions with Tenax and cyclodextrin, most probably due to artefacts. Moreover, the hydrocarbon block pattern produced by these methods considerably differed from the pattern observed for worms; an additional reason for disqualification also applying to headspace-SPME. In contrast, the pattern produced by polyoxymethylene solid phase extraction (POM-SPE) closely mimicked the worm pattern and a sediment, hydrocarbon block, and concentration-independent correction factor (17) could be derived, based on which actual bioaccumulation could be predicted within a factor of three. Finally, the model predicted bioaccumulation directly within a factor of two. The accompanying hydrocarbon block pattern however deviated significantly more from the worm pattern than the POM-SPE pattern did. We therefore conclude that POM-SPE may be the overall best approach to predict bioaccumulation of complex hydrocarbon mixtures in aquatic worms, all the more since an experimental approach will implicitly capture all factors determining bioavailability, which may prove difficult through a modelling approach.

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Introduction

It is well-known that risks of hydrophobic organic contaminants present in soils and sediments may be overestimated on the basis of total, solvent extraction-derived concentrations (*1,2*). Instead, actual risks are believed to be reflected more closely by bioavailable concentrations or rapidly-desorbing sediment-bound fractions (*3-5*). Research during the past decades has demonstrated that the bioavailability of chemicals may be lower than assumed within the existing risk assessment models, due to time-dependent sorption (*1,6*) or strong sorption to specific, carbonaceous soil or sediment constituents (*7,8*). Most bioavailability studies so far have focused on individual compounds, possibly as representatives of mixtures, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). To assess the bioavailability of these chemicals in environmental samples, several chemical–analytical methods, such as solid phase micro extraction (SPME), polyoxymethylene solid phase extraction (POM-SPME), extractions with Tenax, supercritical fluid extractions, and extractions with cyclodextrin have been developed and applied (*5,9-12*). Because of the primary focus on specific, individual chemicals, very little information on the bioavailability of complex chemical mixtures in soils and sediments is available at present. Studies performed within the scope of complex mixtures moreover are often focused on specific marker compounds, such as PAHs (*13,14*). Oil (petroleum hydrocarbons) is an important example of a complex mixture frequently occurring in the environment. It is ubiquitous due to spills, natural seeps, shipping, offshore drilling, and other human activities (*15*). The mixture consists of thousands of hydrophobic hydrocarbons (*16*), which have been demonstrated to be bioaccumulative (*17*, chapter 4) and toxic (*15,18*). A recent study has shown that bioavailability of complex petroleum mixtures in sediments is an important issue, as accumulation in aquatic worms was observed to be lower than predicted on the basis of total sediment-associated concentrations (chapter 4). As such, including bioavailability in the risk assessment of oil-contaminated soils and sediments would be recommendable (*19*), since it may help judging the need for remediation and/or prioritizing clean-up activities.

In the present study, four of the above-mentioned bioavailability methods (SPME, POM-SPE, and extractions with Tenax and cyclodextrin, i.e., the ones most frequently-applied and well-characterized), as well as a headspace (hs) SPME approach were applied to predict the bioavailability of complex petroleum hydrocarbon mixtures in a series of 14 field-contaminated sediments. Petroleum hydrocarbons were quantified in the resulting extracts using GC with Flame Ionization Detection (FID) and characterized in terms of linear alkane boiling point fractions based on their retention times between decane (C_{10}) and tetracontane (C_{40}). To our knowledge, none of the above methods has been applied before to oil within a bioavailability framework. Part of the study was therefore devoted to method development, i.e., optimizing extraction parameters, such as kinetics. All results were compared to actual bioaccumulation measurements with aquatic worms (*Lumbriculus variegatus*), which had been exposed to the same sediments (chapter 4). In addition to the experimental methods, a recently-published equilibrium partitioning model for oil (*20*) was used to assess bioaccumulation in the

worms. The objectives of the present study were to optimize and evaluate the performance of the different approaches and to select the most promising approach for assessing the bioavailability of complex petroleum hydrocarbon mixtures in sediments. The overall best method is not simply the method that predict bioaccumulation best, but that extracts petroleum hydrocarbons from sediments as worms do.

Experimental section

Chemicals, extraction materials, and sediments

Solvents used (hexane, heptane, and acetone; all Pestiscan grade) were obtained from Lab-Scan (Dublin, Ireland). Aluminium oxide (50-200 µm; Super I) was purchased from MP Biomedicals (Eschwege, Germany) and was deactivated with 10% of Millipore water. Tetracontane (C_{40}; >95%) was purchased from Fluka (Steinheim, Germany). Other alkanes with even carbon numbers between C_{10} and C_{36}, and C_{11} (all >98 %) were from Sigma Aldrich (Steinheim, Germany) or Fluka. A certified standard oil (RIVM-LOC-001) was obtained from NMi (Nederlands Meetinstituut; Delft, The Netherlands). The aqueous solution used during most extractions (hereafter and in the Supporting Information referred to as the aqueous solution) was made by solving 1.47 g calcium chloride and 50 mg sodium azide (both from Merck; Darmstadt, Germany) per L of Millipore water. Disposable SPME fibre (glass fibre core diameter 110 µm, polydimethylsiloxane (PDMS) coating thickness 28.5 µm; Poly Micro Industries; Phoenix, AZ) was cut into pieces of desired length, which were then washed three times with heptane, air-dried (1 h), and stored in either Millipore water (for SPME measurements) or a dry vial (for hs-SPME) until use. A polyoxymethylene sheet (POM; thickness 76µm; CSHyde company; Lake Villa, IL, USA) was cut into pieces of about 250 mg, which were Soxhlet-extracted for 6 h with acetone:hexane (1:3 v/v) and air-dried (1 h) before use. Tenax-TA (60-80 mesh; Chrompack; Middelburg, The Netherlands) was Soxhlet-extracted for 2 h with hexane and acetone, successively, and dried overnight at 80°C. 2(Hydroxyl)propyl-β-cyclodextrin (hereafter referred to as cyclodextrin) was from CycloLab R&D (Budapest, Hungary).

Sediments were sampled from 16 different Dutch locations with a known history of oil pollution (see appendix IV, table S1 for details). They were sieved (1 mm), homogenized, and stored at 4°C. Dry weights were determined gravimetrically on dried (105°C) sediments. Two of the sediments (ADPH and ROSL) appeared toxic to *L. variegatus* and were therefore only used for method characterization purposes (in case of SPME only).

SPME

SPME determinations were performed as described before (*2,3*). In short, amber-coloured 7 mL vials were filled with sediment (5 g of wet weight) and 5 mL of aqueous solution. After homogenization, 15 to 20 cm of fibre was added, vials were closed and put on a rock and roll shaker (Snijders Scientific; Tilburg, The Netherlands) at 20°C. Upon finishing the exposures, fibres were collected, cleaned with a wet (Millipore

water) tissue, measured, cut, and transferred to autosampler vials, containing inserts filled with 200 µL of heptane with 60 mg/L tetracontane as internal standard. Uptake kinetics were determined for HIJG, LERI, and ROSL sediment, to which fibres were exposed in quadruplicate for 1, 3, 7, 14, 28, 42, and 70 days. For the other sediments, fibres were equilibrated in quadruplicate for 42 days only.

Headspace-SPME

Headspace extractions were performed in custom-made glassware, which consisted of glass baskets attached to the bottom of glass stoppers that were placed on 50 mL Erlenmeyer flasks. Wet sediments were weighed into the flasks to an equivalent of 2 g of dry weight (dw) and water volumes were adjusted to 10 mL with Millipore water. Five 2 cm SPME fibres were then put in the baskets, and the stoppers were placed and secured (with stainless steel rings) on the Erlenmeyers. The flasks were placed in a water bath shaker (operating at 75 rpm) in which the water level reached halfway the Erlenmeyers. Kinetics were determined at 30, 50, 70, and 90°C. Final headspace-extractions were performed at 95°C for 16 h in quadruplicate. After exposure, fibres were collected and extracted as described above.

POM-SPE

Sediments were extracted with POM as described previously (*3,10*). In short, wet sediments were weighed into 250 mL bottles, which were then filled with aqueous solution, leaving a headspace of about 10 mL. Subsequently, POM strips (about 250 mg) were weighed and added to the suspensions. For individual compounds (PAHs, PCBs), a 4-week exposure period under intensive shaking conditions appears to be sufficient for reaching equilibrium in a sediment-water-POM system (*3,10*). To be on the safe side, in the present study POM was shaken (150 rpm, 20°C) for the same length of time as SPME, i.e., 42 days. Then, POM strips were collected, thoroughly wiped with wet tissue to remove any particles or oil stains, cut, and Soxhlet-extracted for six hours with 80 mL of acetone:hexane (1:3 v/v). The extracts were concentrated, cleaned-up through aluminium oxide columns, concentrated again, and solvent-exchanged to 0.4 mL of heptane. Internal standard solution (100 µL of 300 mg/L tetracontane in heptane) was added and after vortexing, the extracts were transferred to autosampler vials. POM extractions were performed in triplicate or singularly in case SPME experiments demonstrated that available oil concentrations were close to detection limits.

Extractions with Cyclodextrin

The experimental setup for the extractions with cyclodextrin was based on previous studies (*3,12,21*). Wet sediments were weighed into 20 mL vials to an equivalent of 1 g dw and water volumes were adjusted to 5.5 mL with Millipore water. Then, 10 mL of aqueous solution additionally containing dissolved cyclodextrin was added and the slurries were shaken at 150 rpm and 20°C for a specified length of time (see below). Subsequently, the systems were centrifuged (3000 rpm, 7 minutes) and 10 mL of the supernatant was weighed into another set of 20 mL vials. These solutions were

extracted with 5 mL of hexane by shaking mechanically at 150 rpm for 1h. After centrifugation (2500 rpm, 7 minutes) the hexane phases were transferred to pointed flasks. The supernatant phases were extracted another two times and the pooled hexane phases (about 15 mL) were evaporated under nitrogen to about 1.5 mL. Extracts were purified, concentrated, and solvent-exchanged as described above, but the final volume was 1 mL, including 200 µL of internal standard solution. Possible effects of the cyclodextrin concentration on the extraction yield were determined for the JHWE, LERI, and WOGR sediment, which were extracted for 16 h with a solution containing 31, 61, 123, or 245 mM of cyclodextrin (actual concentrations). Extraction kinetics were determined by extracting these sediments for 3, 6, 12, 24, 48, and 96 hours with a 50 mM cyclodextrin solution.

Extractions with Tenax

Sediments were extracted with Tenax powder according to a slightly modified version of the method previously reported (*3*). Wet sediments were weighed in triplicate into 100 mL separation funnels to an equivalent of 1 g dw. Aqueous solution (70 mL) and Tenax (0.6 g) were added and the funnels were shaken (125 rpm, 20°C) for 10 h. Tenax was separated from the sediment suspensions by opening the funnels and the beads were washed twice with Millipore water. They were subsequently extracted with hexane (30 mL) on a shaker (225 rpm, 1 h), after which the hexane was transferred to 250 mL pointed flasks through quartz wool filters. Tenax beads were then extracted another two times with hexane (30 mL) by manually shaking (2 minutes). The hexane phases were pooled, the filters were flushed with hexane (5 mL), and the collection flasks were left overnight. Any water in the extracts was removed the next day with a glass pipette and the extracts were concentrated, purified, and solvent-exchanged as described above.

Total extractions

Oil concentrations in all sediments were determined as described previously (chapter 2) by extracting anhydrous sodium sulphate-dried sediments with acetone:hexane (1:3 v/v) in a Soxhlet apparatus. The extracts were cleaned-up through deactivated aluminium oxide and solvent-exchanged to heptane, after which internal standard solution was added.

Bioaccumulation experiments

Oil accumulation in worms was determined as reported previously (chapter 4). Briefly, the organisms were exposed to the sediments for four weeks in 1L bottles filled with sediment and tap water in a climate-controlled room (20°C). Worms were then separated from the sediment, gut-purged, freeze-dried, and extracted and analyzed as described above, but acid/base-treated silica gel columns (chapter 2) were applied for extract purification.

GC analyses

Extracts were analyzed on a Carlo Erba 8065 gas chromatography system (Milan, Italy). All samples resulting from the bioavailability methods were analyzed using Large Volume Injection (30 µL); for total and worm extracts only 1 or 3 µL was injected. Extracts were injected on-column on a 5 m pre-column (J&W Scientific, Folsom, CA), coated with deactivated silica, which was connected to a 15 m 0.25 mm i.d. DB5.625 column (J&W Scientific). The oven was programmed as follows: 5 minutes at 98°C, increasing with 20°C/min, and held at 300°C for 20 minutes. Hydrocarbons were detected with a Flame Ionization Detector (FID) operating at 320°C, quantified by using calibration standards containing the certified oil, and divided in hydrocarbon blocks based on the stable retention times of 16 *n*-alkanes. All integrated areas were normalized to the internal standard peak areas. Additional information on the oil quantification can be found in chapter 2.

Quality assurance

To minimize losses of compounds by photodegradation, only amber-coloured or aluminium foil-covered glassware was used. Microbiological degradation was prevented by the addition of sodium azide in the aqueous solutions. Contaminant losses by sorption to containers were minimized by using glass only for extractions and storage, and aluminium-lined caps for exposure (not autosampler) vials. Final oil concentrations were corrected for procedural blanks by extracting the aqueous solution only, and for procedural recoveries by adding a pre-weighed volume of standard oil solution to the extraction solvent (hexane or acetone/hexane) or the cyclodextrin solution. Oil concentrations were recovery-corrected when significant losses were observed (t-test), i.e., in case of the lowest boiling point fractions (C_{10}-C_{16}) only, for which recoveries were about 90%. No recoveries were determined for SPME and hs-SPME experiments. Extracts were stored at room temperature until analysis to prevent precipitation of tetracontane.

Data analysis

Data resulting from bioavailability/bioaccessibility methods can either directly be compared to concentrations accumulated in organisms (comparison approach) or indirectly by applying equilibrium partitioning calculations (calculation approach) (*3*). The latter approach requires bioaccumulation factors and sampler-water or sediment-water partition coefficients, which unfortunately are not available for oil. A preliminary attempt to measure oil sampler-water partition coefficients resulted in obscured data and indicated both the complexity of the process and the need for novel measuring approaches. Therefore, all data in the present study were analyzed according to the comparison approach only. In addition to bioaccumulation predictions based on the experimental methods, oil accumulation in worms was also predicted by using model calculations. The calculations were performed with a partitioning model recently published by Verbruggen et al. (*20*), which estimates oil accumulation in membrane lipids upon exposure to oil-contaminated sediment. The model assumes that petroleum hydrocarbons partition between sediment organic carbon, pore water, the liquid oil

phase, and membrane lipids. Modelling is performed based on model-derived sediment-water and membrane-water partition coefficients and Raoult's law, starting from measured TPH-concentrations in sediments. The model requires oils to be separated into aliphatic and aromatic fractions. Because this information was lacking for the sediments under investigation, we assumed the oils to be composed of 70% aliphatics, an average percentage that has been reported for various oil-contaminated soils (*22*).

Results and discussion

Oil uptake in worms and extraction phases

As reported and discussed previously (chapter 4), petroleum hydrocarbons up to C_{34} accumulated in the worms. Therefore, the ability of the five chemical-analytical methods to assess bioaccumulation of the fraction C_{11}-C_{34} was evaluated. This fraction hereafter will be referred to as bioaccumulative petroleum hydrocarbons (BPH), in order to distinguish it from the C_{11}-C_{40} fraction, which is often referred to as total petroleum hydrocarbons (TPH). Because the C_{34}-C_{40} fraction did not accumulate, its inclusion resulted in increased data scatter. Actually, the best correlations between bioaccumulation and concentrations extracted with the chemical methods applied were obtained when focusing on the C_{11}-C_{20} range and relationships got weaker when higher boiling point fractions were included. One explanation for this observation might be the differences in dynamics between systems with worms and artificial extraction phases. Uptake in worms is relatively slow and high boiling point fractions may not have equilibrated completely within 4 weeks (chapter 4), whereas accumulation in the extraction phases is accelerated by thorough shaking and equilibrium conditions are more plausible also for the higher fractions. Furthermore, in case the higher boiling point fractions are predominantly associated with the liquid oil phase itself, bioaccumulation might be reduced due to the possible ability of worms to avoid liquid oil (chapter 4), while extensive shaking may cause the fractions to be available for extraction with artificial phases. This issue will be discussed further below.

SPME

BPH equilibrated with PDMS-coated SPME fibres within 30-40 days for the three sediments selected for the kinetic test (appendix IV, figure S1). Although kinetics for more hydrophobic compounds are usually observed to be slower (*2,4,23*), uptake rate constants in the present study did not significantly vary between the hydrocarbon blocks. To ensure full equilibration in subsequent experiments with all other sediments, the final exposure duration was set at 42 days. Although concentrations in PDMS are generally lower than in organisms, it has been suggested that PDMS-SPME would be a good surrogate for bioaccumulation measurements by using correction factors (*23*). These correction factors would be chemical group-specific (*3,4*), and were recently demonstrated to be also dependent on testing conditions and the specific compound within a chemical group (chapter 3). The results in figure 1B add to the complex nature of such correction factors, as for BPH they even appeared to be dependent on the oil

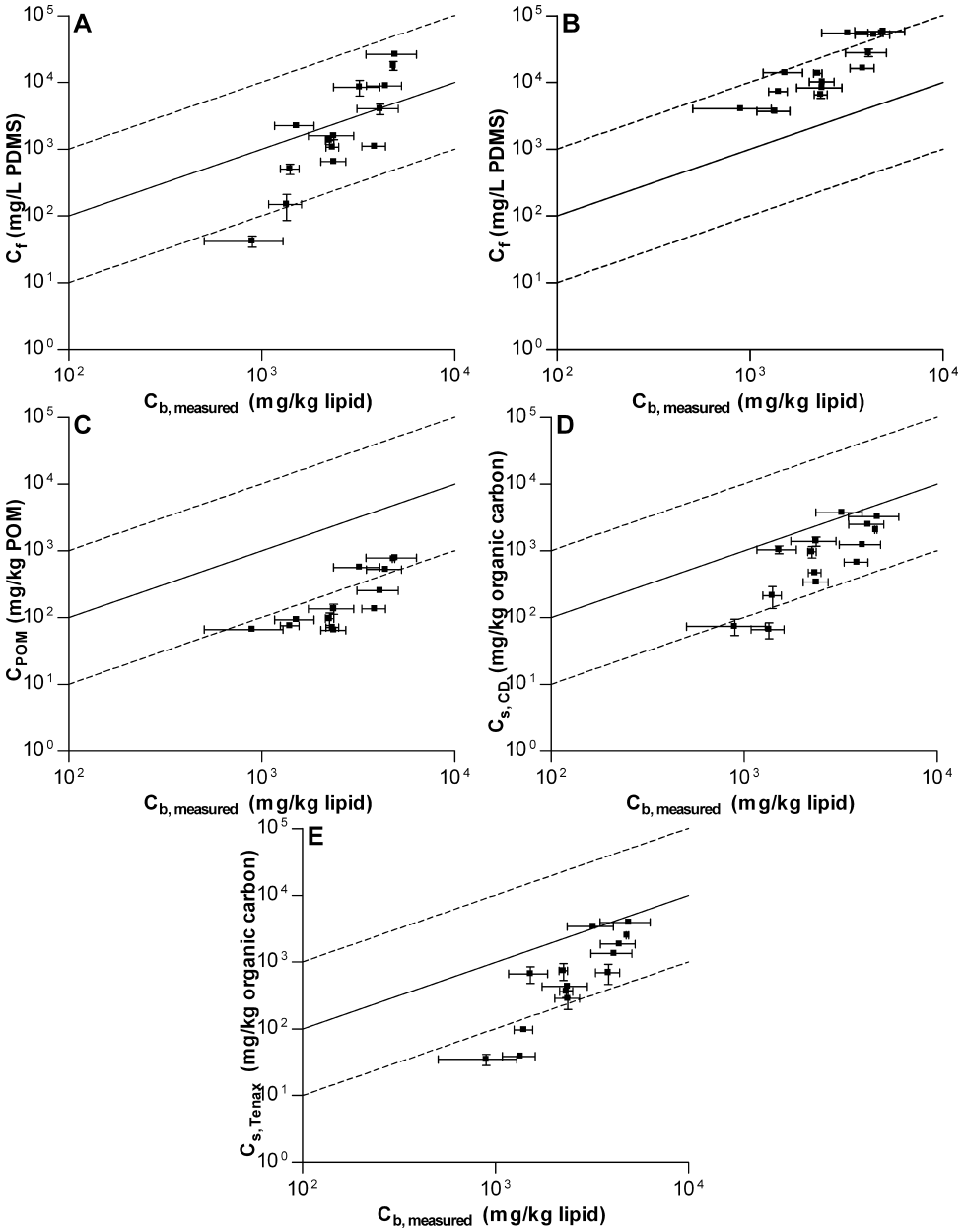

Figure 1. Relationships between BPH (C_{11}-C_{34}) concentrations measured in *Lumbriculus variegatus* exposed to 14 field-contaminated sediments and BPH concentrations extracted with (A) solid phase micro extraction (SPME), (B) headspace-SPME, (C) polyoxymethylene solid phase extraction, (D) cyclodextrin [CD], and (E) Tenax. Values are averages ± standard deviations. The solid lines represent the 1:1 relationships, whereas the dotted lines delimit the one order of magnitude deviation intervals.

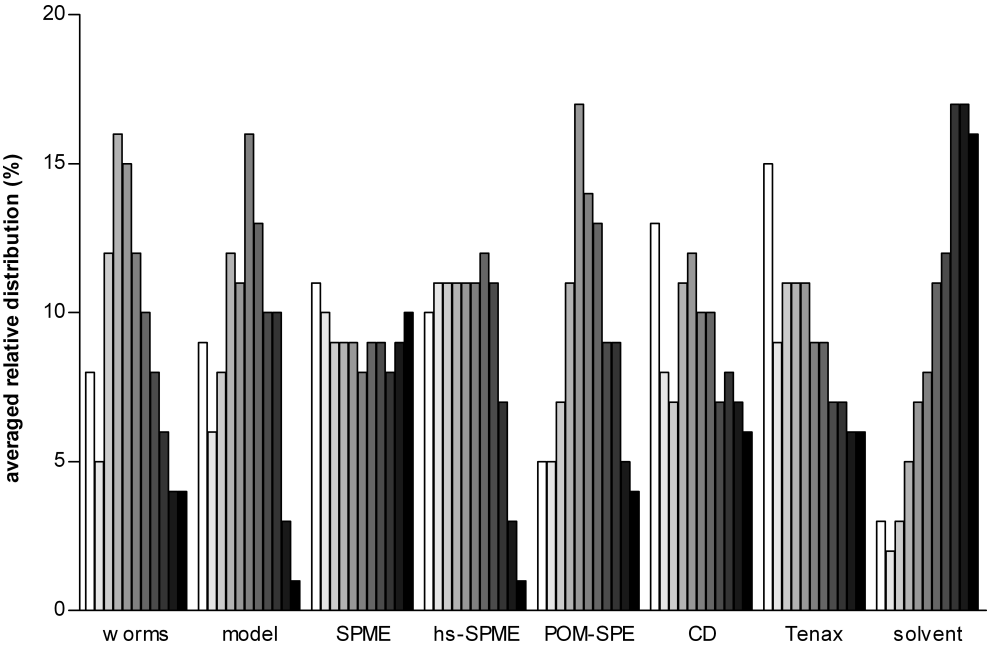

Figure 2: Averaged (n = 14 sediments) relative BPH boiling point fraction distribution as observed in worms (*L. variegatus*), model outcomes, extracts produced by the different experimental methods (SPME, hs-SPME, POM-SPE, cyclodextrin [CD], and Tenax), and extracts from sediments as obtained by Soxhlet extractions. The consecutive bars (from light to dark) represent the following hydrocarbon blocks: C_{10}-C_{14}, C_{14}-C_{16}, C_{16}-C_{18}, C_{18}-C_{20}, C_{20}-C_{22}, C_{22}-C_{24}, C_{24}-C_{26}, C_{26}-C_{28}, C_{28}-C_{30}, C_{30}-C_{32}, and C_{32}-C_{34}. Note that the alkanes mentioned after the hyphens are not included in that block; they are part of the next block, which starts with the respective alkane.

concentration in sediment: at low concentrations, bioaccumulation was underestimated by concentrations in PDMS, whereas for heavily polluted sediments an overestimation was observed. Interestingly, oil concentrations in the latter cases exceeded the critical separate phase concentration (CSPC) (*6*, chapter 4), i.e., the concentration above which oil will form separate oil phases, such as oil droplets and films; also referred to as non-aqueous phase liquids (NAPLs). The observed overestimation at high concentrations therefore might be caused by the extraction of (unavailable) pure oil, possibly being present as fouling on the fibres. Fouling of SPME fibres with pure sedimentary oil has been suggested before (*3*) and seems plausible considering the tiny fibre phase and the sticky nature of PDMS and oil. Note that apart from any undesirable fouling and the fact that concentration-dependent correction factors are not practicable, the SPME results were relatively scattered (r^2=0.59; figure 1B). Moreover, as demonstrated by figure 2, the accumulation pattern of separate BPH blocks (boiling point fractions) in PDMS clearly differed from that in worms. Because different BPH blocks may represent different toxic potencies, the ultimate

bioavailability method should also closely mimic uptake at the hydrocarbon block pattern level. Data points in figure 1 being close to the 1:1 line therefore do not straightforwardly imply a high-quality bioavailability assessment.

Headspace-SPME

In order to avoid fouling, SPME fibres were also exposed in the headspace above the sediments. Headspace SPME (hs-SPME) was initially introduced for the quantification of organic compounds in (ground) water (*24*) and soils (*25,26*), but to our knowledge has not been applied so far to assess bioavailability in sediments. The basic principle of hs-SPME is evaporation and increasing the temperature will therefore result in the extraction of compounds with higher boiling points (*27*). In the present study, compounds up to C_{22} were extracted at 30°C and increasing the temperature to 90°C resulted in the extraction of compounds with retention times similar to that of C_{30} (appendix IV, figure S2). Because BPH included C_{34}, the temperature was increased to 95°C for the final extractions. Although this temperature only resulted in the extraction of compounds up to C_{32}, higher temperatures (extraction of C_{34} requires temperatures >100°C (*27*)) were not feasible with the current setup. As demonstrated in figure S2, increasing the temperature resulted in higher BPH concentrations, but mainly due to an increased contribution of higher boiling point fractions; the concentrations of lower boiling point fractions remained constant. This phenomenon has also been demonstrated for headspace extractions of individual compounds from water and spiked animal tissue (*24,28*) and is explicable because even though concentrations in the headspace will increase with temperature, fibre-air partition coefficients will decrease (*24*). Uptake kinetics were fast using the present setup (appendix IV, figure S3), with equilibrium conditions being attained within 10 h for all temperatures. Still, this is remarkably slower than for PAHs, benzenes, and alkanes (nonane and decane) extracted from liquids at 25-28°C under static conditions (*25*).

Figure 1C demonstrates that hs-SPME yielded results showing a relatively strong relationship with bioaccumulation (r^2=0.70), i.e., with a relatively low degree of variation. However, bioaccumulation was consistently overestimated with up to a factor of 10. As such, equilibrium BPH concentrations in PDMS were higher than those observed in fibres directly exposed to sediments (c.f. figure 1B). The difference in exposure temperature probably explains this discrepancy (in case of equal temperatures, these concentrations theoretically should be the same (*27*)), partly through increased partitioning of compounds from the sediment organic matter into water (*29,30*). Furthermore, hs-SPME resulted in a BPH block pattern that considerably differed from the pattern observed in worms (figure 2).

POM-SPE

Another way of circumventing bias by fouling may be to use POM. Because of its hard and smooth surface, particles and oil phases generally can be easily wiped off this plastic, as discussed and demonstrated in (*6,10,31*). Also, bias by the presence of interfering matrix compounds (e.g., sediment organic matter-derived compounds) that

will add to the overall GC response (chapter 2) can be minimized by cleaning-up the extracts; a procedure which is omitted for SPME.

Figure 1D shows a positive relationship between BPH concentrations in POM and in worms. The correlation has a similar significance as observed for hs-SPME (r^2=0.69), but for POM-SPE bioaccumulation appears to be underestimated instead of overestimated. As discussed for SPME, a correction factor would be needed in order to accurately assess bioaccumulation. Pooling the data from all sediments, the averaged correction factor was derived to be 17.7 ± 10.2. Interestingly, the factors for separate boiling point fractions overall were similar (appendix IV, table S2), with only the factors for the lowest boiling point fraction (C_{11}-C_{14}) generally being elevated. The concentrations of this fraction in the extracts of both worms and POM were however usually close to the detection limit, which might explain the deviating behaviour. Correction factors for the sediments in which oil concentrations exceeded the CSPC (HIJG, LERI, S16O, SCSL, and WOGR) were lower than the average. This may suggest that POM is able to extract petroleum hydrocarbons, which are not available for or are being avoided by worms as discussed previously. Still, the application of the averaged correction factor to the data resulted in a relationship being very close to the 1:1 line (appendix IV, figure S4). Note that the application of a correction factor does not turn a method into an empirical one *per se*. Passive sampling with POM is a mechanistic approach: the sampler material extracts the freely dissolved aqueous concentration via a POM-water partition coefficient (K_{POM}); the concentration that is assumed to be available for uptake in biota according to a *BAF*. As such, the correction factor should be considered the ratio of the unknown mechanistic parameters *BAF* and K_{POM}. Interestingly, for 13 individual PAHs, this ratio equals on average 17.2 (unpublished data). Finally, an obvious advantage of POM-SPE is illustrated in figure 2: the distribution of separate BPH blocks accumulated in POM was very similar to that observed for worms. POM therefore seems to closely mimic the uptake of BPH in at least a qualitative way. Figure 2 also shows that the relative fraction distribution for POM was completely different from the distribution observed for sediments (Soxhlet extractions). As discussed in chapter 4 for worms, such a different pattern strongly suggests the absence of fouling, as the presence of pure oil on POM (or worms) would yield chromatograms and fraction distributions more closely resembling those of sediment (with a higher abundance of the high boiling point fractions).

Extractions with cyclodextrin

In contrast to the above-discussed methods, for which the extraction is based on equilibrium sampling, extraction with cyclodextrin relies on capturing the rapidly desorbing fraction (F_{rap}) (*12*). At least for individual chemicals, this fraction is assumed to be the bioaccessible fraction of the total solid phase-associated quantity (*5*). In the present study, cyclodextrin extracted petroleum hydrocarbons as well as interfering matrix compounds (i.e., dissolved organic matter). Because the presence of the latter compounds in extracts would cause an over estimation of the F_{rap}, we applied a clean-up procedure. Although increasing concentrations of cyclodextrin resulted in higher BPH concentrations, the lowest cyclodextrin concentration tested (50 mM) was applied in subsequent experiments, as this setup resulted in a negligible contribution of

interfering compounds. At higher cyclodextrin levels, the clean-up applied (elution through 4 g of aluminium oxide) appeared insufficient, as indicated by the increasing presence of interfering peaks in the chromatograms (appendix IV, figure S5). After 3 h of extraction, the BPH concentration in the extracts did not increase further (appendix IV, figure S6), which makes the extraction of these compounds remarkably faster than that of pesticides (*21*). Final extractions were however performed overnight (16 h) to ensure complete inclusion of the F_{rap} for all sediments. Note that usually cyclodextrin extractions last for about 24 h (*3,12*).

Cyclodextrin extractions underestimated actual bioaccumulation only slightly, with a factor of about 5. A similar underestimation was recently reported for PAH bioaccumulation in *L. variegatus* (*3*), but cyclodextrin-extracted pesticide concentrations showed an almost 1:1 relationship with bioaccumulated concentrations in the earthworm *Eisenia fetida* (*21*). The presently-observed underestimation might be related to a 'size-exclusion' phenomenon: the interior of cyclodextrin molecules may not be big enough to encapsulate large petroleum hydrocarbons, not even in case 2:1 complexes (*21*) are formed. This hypothesis is supported by the observation that linear alkanes $>C_{14}$ were absent in the cyclodextrin extracts. If true, the petroleum hydrocarbons in the higher boiling point fractions extracted probably mostly concern aromatics, i.e., compounds with a lower molecular volume, yet a high boiling point. Anyhow, the resulting BPH fraction distribution (figure 2) did not resemble very well the pattern observed in worms. Additionally, the regression between extracted and actually bioaccumulated concentrations was poor (r^2=0.49; figure 1E) and similar to the SPME results, the slope of the relationship was > 1, i.e., any correction factor would be concentration-dependent. In this case, the concentration-dependent behaviour cannot be attributed to fouling, but NAPLs released from the sediment upon shaking might contribute to the explanation. Such separate oil phases will float on the aqueous solution and may be extracted along with cyclodextrin-encapsulated hydrocarbons during the extraction of the supernatant with hexane.

Extractions with Tenax

The F_{rap} is believed to be captured by Tenax within 10 h (*5*). Still, the optimal extraction duration is a point of discussion: times between 6 and 30 h have been applied for individual compounds (*5,32,33*). In the present study we chose not to investigate Tenax extraction kinetics in detail, but extracted the sediments for 10 h. The results underestimated BPH concentrations in *L. variegatus* in a similar fashion as cyclodextrin, but the relationship observed was somewhat more significant (r^2=0.60; Figure 1F). Again, the correction factor needed to properly assess actual bioaccumulation would be concentration-dependent, as the slope of the relationship observed in figure 1F is > 1. Also in this case, the explanation might be fouling of the Tenax beads when shaken in the presence of NAPL-containing sediments or the ability to extract BPH from non-bioavailable phases. On the other hand, Figure 2 suggests fouling is not plausible, because Tenax, unlike worms, showed a preference for the low boiling point fractions. Anyhow, precautionary cleaning of the Tenax beads (powder) to remove any fouling is impossible.

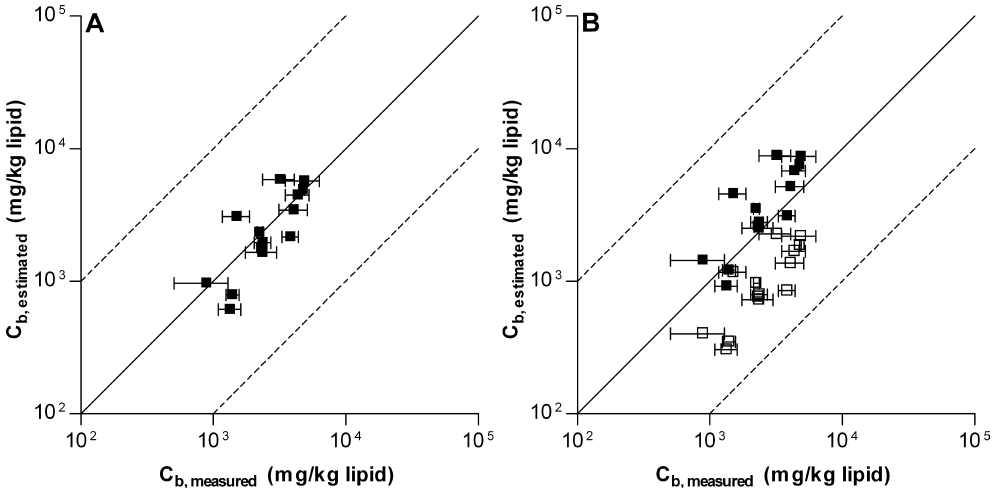

Figure 3. Relationships between BPH (C_{11}-C_{34}) concentrations measured in *Lumbriculus variegatus* exposed to 14 field-contaminated sediments and BPH concentrations in the worms as predicted by the model from Verbruggen (*20*), assuming aliphatic compounds to represent either 70% (A), 50% (B, closed symbols), or 90% (B, open symbols) of the total oil mass. The solid lines represent the 1:1 relationships; the dotted lines delimit the one order of magnitude deviation intervals. Values are averages ± standard deviations.

Modelling oil bioaccumulation

The application of the model from Verbruggen (*20*) resulted in predicted lipid-normalized BPH concentrations in worms that closely agreed (within a factor of two) with observed concentrations (figure 3A). In addition, for two sediments that appeared lethal to the worms (see Material and Methods section) and thus were excluded from the present data set, the model predicted lipid-normalized concentrations that exceeded the critical body residue for nonpolar narcosis (i.e., 50-200 mmol/kg lipid (*34*)), and theoretically thus should be lethal. As mentioned, the model predictions were based on the assumption that the oils in the different sediments all consisted of 70% aliphatic compounds. Lowering this percentage to 50% resulted in an over prediction, whereas increasing the percentage to 90% yielded an under prediction of actual accumulated oil concentrations (figure 3B). This model response is explicable because the aromatic fraction is assumed to be more soluble than the aliphatic one. The latter fraction hardly dissolves in the pore water and predominantly is present in liquid oil phases (*20*). Figure 3A suggests that the presently-assumed fraction distribution (70% aliphatic: 30% aromatic), as derived from the analyses of different oil-polluted soils (*22*) is realistic for sediments as well and that additional oil fractionations may not strictly be necessary when assessing oil bioaccumulation. However, the relationship in figure 3A shows quite some scatter (r^2 = 0.63), which could be related to differences in the fraction distribution between different oils (sediments). In terms of the BPH block pattern (figure 2), the model relatively closely mimicked the pattern

observed in worms as well and the model therefore seems quite promising. Nevertheless, several comments should be given. First of all, the model assumes the existence of NAPLs independent of the oil concentration. The presence of such phases probably considerably affects the sorption and availability of hydrocarbons (*6*, chapter 4). Several previous studies have however suggested that such separate oil phases may occur only above the CSPC, which should correspond to the about 30% saturation level of the organic carbon phase with oil (*6,31,35*, chapter 4). In the present sample set of 14 sediments, NAPLs are therefore only expected in the 5 most polluted sediments with low organic carbon contents (chapter 4). Second, due to the assumption of a nonlinear relationship between hydrophobicity and uptake (i.e. a hydrophobicity cut-off), bioaccumulation of very hydrophobic petrochemicals such as alkanes > C_{13} is modelled being negligible. Yet, these chemicals were observed in the worms in considerable quantities. Additionally, for aromatic compounds, the applied membrane-water partition coefficients are about 1-2 log units lower than those recently reported for PAHs (*36,37*). Despite this, the model over predicts the actual uptake of the fraction C_{24}-C_{30} (figure 2). Third, the model does not consider other sorption phases such as black carbon, which will often co-occur with oil contamination and might considerably lower the bioavailability of (specific) petroleum hydrocarbons. Fourth, the model does not take into account the weathering status of the oil, which can also be assumed to affect bioavailability (*6*). Considering the chromatograms of the sediment extracts (chapter 4), the present oils will have been relatively fresh and it therefore remains to be seen whether the model is able to predict BPH uptake from highly weathered oil-contaminated sediments equally well.

Overall method evaluation and selection

None of the five tested experimental methods was able to directly and accurately predict uptake of BPH in *L. variegatus*. In all cases, correction factors would be needed to match the extracted with actual bioaccumulated concentrations, i.e., in order to shift the observed relationships in figure 1B-F towards the 1:1 line. For SPME, cyclodextrin, and Tenax, such correction factors would be clearly dependent on the sedimentary oil concentration. A mechanistic basis for a similar correction is however lacking and the observed concentration-dependent behaviour is probably due to artefacts. Therefore, based on the present results these three methods should be concluded unsuitable for predicting the bioavailability of BPH in sediments. Another important reason for rejecting the methods is that the fraction distribution of the extracted BPH does not resemble the distribution observed in worm extracts. Any 1:1 predictions of the overall BPH values should therefore rather be considered a 'coincidence'. This certainly also applies to the hs-SPME approach, which is unfortunate because of this method's superiority in terms of speed, absence of bias by fouling, and significance of the observed correlation with BPH in worms. The experimental method that produced the second best correlation, did not require concentration-dependent correction factors, does not suffer from fouling, but did show a fraction distribution pattern that closely mimicked the biological pattern, is POM-SPE. Although this method is slow as compared to hs-SPME, cyclodextrin, and Tenax, and laborious as compared to (hs-)SPME, its (scientific) advantages in our opinion are outbalancing these practical

aspects. With the help of fraction-dependent correction factors, BPH concentrations in worms could be predicted within a factor of 3 (appendix IV, figure S4). Overall, the present study therefore suggests that POM-SPE would be the best experimental technique to assess the bioaccumulation of BPH in aquatic worms. By actually sampling the freely dissolved aqueous concentration, the method takes into account all conditions/processes controlling bioavailability, i.e., the weathering status of the oil and sorption to specific sedimentary phases. Recommendations for future applications of POM-SPE could be to lower the shaking intensity during equilibration in order to avoid breaking up NAPLs and thus the inclusion of unavailable fractions (although this may slow down kinetics and thus may require longer equilibration times), and to (re)try to measure K_{POM} and *BAF* values of BPH fractions in order to enable the mechanistic calculation approach.

As an alternative to the experimental approach, the model could be a potential tool for predicting bioaccumulation of complex hydrocarbon mixtures from sediments. Modelling is often considered preferable because of the convenient desktop-related activity involved; however, one should realize that the model actually requires input of (two or) three independent chemical analyses (total extraction, fractionation of total extracts into aliphatics and aromatics, and organic carbon determination), whereas POM-SPE only requires one measurement. Additionally, the BPH block pattern predicted by POM-SPE resembled the worm pattern more closely (figure 2 and appendix IV, figure S7). Recommendations for future applications of the model could be to adapt it based on the comments mentioned above. However, because mathematically modelling (the different processes influencing) bioavailability of complex petroleum hydrocarbon mixtures may not be easy or fully possible, a site-specific approach based on chemical-analytical (POM-SPE) measurements of bioavailability may be needed to obtain the most reliable predictions. In a follow-up paper, the potential of the model and the POM-SPE technique to predict actual *in situ* bioaccumulation will be tested.

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Chapter 6

Testing the potential of passive samplers to assess actual in situ bioaccumulation of PAHs and petroleum hydrocarbon mixtures

Barry Muijs, Michiel T.O. Jonker

Institute for Risk Assessment Sciences, Utrecht University

Abstract

Over the past couple of years, several analytical methods have been developed for assessing the bioavailability of environmental contaminants in sediments and soils. Comparison studies suggest that passive sampling methods generally provide the better estimates of internal concentrations in organisms and thus of subsequent risks. However, field studies to validate the potential of passive sampling to predict actual in situ exposure are scarce and information on mixtures is lacking, even though organisms are never exposed to single chemicals in the real world. The present study therefore investigated whether bioaccumulation of PAH and petroleum hydrocarbon mixtures in field-exposed aquatic worms could be predicted properly with passive samplers. To this end, in situ bioaccumulation at 9 PAH-contaminated locations and 9 petroleum hydrocarbon (oil)-contaminated locations was compared with the results of in situ solid phase micro extraction (SPME) applications. For the oil-contaminated sites, internal exposure was also assessed with polyoxymethylene solid phase extraction (POM-SPE) in the lab. Actual PAH bioaccumulation was generally predicted within a factor of 4 with in situ SPME, using temperature-adjusted SPME fibre-water partition coefficients and bioaccumulation factors (BAFs), demonstrating the method's validity under field conditions. In situ SPME could however not predict internal exposure of oil properly, in contrast to POM-SPE in the lab, which assessed in situ oil bioaccumulation within a factor of 3, while also closely reflecting the actual hydrocarbon block profile of the mixtures as accumulated by the worms. All in all, the results indicate that (specific) passive samplers, either applied in the field or the lab, have great potential for assessing internal exposure to environmental contaminant mixtures under real field conditions.

Submitted

Introduction

Organisms in the environment are exposed to a complex mixture of numerous contaminants. Assessing the risks associated with these chemicals requires detailed knowledge on their (internal) exposure concentrations, i.e., concentrations that have accumulated in the organisms. After all, these are the concentrations that are actually available for causing effects (*1*). The most straightforward way of measuring internal exposure is to perform bioaccumulation experiments with organisms and to subsequently determine the concentrations of the chemicals of interest by extracting the organisms. However, because such tests are laborious and expensive, and because of restriction and replacement considerations regarding animal testing, considerable effort has been put into developing alternative, chemical methods for assessing the internal dose. For organisms exposed to sediment or soil-associated contaminants, examples of such methods are Tenax extractions (*2*), cyclodextrin extractions (*3*), polyoxymethylene solid phase extraction (POM-SPE) (*4*), and solid-phase microextraction (SPME) (*5*). Recent comparison studies (*6,7*, and chapter 5) have indicated that among these methods the passive sampling approaches (e.g. SPME and POM-SPE), which determine freely-dissolved aqueous concentrations in the exposure medium, seem to produce more reliable estimates of internal concentrations than methods like Tenax, cyclodextrin, and mild solvent extractions, which are based on estimating the rapidly desorbing fractions of chemicals from sediments or soils (*2,3*). For instance, in situ bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in aquatic worms was best and closely predicted with in situ application of SPME (*6*). The technique performed slightly better than POM-SPE in the lab, and was clearly superior to Tenax and cyclodextrin extractions. Likewise, passive samplers predicted PAH uptake from soils into earthworms and plants better than methods based on capturing the rapidly desorbing fraction (*7*). Furthermore, internal concentrations of petroleum hydrocarbon mixtures (oil) in aquatic worms exposed to oil-contaminated sediments in the lab were most closely predicted with POM-SPE (chapter 5), whose estimates were outachieving those of (headspace) SPME, Tenax, and cyclodextrin extractions. The results of these studies all suggest that it is very well possible to use passive samplers for predicting the internal dose resulting from exposure to contaminated environmental samples. Still, information on the methods' potential for predicting internal concentrations under actual field conditions is scarce, as most work has been done in the lab and field studies investigated in situ PAH bioaccumulation at 2 locations only (*6*). For complex hydrocarbon mixtures no field data are available at all. As such, the question remains to which extent passive samplers are capable of closely predicting actual internal concentrations in organisms inhabiting the real world, i.e., at various locations under field conditions, where factors like e.g. temperature, substrate, the presence of co-contaminants, and food availability may vary.

In an attempt to answer this question, we performed in situ bioaccumulation experiments with aquatic worms (*Lumbriculus variegatus*) at nine PAH- and nine oil-contaminated locations in the Netherlands. The results were compared to those of the applications of the passive sampling techniques that were previously selected as best methods for predicting internal exposure, i.e. in situ SPME for PAHs (*6*) and lab-based

POM-SPE for petroleum hydrocarbons (chapter 5). Additionally, as hardly any knowledge exists on (in situ) exposure assessment of petroleum hydrocarbons, for these contaminants measurements were completed with in situ SPME and a desktop application of a model to predict petroleum hydrocarbon bioaccumulation (*8*).

Experimental section

Chemicals and extraction materials

Solvents used (hexane, heptane, acetonitril, and acetone) were obtained from Lab-Scan (Dublin, Ireland) and were of Pestiscan grade. Aluminium oxide (50-200 µm; Super I) was from MP Biomedicals (Eschwege, Germany) and was deactivated with 10% w/w of Millipore water. Silica gel (63-200 µm; for column chromatography) was from Merck (Darmstadt, Germany) and was impregnated with 7% w/w of sulphuric acid (Baker; Deventer, The Netherlands) or 33% w/w 1M sodium hydroxide (Merck). , Anhydrous sodium sulphate (Merck) was heated at 600°C and cooled in a desiccator. Calcium chloride, sodium azide were from Merck. Hydrochloric acid and atropine were purchased from Merck and Sigma Aldrich (Steinheim, Germany), respectively. PAHs (phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*e*]pyrene (BeP), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), benzo[*g,h,i*]perylene (BghiP), dibenz[*a,h*]anthracene (DahA), and indeno[1,2,3-*c,d*]pyrene (InP); all > 98%) were from Sigma Aldrich and 2-methylchrysene (99.2%) was from BCR (Geel, Belgium). Tetracontane (C_{40}, >95%) was from Fluka (Steinheim, Germany) and other alkanes with even carbon numbers between C_{10} and C_{36}, and C_{11} (all >98 %) were from Sigma Aldrich or Fluka. Certified standard oil (RIVM-LOC-001) was from NMi (Nederlands Meetinstituut, Delft, The Netherlands). Disposable SPME fibre (glass fibre core diameter 110 µm, poly(dimethylsiloxane) (PDMS) coating thickness 28.5 µm) was from Poly Micro Industries, Phoenix, AZ. It was cut into pieces of 4 cm length, which were shaken three times with methanol and three times with Millipore water for PAH analyses. For oil analyses, fibres were washed three times with heptane, after which they were air-dried (1 h). A polyoxymethylene sheet (POM; thickness 76µm; CS Hyde Company; Lake Villa, IL, USA) was cut into pieces of about 250 mg, which were Soxhlet-extracted for 6 h with acetone:hexane (1:3 v/v) and air-dried (1 h) before use.

Organisms

Aquatic worms (*Lumbriculus variegatus*) were cultured in our laboratory in 45 L aquaria on pulverized cellulose and cupper free water (23±1°C). The water was continuously refreshed and aerated, and worms were fed once a week with dried fish flake food (*6*). One day before the start of the field exposures, worms were scooped out of their aquaria and after the removal of cellulose they were gut-purged overnight under running tap water.

Sample locations

A first selection of sampling locations was made based on information provided by local water authorities and expected PAH or oil concentrations, resulting from adjacent activities. All potential locations were sampled and screened in the lab for bioavailable PAH and oil concentrations (with SPME) and worm survival. Nine sampling sites for PAHs and nine for oil (distributed over in total eleven different locations) were finally selected, not only based on worm survival and contaminant bioavailability, but also on accessibility, water depth (1 m max), and risk of vandalism. Information on the locations is provided in appendix V, tables S1 (PAHs) and S2 (oil).

In situ bioaccumulation and SPME

Field bioaccumulation tests and *in situ* SPME exposures were based on a previous study (6). In short, one custom-made bottomless stainless steel enclosure (l×w×h: 16 × 16 × 25 cm) was penetrated 18 cm in the sediment and one stainless steel SPME in situ exposure unit containing five 4 cm fibres was placed inside the enclosure, in the upper 4 cm of the sediment. Worms (20 g of fresh weight) were then carefully released into the enclosures and the enclosures were closed with stainless steel mesh. After four weeks of exposure, the water temperature inside the enclosures was measured and the SPME exposure units were collected. SPME fibres were immediately removed, wiped with a wet tissue (Millipore), cut, and transferred to autosampler vials with 250 µL inserts containing 180 µL of acetonitril for PAH analysis or 200 µL internal standard solution (60 mg/L tetracontane in heptane) for oil analysis. In the lab, 20 µL of PAH internal standard (10 mg 2-methyl-chrysene/L acetonitril) was then added to the PAH vials. Five sediment samples were taken randomly from the upper 4 cm of the sediment inside the enclosure, after which they were pooled for chemical analyses and/or POM-SPE experiments. The sediments inside the enclosures were subsequently excavated and transferred to plastic 5 L buckets. Autosampler vials and sediment samples for chemical analyses were kept on ice and all samples were transported immediately to the lab. Sediments with worms were stored in the laboratory at a temperature close to that in the field, and worms were removed manually the next day. They were gut-purged for 6 h in 100 mL of tap water while gently shaking to prevent re-ingestion of the faeces. Water was changed two times during the gut-purging process. Worms were subsequently collected, frozen, and freeze-dried.

POM-SPE

Oil bioaccumulation was assessed with POM-SPE as described in chapter 5. In short, wet sediments were transferred to 250 mL all-glass bottles and about 240 mL of an aqueous solution containing 0.01 M calcium chloride and 50 mg/L sodium azide was added, along with one POM strip. Bottles were then shaken for six weeks (150 RPM, 20°C) in the dark. POM strips were subsequently collected, cleaned with a wet tissue (Millipore), and Soxhlet-extracted with acetone:hexane (1:3 V/V) for 6 hours. Extracts were cleaned-up through aluminium oxide and the concentrated eluates were solvent exchanged to heptane. Tetracontane (300 mg/L heptane) was finally added as internal standard.

PAH and oil analyses

Detailed analytical procedures for PAHs and oil are given in references (9) and chapter 4, respectively. In short, sodium sulphate-dried sediments and freeze-dried worms were Soxhlet-extracted with acetone:hexane (1:3 V/V) for 16 hours. Extracts were purified through aluminium oxide in case of sediments and PAH-exposed worms. Oil extracts from worms were purified through multi-layered columns, containing basic- and acid-impregnated silica gel (chapter 2). After volume reduction, PAH extracts were solvent-exchanged to acetonitril and 2-methyl-chrysene was added as internal standard. Oil extracts were solvent-exchanged to heptane and tetracontane was added as internal standard. All extracts were transferred to brown autosampler vials. PAHs were analyzed with HPLC (*10*) and oil was analyzed with GC-FID (chapter 2), using large volume injection in case of SPME and POM extracts.

Lipid and organic carbon analyses

Lipid contents of worms were determined gravimetrically by evaporating acetone:hexane-extracted freeze-dried worm tissue to dryness (chapter 3). Nitrogen and organic carbon fractions of freeze-dried sediments were determined with an elemental analyzer (Carlo Erba Na 1100) using atropine as a standard, after inorganic carbon had been removed with hydrochloric acid (chapter 4).

Data analysis

Biota-to-sediment accumulation factors (BSAFs) for PAHs and separate petroleum hydrocarbon blocks (chapter 4) were determined according to:

$$BSAF = \frac{C_b / f_{lip}}{C_s / f_{oc}} \quad (1)$$

in which C_b is the concentration in the worms (mg/kg), C_s the concentration in the sediments (mg/kg dw), f_{lip} the lipid fraction of dry worms, and f_{oc} the fraction of organic carbon in dried sediments. PAH concentrations in *L. variegatus* were estimated (and compared to measured concentrations) from the concentrations in the in situ-exposed SPME fibres (C_f, mg/L PDMS) according to:

$$C_b = C_f \frac{BAF}{K_f} \quad (2)$$

where BAF is the lab-derived lipid-normalized bioaccumulation factor (chapter 3) and K_f the fibre-water partition coefficient. K_f and BAF values were taken from and temperature-corrected (chapter 3). In-situ organic carbon-water distribution coefficients (in situ K_{oc}s) and BAFs were calculated for PAHs from the measured concentrations in sediments, worms, and fibres, and temperature-corrected K_f values according to:

$$in\ situ\ K_{oc} = \frac{C_s / f_{oc}}{C_f / K_f} \quad (3)$$

$$in\ situ\ BAF = \frac{C_b / f_{lip}}{C_f / K_f} \quad (4)$$

Results and discussion

Field work evaluation

Field studies are costly, laborious, and sometimes difficult to control, due to unexpected weather events, mortality of organisms, or vandalism. Developing and applying simple tests or proxies that closely reflect actual exposure in the field is therefore highly advantageous. However, for validation purposes, as in the present study, field studies are still required. The present field experiment clearly illustrated the above-mentioned downsides of in situ exposures. First of all, although preliminary tests had indicated that sediments taken from locations 2, 3P, and 15 were not toxic to *L. variegatus*, survival rates at these locations were low (appendix V, table S3). This may for instance be related to the abnormal hot weather conditions during the exposures or possible inclusion of predators in the enclosures during installation. High temperatures and low oxygen concentrations may have killed the majority of the worms at the shallow location 2, and at locations 3P and 15 the surface was completely covered with duckweed. Second, test systems at three other locations were partly destroyed. Worms at location 8 escaped, because the enclosures were slanting due to settling of the peaty sediments, and strong water currents caused by shipping and tide at location 12 flushed away the enclosures for a couple of meters. Fortunately, fibres were recovered at both locations and some worms as well at location 12 by collecting sediment at the original location of the enclosures. The enclosure at location 14P was replaced after three weeks and new worms were added, because vegetation removal activities by local water authorities destroyed the field plot. The fibres were however recovered and placed back together with a new set, yielding a four and seven week exposure for this location.

Worms recovered from all locations appeared healthy, showed no signs of toxicity (i.e. protusions), and their physical condition (colour, length, activity) was similar to unexposed animals. Lipid contents varied between 10.8 and 19 % (see appendix V, tables S1 and S2), which is similar to or higher than that of unexposed *L. variegatus*, suggesting that substrate and food quality/quantity was at least sufficient. The field experiment eventually resulted in eight complete oil data sets and six data sets for PAHs (see appendix V, tables S1 and S2).

Predicting in situ PAH exposure

The objective of the present study was to investigate how actual in situ exposure would be predicted with passive samplers at different field locations with different characteristics. Appendix V, table S1 shows that the selected PAH locations were quite different, as for instance ΣPAH concentrations in sediments ranged from 0.28 to 59 mg/kg dry weight (dw) and the sediment carbon-nitrogen (C/N) ratio varied between 12 and 79. Also, the sorption strength of the PAHs to the different sediments varied

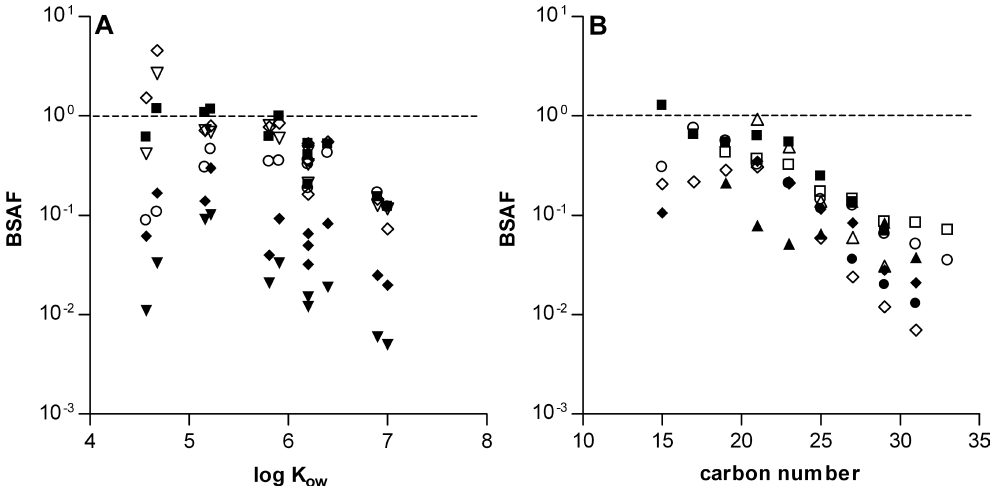

Figure 1. Biota-to-sediment accumulation factors (BSAFs) as a function of octanol-water partition coefficients (log K_{ow}) for PAHs (A) and as a function of the averaged carbon number of hydrocarbon blocks for petroleum hydrocarbons (B). Different markers indicate different sediments and dotted lines represent the theoretical BSAF of 1.

greatly, showing differences of more than 2 orders of magnitude between locations. This is illustrated in appendix V, figure S1 by site-specific in situ water organic carbon-partition coefficients (K_{oc}) values that were derived from the freely-dissolved aqueous concentrations determined with in situ SPME. The underlying data in appendix V, table S1 show that some in situ K_{oc}s are very high, with values up to 7 for Phe and almost 10 for InP. Such high values are quite exceptional for sediments, but the large variation in K_{oc}s for different locations has been observed before (*11,12*). It should be emphasized though that the present data set is one of the very few presenting true in situ K_{oc} values. Obviously, the differences observed in sorption strength will cause considerable variation in exposure concentrations (bioavailability). This is indeed demonstrated in figure 1A, which shows that in situ biota-to-sediment accumulation factors (BSAFs) derived for the different PAHs and locations varied with a factor of 10 to as much as 1000. Although some values were close to the theoretical value of 1-2 (*13*), most were far below 1. All locations however demonstrated the same trend of decreasing BSAFs with increasing hydrophobicity. This phenomenon has been reported before (*6,14,15*) and was attributed to sorption to sediment becoming stronger relative to partitioning into biota as the hydrophobicity of the chemicals increases. Although non-equilibrium conditions theoretically could explain the trend in figure 1A as well (*14,15*), this explanation is not plausible here as in situ-exposed *L. variegatus* and SPME fibres can be assumed to have equilibrated within 4 weeks (*6*). The present data set supports this hypothesis by showing linear correlations between $\log K_{oc}$ and $\log K_{ow}$ (*16*) and by the observation that the in situ SPME-derived exposure concentrations on location 14 for four and seven weeks of exposure were indistinguishable.

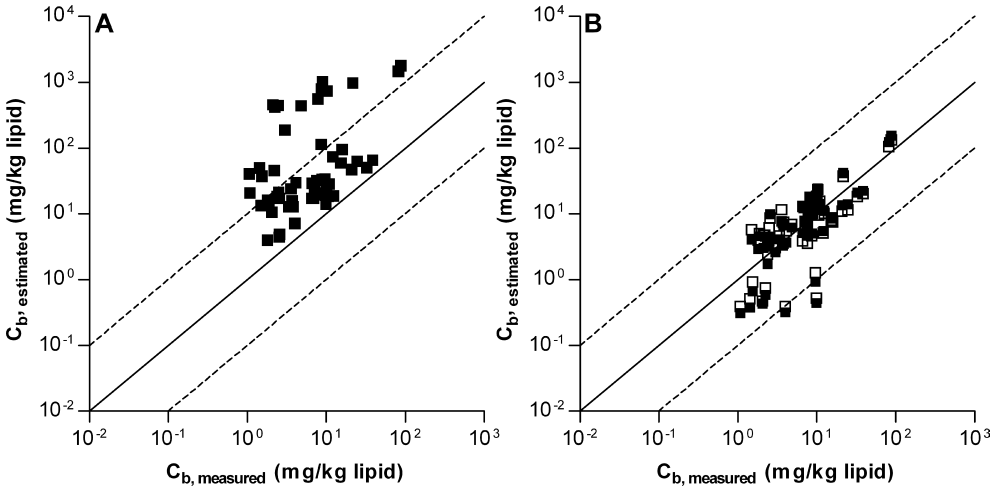

Figure 2. Relationships between measured and predicted concentrations of PAHs in in situ-exposed *Lumbriculus variegatus*. Figure A represents the current risk assessment practice (predicted concentrations are based on total organic carbon-normalized concentrations); Figure B shows predicted PAH concentrations based on in situ SPME measurements, with solid squares representing non-temperature-adjusted data and open squares being temperature-adjusted data. The solid lines are the 1:1 relationships, whereas the dotted lines delimit the one order of magnitude deviation intervals.

Predicted internal PAH concentrations (C_b; mg/kg lipid) in worms exposed at the different locations are presented in figure 2. According to expectations based on the above and the literature (*6,9*), concentrations were overestimated by a factor of 2-200 when using total concentrations in sediments and literature K_{oc} and BAF values (i.e., following the standard generic risk assessment procedure; here with log K_{oc} = 1,12 log K_{ow} – 0.86 (*6,17*) and BAF=K_{ow}) (see figure 2A). In contrast, accounting for site-specific differences in bioavailability, i.e., by using in situ SPME-derived freely-dissolved aqueous concentrations and multiplying them with literature BAFs (see eq. 2), internal concentrations in worms were generally predicted within a factor of 4 (figure 2B). The three lower outliers are data points for Phe and Ant for which we do not have an explanation. The K_f and BAF values used for the calculation of internal concentrations are however temperature-dependent (chapter 3) and the most accurate predictions of C_b should therefore be obtained by applying temperature-adjusted coefficients and factors. Doing so (figure 2B) resulted in only a minor improvement of the predictions (r^2 increased from 0.50 to 0.56 and the sum of absolute residuals (C_b measured–C_b predicted) decreased with 4.4 %). This is explicable though, because exposures were performed during late summer and actual in situ temperatures were close to 20 °C (see appendix V, table S3), i.e., the temperature at which the standard K_f and BAF values used for the non-temperature-corrected calculations were determined.

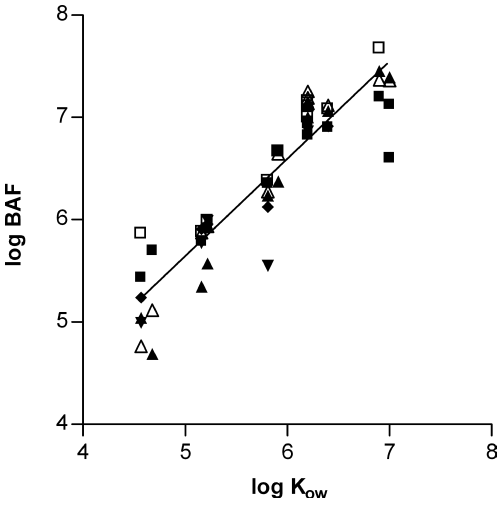

Figure 3. Logarithmic in situ (SPME-derived) bioaccumulation factors (log BAF) for *Lumbriculus variegatus* against logarithmic octanol-water partition coefficient (log K_{ow}) for PAHs. The solid line represent the linear regression curve. Different symbols represent different locations (■ 2P, ▲ 6P, ▼ 7P, ◆ 14P, □ 15P, and △ 16P).

Although the predictions of internal exposure concentrations via in situ SPME measurements are not bad and certainly should be considered acceptable for risk assessment purposes (they at least provide a considerable improvement as compared to the current generic procedure), figure 2B still shows somewhat more variation than expected based on previous work (*6*). The reason for this higher-than-expected variation lies in the fact that lab-derived BAF values were applied for the calculations. Although they were temperature-adjusted, the factors still represent one single value for a specific PAH. Figure 3 shows, however, that actual in situ BAFs (as calculated according to eq. 4 from in situ SPME-derived freely-dissolved aqueous concentrations and measured C_b values) are location-specific to some extent. The variation observed in these factors causes the variation in predicted concentrations observed in figure 2B. Question however is why in situ BAFs are not constant for one chemical and differ for different locations. Obviously, part of the variation will be caused by measurement errors. In particular the PAH concentrations in SPME fibres were generally low, which increases uncertainty in the data. This experimental variation is illustrated by the fact that in several cases BAFs seem to vary randomly. On the other hand, some locations consistently had higher or lower BAF values than others, which may point to a mechanistic cause of part of the variation. Because the BAF values are based on freely-dissolved aqueous concentrations and bioavailability issues are thus ruled out, such a mechanistic cause should have its origin in the organisms. Bioaccumulation may be affected by a series of biotic and abiotic factors, including organism size, age, sex, lipid composition, metabolism, avoidance behaviour, temperature, salinity, pH, feeding regime, food quality, growth rate, exposure concentration, and presence of other chemicals and stressors (*18-20*). Many of these factors do however not apply in the present study, as the organisms used at all locations originated from the same lab

culture, BAF data were temperature-adjusted and suggest that worms were equilibrated (see above), and bioaccumulation of PAHs can be expected to be independent of pH, small variations in salinity, and exposure concentration. Possibly, any systematic variation in BAF values may therefore stem from differences in substrate or food quality, causing e.g. differences in lipid composition (*19*). No consistent trends were however observed between BAF values and sediment organic carbon and nitrogen content, C/N ratio, or worm lipid content (results not shown), leaving the observed variation in figures 3 and 2B unexplained. The variation in figure 3 does however question the implicit assumption in risk assessment that BAFs are constant species-independent values. Apart from interspecies variation and temperature-induced intraspecies variation (*19*), the present data suggest that BAFs may differ as a result of location-specific factors.

Finally, the data in appendix V, figure S2 demonstrate that field-derived BAFs are not consistently higher than lab-derived values (except for Phe), as was observed for literature BAF data by Arnot and Gobas (*20*). Most probably, the discrepancy in literature values is caused by the differences in exposure time between lab and field studies (i.e., non-equilibrium conditions in short-term lab experiments may underestimate actual BAF values). The present study at least demonstrates that BAFs derived on the basis of freely-dissolved concentrations and measured with organisms that are equilibrated sufficiently long, are comparable in the lab and the field.

Predicting in situ bioaccumulation of oil

The locations selected for oil bioaccumulation assessments also varied in their total petroleum hydrocarbon (TPH; i.e., C_{11}-C_{40}) concentrations and bioavailability of oil. TPH concentrations in the sediments ranged from 100 to 1000 mg/kg dw (appendix V, table S2), showing a relative hydrocarbon block profile dominated by high boiling point fractions (C_{28}-C_{34}), which suggests the presence of relatively heavy and/or weathered oils (*21,22*). As also observed previously in the lab (chapter 4), worms accumulated petroleum hydrocarbons from sediments up to C_{34}, but showed a preference for lighter fractions (appendix V, table S2). These 'bioaccumulative petroleum hydrocarbons (BPH, i.e., C_{11}-C_{34}, chapter 5) reached concentrations of 440 to about 3000 mg/kg lipid. BSAF values for separate hydrocarbon blocks calculated from the concentrations in worms and sediments (see figure 1B) indicated that BPH bioavailability at the different locations differed with a factor of about 15. Similar to the trend observed for PAHs, BSAFs decreased with increasing hydrocarbon block (boiling point fraction). In contrast however to the explanation given above for PAHs, the boiling point-dependent BSAFs for petroleum hydrocarbons might be the result of enhanced sorption of increasingly hydrophobic/highly-boiling petrochemicals to hydrophobic phases (including oil phases), non-equilibrium conditions, and/or size exclusion (chapter 4). Because BSAF values were <1 even for the lowest hydrocarbon blocks and thus did not follow the theoretical value of 1-2, the present results clearly indicate reduced bioavailability at all locations. This reduced bioavailability might be caused by the presence of strongly sorbing carbonaceous sorbents (*23*) like soot (from e.g., pumping stations or ships) or car tire grindings (*24*), but their presence was not confirmed. The presence of oil was however confirmed, and separate oil phases are known to provide strongly sorbing

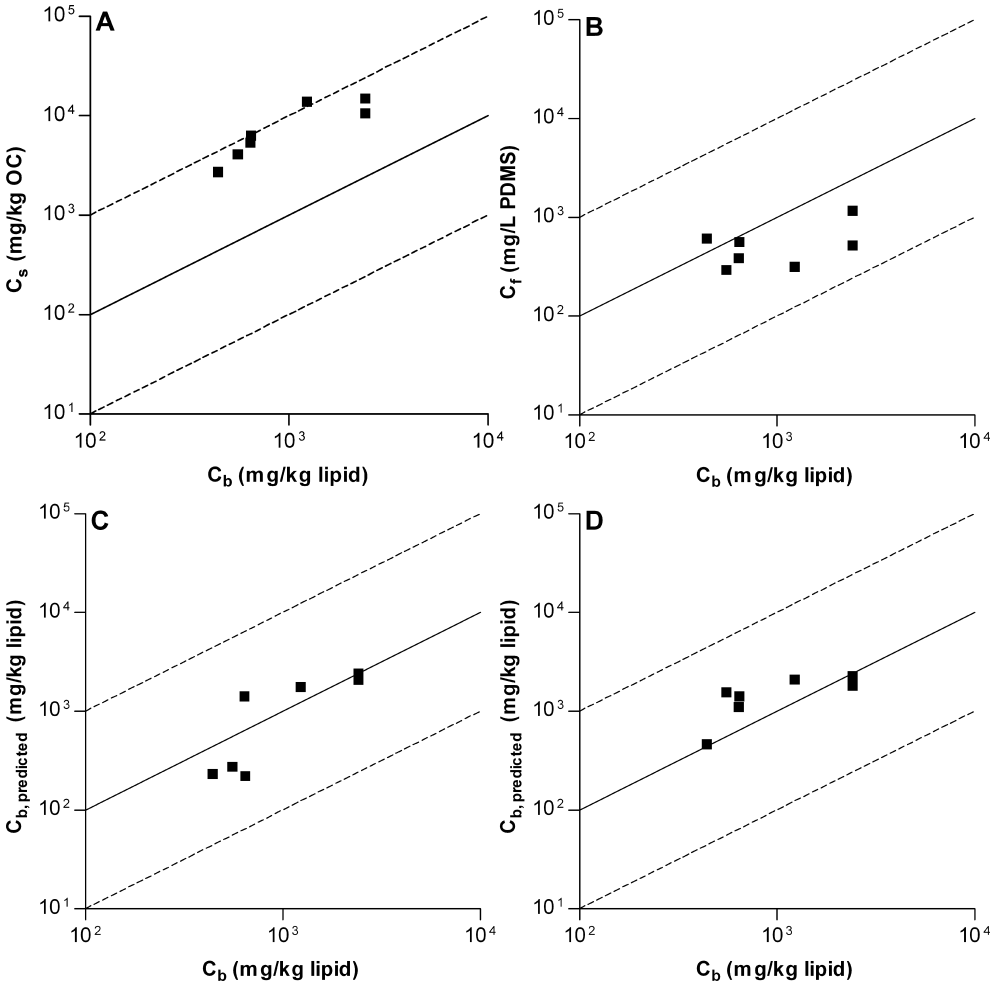

Figure 4. Relationships between BPH (C_{11}-C_{34}) concentrations measured in *Lumbriculus variegatus* exposed in situ to oil-contaminated sediments (x-axes) and BPH concentrations predicted (y-axes) using (A) organic carbon-normalized oil concentrations in sediments, (B) in situ SPME, (C) ex situ POM-SPE, and (D) a partitioning model (*8*). The solid lines represent the 1:1 relationships, whereas the dotted lines delimit the one order of magnitude deviation intervals.

domains for hydrophobic chemicals as well (*25,26*), thereby possibly reducing the availability of 'their own' petrochemicals (chapter 5).

Because of the reduced bioavailability, predicting BPH concentrations in worms based on total concentrations in sediments (i.e., assuming concentrations in lipids to be equal to organic carbon-normalized concentrations in sediments) makes no sense; it resulted in overpredictions with a factor of about 10 (figure 4A). In contrast, the passive samplers applied predicted BPH uptake within a factor of 3 (lab POM-SPE) or 5

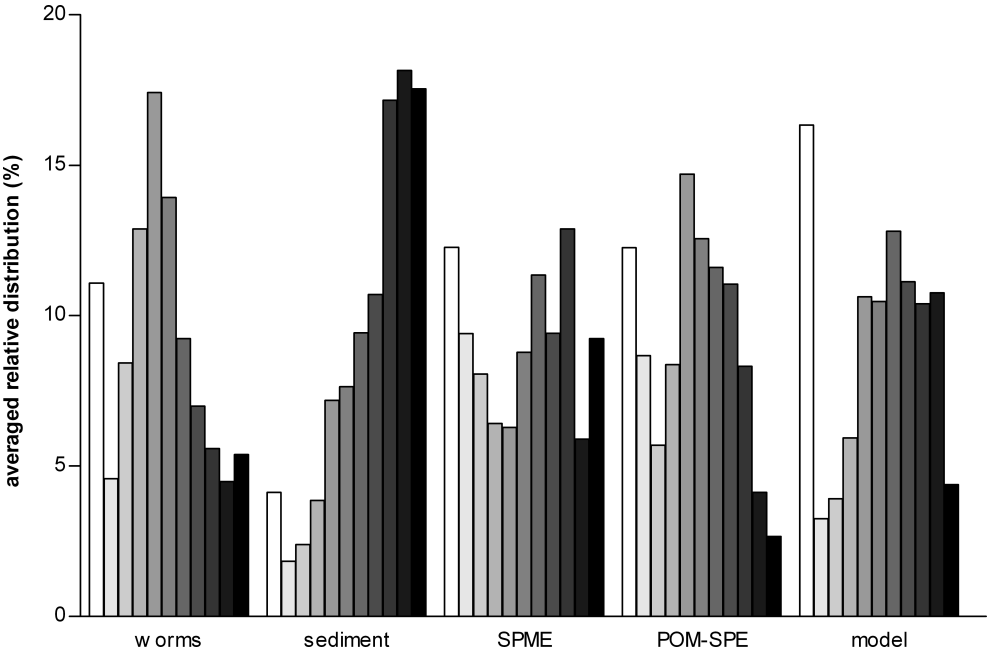

Figure 5. Averaged relative BPH boiling point fraction distributions as observed in extracts of worms (*L. variegatus*), sediments, in situ-exposed SPME fibres, and lab-exposed POM strips; and as obtained by the partitioning model applied. The consecutive bars (from light to dark) represent the following hydrocarbon blocks: C_{10}-C_{14}, C_{14}-C_{16}, C_{16}-C_{18}, C_{18}-C_{20}, C_{20}-C_{22}, C_{22}-C_{24}, C_{24}-C_{26}, C_{26}-C_{28}, C_{28}-C_{30}, C_{30}-C_{32}, and C_{32}-C_{34}. Note that the alkanes mentioned after the hyphens are not included in that block; they are part of the next block, which starts with the respective alkane.

(in situ SPME) (figure 4B-C). For SPME, BPH concentrations determined in the fibre coatings (mg BPH/L PDMS), however, tended to underpredict internal concentrations in worms (figure 4B). This underprediction might be explained by non-equilibrium conditions, since for dynamically-exposed fibres in the lab, full equilibration took 30-40 days (chapter 5) and the present fibres were statically exposed in situ for 28 d only. In the lab, the method either under or overpredicted bioaccumulation and a clear concentration-dependent behaviour was observed, with higher concentrations in sediments yielding higher concentrations in the fibres (chapter 5). This observation was hypothetically explained by fouling of the fibres with oil and it was concluded that the approach therefore was unsuitable for exposure assessment of BPH. Still, we applied the technique in the present study, because fibres were exposed statically in situ, thereby reducing the contact between fibres and any separate oil phases, thus reducing fouling possibilities. The absence of a concentration dependency (i.e., the slope in figure 4B not being >1) and the underprediction of C_b values suggests that fouling most probably was not an issue in situ. Yet, the r^2 value of the relationship in figure 4B is low (0.20) and the relative distribution pattern of separate BPH blocks did not resemble the pattern observed for worms well (see figure 5). Hence, although in

situ SPME seems reasonably capable of assessing *total* BPH concentrations in in situ-exposed worms, its use for assessing BPH exposure is not recommended, neither under dynamic conditions in the lab, nor under static conditions in the field.

POM-SPE in the lab clearly underpredicted concentrations in the field-exposed worms. This was expected however based on the works described in chapter 5 in which hydrocarbon block-dependent correction factors (on average about 17) were derived with which concentrations in POM were multiplied to obtain more accurate predictions of lipid-normalized concentrations in worms. Application of these previously lab-derived correction factors to the present data resulted in close predictions of BPH concentrations in in situ exposed *L. variegatus* (figure 4C). Moreover, the relative hydrocarbon block pattern in POM closely resembled the pattern observed for worms (figure 5). In other words, in addition to being the best method for assessing BPH bioaccumulation in the lab, POM-SPE also seems to be a very good lab-based passive sampler for predicting internal exposure in the field. At least, the method is preferred over SPME, all the more since fouling issues are not expected for POM, due to the material's smooth surface (*4,* and chapter 5). It should however be stressed once more that the exposure temperatures in the field were close to the lab temperature. Therefore, whether POM-SPE is capable of accurately assessing internal BPH exposure concentrations at other (much lower) temperatures remains to be investigated.

Finally, in situ BPH concentrations in worms were assessed with the model recently published by Verbruggen *et al*., which assumes petroleum hydrocarbons to be distributed over sediment organic matter, a liquid oil phase, pore water, and organism lipids, according to modelled equilibrium partition coefficients (*8*). Because the model needs the ratio of aliphatic to aromatic compounds as input and fractionations were not performed on the present oils, we assumed that all oils contained 70% aliphatic compounds, which previously proved to be a plausible assumption (*27*, and chapter 5). Although POM-SPE was previously selected as preferable method for predicting bioavailability in the lab (chapter 5), the model was a close runner-up. The same conclusion can be drawn on the basis of the present field data, as the model also predicted actual BPH concentrations in worms within a factor of 3, but the r^2 value of the relationship in figure 4D was somewhat lower than for POM-SPE (0.54 vs. 0.69) and the relative hydrocarbon block distribution pattern deviated more from the worm pattern than the POM pattern did (figure 5) (based on sum of squares calculations as previously (chapter 5); results not shown). Therefore, POM-SPE would be the preferred method when trying to assess actual internal exposure concentrations in the field as well, all the more because (i) application of the model requires 2-3 experimental measurements (TPH concentration in sediment, f_{oc} determination, and preferably a separation of TPH into an aliphatic and aromatic fraction), whereas POM-SPE requires only one, and (ii) occasional excessive reductions in bioavailability (because of the presence of e.g. high coal or tar contents) will be reflected by POM-SPE measurements, but will probably be missed by the model.

In summary, this study showed that passive samplers are very useful tools for the in situ exposure assessment of individual chemicals, but also of complex hydrocarbon mixtures ubiquitously present in the environment. The samplers can integrate all

factors influencing the bioavailability of such compounds and mixtures, thereby providing accurate predictions of the internal dose and subsequent risks of environmental contaminants. As such, they are very helpful techniques when taking remediation decisions and setting cleanup priorities.

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Chapter 7

Summary and implications

Summary

Aquatic organisms are exposed to a cocktail of hydrophobic organic compounds released both as single compounds from multiple sources and as multiple compounds from single sources. Adequate risk assessment for these compounds is of vital importance, but often focuses on single compounds rather than complex mixtures, despite the fact that single compounds rarely occur outside of the laboratory. Bioaccumulation plays a major role in risk assessment as it determines the effects of contaminants on directly exposed organisms, as well as the effect on organisms, such as humans, which may be indirectly exposed via the food chain. The goal of this thesis was to improve the assessment of bioaccumulation of complex mixtures in aquatic (benthic) organisms, focusing on polycyclic aromatic hydrocarbons (PAHs) and petroleum hydrocarbons (oil), contaminants that are ubiquitously present in fresh water and marine sediments.

Current approaches have several shortcomings. Firstly, they assume that exposure concentrations in sediments are completely controlled by the amount of organic carbon in sediments. During recent years, however, it has been demonstrated that the bioavailability of organic compounds varies enormously amongst sediments due to the differences in organic matter composition, time-depended sorption (aging), and the presence of hydrophobic sorption phases other than organic carbon, such as black carbon, soot, and even oil phases. Moreover, current approaches rely strongly on

parameters which were determined in laboratories at standardized conditions, and are not necessarily representative for actual bioaccumulation as observed in the field. Current risk assessment might, therefore, result in erroneous predictions of actual risks.

Bioaccumulation factors (BAFs) play a key role in risk assessment of organic chemicals in soils and sediments. These factors are, however, difficult to determine experimentally and therefore values are mostly obtained by modelling. Apart from the lack of reliable data, the accuracy of field applications of bioaccumulation factors derived in the laboratory at standardized conditions is unknown. Therefore, the effect of temperature on the bioaccumulation of a series of moderate to very hydrophobic PAHs in the aquatic worm *Lumbriculus variegatus* was studied in a three-phase system that consisted of water, cellulose, and worms, It was demonstrated that this approach resulted in more reliable bioaccumulation factors, especially for more hydrophobic PAHs (Chapter 2). As freely-dissolved, bioavailable PAHs were determined using solid phase micro-extraction (SPME) fibres coated with polydimethylsiloxane (PDMS), the effect of temperature on fibre-water partition was determined as well. Accumulation of non-metabolizable hydrophobic organic chemicals can be expected to decrease with increasing temperature, because sorption to storage and membrane lipids being the major partitioning sites for these chemicals is an exothermic, enthalpy-driven process. This was demonstrated for PAHs (chapter 2). Bioaccumulation of PAHs may be therefore several times higher in winter than during the summer, which might have ecotoxicological consequences. Based on the results of Chapter 2, bioaccumulation of PAHs can be predicted from the temperature and the compounds' hydrophobicities. Chapter 2 also demonstrated that PDMS-coated SPME-fibres cannot be used as a surrogate for bioaccumulation of PAHs as suggested before because (temperature-dependent) sorption to lipids and PDMS is different. Still, SPME provides an appropriate analytical tool for the measurement of aqueous concentrations, from which bioaccumulation can subsequently be estimated by using bioaccumulation factors. As the relationship between temperature and partitioning of PAHs to both PDMS and lipids has been quantified, the technique is now applicable to assess bioaccumulation in *L. variegates* exposed to PAH-polluted sediments at least within a temperature range of 4 to 24°C.

Unlike PAHs, petroleum hydrocarbons are a mixture consisting of thousands of hydrophobic compounds which are not individually detectable. For environmental risk assessment purposes, their concentrations, however, have to be determined in various matrices, including soils and sediments as well as biota. This is frequently done by solvent extractions and subsequent analysis with gas chromatograpy (GC) equipped with flame ionisation detection (FID) or mass spectrometry (MS). As the total GC detector response between the retention times of two marker alkanes (mostly decane (C_{10}) and tetracontane (C_{40})) is labelled as the total petroleum hydrocarbon (TPH) concentration, and matrix interfering compounds such as organic matter and lipids will contribute to this signal, proper clean-up of the extracts prior to detection is crucial. Although petroleum hydrocarbons are analyzed in various matrices frequently, there is still no consensus on the clean-up agents to be used and thus the choice for a specific clean-up is arbitrary. It was demonstrated in Chapter 3 that none of the commonly used

agents fulfils the requirements of complete matrix compound removal and a high TPH recovery. The comparison study performed in this chapter, however, finally resulted in the recommendation of a novel column filled with (top-down) 1 g of 33% (w/w) 1M NaOH-impregnated and 2.2 g of 7% (w/w) H_2SO_4-impregnated silica gel for cleaning-up biota extracts that fully removes extracted lipids and yields acceptable TPH recoveries of around 90%. For sediment extracts, most columns tested resulted in a negligibly low contribution of matrix compounds to the overall detector response. Open column chromatography using 5% deactivated Florisil or 10% deactivated aluminium oxide is preferable for sediments, because these materials yield the highest (± 95%) TPH recoveries.

Although bioaccumulation plays an important role in risk assessment, the current knowledge on bioaccumulation of petroleum hydrocarbons is limited. Therefore, bioaccumulation of petroleum hydrocarbons in the aquatic worm *L. variegatus* exposed to 14 field-contaminated sediments collected from various locations with a known history of oil pollution was studied in detail (Chapter 4). The main focus was on uptake kinetics, the relationship between oil boiling point fraction and uptake, and the effects of sediment characteristics. Uptake kinetics became slower with increasing boiling point fraction, but 70 to 90 percent of the equilibrium situation was reached within the standard exposure duration of 28 d. Worms accumulated sedimentary petroleum hydrocarbons in the range of C_{10} to C_{34}, a range much wider than expected. Biota-to-sediment accumulation factors (BSAFs) for separate boiling point fractions were constant and around the proposed value of 1 - 2 up to C_{22}, but gradually decreased beyond this point. The decrease was probably caused by a combination of non-equilibrium conditions and enhanced sorption of higher boiling point fractions to sediments; the latter possibly due to the presence of strongly sorbing separate oil phases or black carbon. A negative relationship was observed between BSAF and oil concentration in sediment, which was explained by the presence of separate oil phases at high oil concentrations. These strongly sorbing phases may limit their own availability, particularly when being highly weathered. Separate oil phases may also be avoided by worms. Only a weak relationship (r^2=0.59) was observed between organic carbon-normalized TPH concentrations in sediments and bioaccumulation expressed as lipid-normalized TPH concentrations, suggesting that the uptake of petroleum hydrocarbon is not exclusively influenced by sediment organic matter. The observed phenomena have obvious implications for bioaccumulation assessment of oils and suggest that the current risk assessment procedure for oils in sediments may lead to erroneous results.

Because bioaccumulation of oil may be controlled by bioavailability, it should be possible to assess bioaccumulation of petroleum hydrocarbons by analyzing the exposure concentrations or the bioavailable fraction. Several experimental methods have been developed for this purpose, but only for individual organic compounds and so far none of these methods has been applied to complex mixtures, such as petroleum hydrocarbons. The potential of five of these experimental methods and that of a model approach for bioaccumulation assessment was tested in Chapter 5. The sediments used in the bioaccumulation study (Chapter 4) were extracted using SPME, headspace SPME, polyoxymethylene solid phase extraction (POM-SPE), Tenax, and

2(hydroxyl)propyl-β-cyclodextrin. Bioaccumulation assessed by all methods was subsequently compared with bioaccumulation in worms as previously determined (chapter 4). This comparison was based on both total bioaccumulative petroleum hydrocarbon concentrations and the relative distribution pattern of separate boiling point fractions (hydrocarbon blocks). None of the experimental methods was able to directly assess bioaccumulation in *L. variegatus* and correction factors were needed to match predicted and actual concentrations. These factors appeared concentration-dependent for SPME, and extractions with Tenax and 2(hydroxyl)propyl-β-cyclodextrin most probably due to artefacts. Moreover, an additional reason for disqualifying these methods as well as headspace-SPME is that the hydrocarbon block pattern of the extracts differed considerably from the pattern observed for worms. By contrast, the pattern produced by POM-SPE closely mimicked the worm pattern and allowed for a sediment, hydrocarbon block, and concentration-independent correction factor (17) to be derived and actual bioaccumulation to be predicted within a factor of three based on these derivations. Finally, the model directly predicted bioaccumulation within a factor of two. The accompanying hydrocarbon block pattern, however, deviated more from the worm pattern than the POM-SPE pattern did. It was therefore concluded that POM-SPE may be the best overall approach to predict bioaccumulation of complex hydrocarbon mixtures in aquatic worms, all the more since an experimental approach will implicitly capture all factors determining bioavailability, which may prove difficult through a modelling approach.

The comparison studies described in Chapter 5 suggest that passive sampling methods generally provide the better estimates of internal concentrations in organisms and thus of subsequent risks. However, field studies to validate the potential of passive sampling to predict actual in situ exposure are scarce and information on mixtures is lacking, even though organisms are never exposed to single chemicals in the real world. The study presented in Chapter 6 therefore investigated whether bioaccumulation of PAH and petroleum hydrocarbon mixtures in field-exposed aquatic worms could be predicted properly with passive samplers. To this end, in situ bioaccumulation at 9 PAH-contaminated locations and 9 petroleum hydrocarbon (oil)-contaminated locations was compared with the results of in situ SPME applications. For the oil-contaminated sites, internal exposure was also assessed with POM-SPE in the lab. Actual PAH bioaccumulation was generally predicted within a factor of 4 with in situ SPME, using temperature-adjusted SPME fiber-water partition coefficients and bioaccumulation factors (BAFs), demonstrating the method's validity under field conditions. In situ SPME could however not predict internal exposure of oil properly, in contrast to POM-SPE in the lab, which assessed in situ oil bioaccumulation within a factor of 3, while also closely reflecting the actual hydrocarbon block profile of the mixtures as accumulated by the worms. All in all, the results indicate that (specific) passive samplers, either applied in the field or the lab, have great potential for assessing internal exposure to environmental contaminant mixtures under real field conditions.

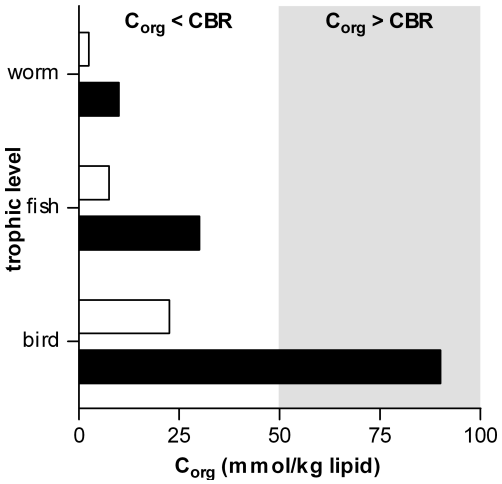

Figure 1. Estimated biotic HOC concentrations (C_{org}; mmol/kg lipid) in an imaginary aquatic food web during summer (white bars) and winter (black bars). CBR is the lipid-normalized molar biotic HOC concentration at which 50% of the organisms die.

Implications

Implications for exposure assessment of PAHs

Measuring exposure concentrations using SPME (*1*) is an adequate tool to assess bioaccumulation of PAHs from soils and sediments (*2,3*), but outcomes are not necessarily representative for contaminant uptake in the field, because partitioning is influenced by exposure conditions (*4*). The observed temperature dependency as shown in Chapter 2 suggests that bioaccumulation of PAHs is higher in cold seasons due to its exothermic behaviour. Bioaccumulation in *L. variegatus* in wintertime is expected to be about four times higher than during summer. A factor of four may be within the confidence intervals of the models used, but can become important when taking account of biomagnification. This is illustrated in figure 1, which presents HOC concentrations in an imaginary aquatic food web. During wintertime, concentrations in aquatic worms will be a factor of four higher than in the summer, but may still be far below the toxic level. In figure 1, this threshold is indicated by the critical body residue (CBR), the molar body residue of HOCs causing mortality: 50-200 mmol/kg lipid (*5*). Assuming an average biomagnification factor of 3, a value based on PCB and pesticide data in Northern American lakes (*6*), body residues in fish feeding on worms may be three times higher but still below the critical limits. However, concentrations in any top predator, in figure 1 represented by a bird, will be 9 times higher than in worms. Whereas during the summer body residues in this top predator may be below the critical threshold, the chance of exceeding the threshold and therefore the likelihood of effects on the ecosystem increases during wintertime (see figure 1). One assumption within this assessment is that CBR values are independent of temperature. For small fish it was shown that CBR values may be higher at lower temperatures; a difference of a factor of 2 was observed between the CBR measured at 20 and 5 °C (*7*). Therefore,

part of the increased concentrations at lower temperatures may be compensated by a higher dose needed for effects. Also, it should be noted that at low temperatures partitioning slows down and organisms are less active in terms of movement and food intake. As such equilibrium conditions may not always occur in the field. Finally, the assessment applies solely to non-metabolizable chemicals that are subject to biomagnification. The presently studied chemicals (PAHs) typically undergo trophic dilution instead of biomagnification (*8*), due to their liability to biotransformation. Figure 1 may therefore not be a realistic scenario for these chemicals, but for compounds such as PCBs it is representative (*6*), assuming that a temperature effect similar to the one observed here for PAHs applies to these chemicals. In summary, despite only a small increase in contaminant concentrations in aquatic worms in wintertime, effects at higher trophic levels cannot be excluded; therefore, it is recommendable to adjust for temperature in risk assessment models.

Because the relationship between temperature and partitioning of PAHs to PDMS-coated SPME fibres and animal lipids was quantified (Chapter 2), SPME is now applicable to determine exposure concentrations and subsequent bioaccumulation in sediments where temperature ranges between 4 and 30 °C. Although the application of SPME *in-situ* by using the temperature-dependent partition coefficients as determined in Chapter 2 results in the most realistic assessment of bioaccumulation, application in the field is technically not always feasible (Chapter 6). An alternative is to extract PAHs from sediment samples taken from the field and compensate for the temperature by applying the temperature-dependent partition coefficients. However, because partition to the sediments' organic phase (*9*) is also influenced by temperature, exposure concentrations are changing and no longer representative for the field. It is therefore advised to transport, store, and extract samples at the temperature of sampling. Because partition of organic contaminants is influenced by other factors such as salinity (*10-13*) and acidity (*14*) as well, the proposed SPME method is currently only applicable to assess exposure concentrations and bioaccumulation in fresh water sediments. However, recent research has revealed the influence of salinity on the partition of PAHs to PDMS-coated SPME fibres (*13*), which may broaden its application to estuarial and marine environments. Because it was demonstrated that lipid composition influences bioaccumulation of hydrophobic organic carbons (*15,16*), the temperature-dependent bioaccumulation factors presented in this thesis might only be applicable to assess bioaccumulation in the species investigated (i.e. *Lumbriculus variegates*). It is therefore advisable to collect bioaccumulation data regarding the dependency on environmental, ecological, and physiological factors of other representative species. With these data, one simple, cheap, and non-laborious SPME-extraction would be sufficient to assess internal exposure concentrations and resulting risks for a wide range of species.

Implications for exposure assessment of petroleum hydrocarbons

Previous attempts to determine bioaccumulation of petroleum hydrocarbons may have been biased as petroleum hydrocarbon concentrations were overestimated by lipids and other interfering matrix compounds insufficiently removed by clean-up prior to analyses on a gas chromatograph. Therefore, various existing and new agents for

open-column chromatography were compared (Chapter 3), resulting in a method that resulted in lipid-free extracts, while recoveries where still high. Unlike previous studies, bioaccumulation data presented in this thesis was therefore truly related to petroleum hydrocarbons. Model calculations based on concentrations measured in sediments were related to oil pollution as well, because the comparison study clearly demonstrated that the contribution of interfering matrix compounds, which are not removable by any of the tested agents, was negligible, which does not seem problematic. Considering the appropriate clean-up method, chapter 4 demonstrated that petroleum hydrocarbons are bioaccumulative and therefore environmental risk of oil contamination cannot be entirely related to physical effects caused by fouling of organisms exposed. Assessing the impact of oil disasters and removal of oil after such a disaster should, therefore, not only focus on oil that floats on the water surfaces, washes up on shores and beaches, and smothers birds and mammals, but also on the water-accommodated and sediment-associated fractions of petroleum hydrocarbons as these fractions are bioaccumulative and toxic (*17*). Although current oil disaster management is focussed on removal of visible oil, adequate removal measures may even result in increased mortality of aquatic organisms, because of an increased bioavailability of petroleum hydrocarbons when oil films on water surfaces are removed by the application of dispersants (*18*).

Exposure assessment based on organic carbon-normalized concentrations in sediments using the generally accepted BSAFs of 1-2 clearly overestimated bioaccumulation (Chapter 4). Petroleum hydrocarbons in sediments appear to be less bioavailable than assumed (Chapter 4), emphasizing once more the need to improve bioaccumulation assessment by including all factors that determine bioavailability. Despite numerous oil spills making oil ubiquitous in aquatic and terrestrial environments, there have been little efforts in this direction. An improvement was made by introducing a model that estimates petroleum hydrocarbon accumulation in phospholipid membranes from the concentration in sediments assuming a distribution of the chemicals over the sediment organic phase, pore water, membranes, and separate oil phases (*19*). However, models are simple mathematical visions of reality and their outcomes are not necessarily representative for actual bioaccumulation. Analytical-chemical methods are therefore preferable, from which POM-SPE was determined to be the best overall measure of bioaccumulation in *L. variegate*s (Chapter 5). POM-SPE also accurately assessed bioaccumulation of field-exposed *L. variegate*s (Chapter 6), suggesting that this method is representative of actual bioaccumulation as observed in the field. Although the POM-SPE method was used to assess bioaccumulation in aquatic worms only, its application is relevant for risk assessment because of the important ecological role worms play as substrate shedders and prey. Despite its success, the application of POM-SPE might be limited to sediments having oil concentration below the critical separate phase concentrations which has been reported in the range of 10 to 30% of the sediment organic carbon content (*20-22*), because it was demonstrated that POM extracted oil from solid and liquid phases that were not available for bioaccumulation in *L. variegate*s (Chapter 5). The same phenomena were also observed for the other methods applied, suggesting that this specific behaviour was not related to POM-SPE only. The suggested limitation

seems, however, only valid for selective feeders such as worms (*23*) because the capability to accumulate petroleum hydrocarbons from separate oil phases may be related to the ecology and physiology of the organisms involved. In conclusion, POM-SPE is a valuable tool to assess internal exposure concentrations in aquatic worms, but future research is needed to broaden its application to other organisms.

Risk assessment of complex mixtures

The basic principle of risk assessment is to link exposure to effects. Risks are expected when predicted exposure concentrations (PECs) are larger than the predicted no-effect concentrations (PNECs) (*24*). This approach is relatively simple for single compounds and mixtures with individually identifiable compounds because it is usually possible to determine the required parameters for exposure and effect assessment. However, a different approach is required for complex mixtures because it is more difficult if not impossible to obtain these parameters. It was demonstrated that organisms selectively accumulate PAHs and petroleum hydrocarbons from sediments (Chapters 4 and 6). Therefore, complex mixtures extracted from the solid phase are not representative for bioaccumulation and related risks. However, as SPME and POM-SPE adequately assessed bioaccumulation of PAHs and petroleum hydrocarbons, respectively, is it possible to compare internal exposure with internal effects considering the non-specific mode of action of the majority of the HOCs. It is generally accepted that organisms die when concentrations of HOCs in membranes lipids are too high, and risks are expected when the internal exposure concentration (predicted with SPME or POM-SPE) exceeds the critical body burden (CBR) of 50-200 mmol/kg lipid (*5*). This approach was previously successfully applied to assess toxicity of worms exposed to PAH-polluted manufactured gas plant soils (*2*). That study demonstrated that no worms survived exposure to soils where SPME predicted bioaccumulation above the CBR.

At the field locations studied in this thesis, worm recovery was low after *in-situ* exposure to PAH-polluted sediments for some locations (Chapter 6), but the previously presented SPME approach demonstrated that this was not related to PAHs, because predicted body burdens were well below 50-200 mmol/kg lipids (the highest predicted exposure concentration was 1.8 mmol/kg lipid). The low survival was therefore attributed to abiotic factors such as inappropriate substrate, high temperature, and low oxygen levels. Effects of other pollutants such as chlorinated organics and/or heavy metals were not plausible, because preliminary toxicity tests demonstrated that none of the sediments were toxic.

A similar approach is applicable for risk assessment of complex organic mixtures, but unfortunately, molar concentrations are not easily obtained from the response of the gas chromatograph. Separation of petroleum hydrocarbons with gas chromatography is mainly based on boiling point, which is not related to molar weight (*19*). Additional fractionation into aliphatic and aromatic compounds (*19*) or the application of sophisticated analytical techniques such as 2-dimensional gas chromatography (*25,26*) would be required to tackle this problem, but such detailed analyses were outside the scope of this study. Therefore, it was assumed that the oil in the oil-

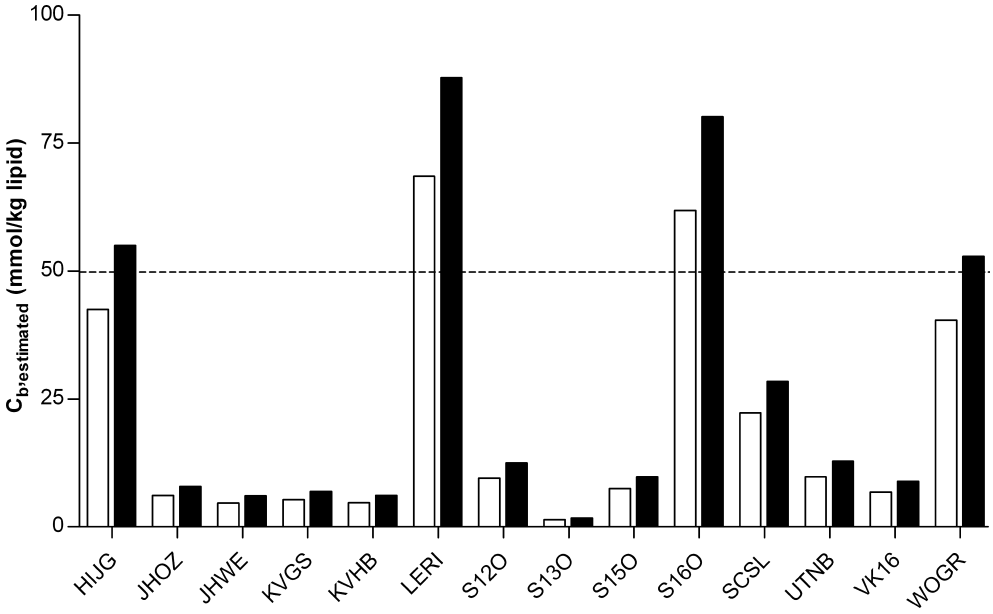

Figure 2. Estimated molar TPH concentrations in *L. variegatus* based on POM-extracted sediment concentrations, a correction factor of 17, and 70% (open bars) and 0% (closed bars) of aliphatic compounds. Dashed line represents the lower limit of the critical body burden (50 mmol/kg lipid)

contaminated sediments studied was made up of 70% of aliphatic compounds (*25*). Molar TPH concentrations per hydrocarbon block were subsequently calculated for both aliphatic and aromatic compounds by applying the relationships presented in Verbruggen *et al*. (*19*) and subsequently summarized. POM-SPE-predicted molar TPH concentrations were slightly above 50 mmol/kg lipid for several sediments (figure 2), but mortality was however not observed in any of these sediments. This may suggest that POM-SPE is suitable to assess risks for organisms exposed to oil-contaminated sediments by comparing bioaccumulation with critical body burdens, but unfortunately not verified as POM-SPE was not performed on the two sediments that were highly toxic to *L. variegates* (Chapter 4) due to logistic reasons. Also note that environmental risks may include fouling of the exposed organisms, but physical effects due to exposure to oil pollution are not predictable using SPME or POM, because fouling of organisms strongly depends on their ecology and physiology.

The assessment of exposure concentrations using SPME or POM lasts at least four weeks, making these tools rather slow as a screening tool for polluted sediments. Simple toxicity screenings or modelling when animal testing is not desirable may be alternatives. Models, however, require data on sediment concentrations and organic carbon contents, but these data are usually present as research on polluted soils and sediments within the framework of environmental legislations often begins with analyses of the concentrations. However, when sophisticated insight in environmental risks is required, SPME and POM become valuable, such as for remediation of soils and

sediments. Remediation is nowadays based on risk reduction instead of recovery of the multifunctional use of soils and sediments. As such, it is no longer standard practice to excavate and replace polluted soils and sediments with clean ones; however, adequate measures are taken in order to prevent negative effects for both the environment and human health. This may occasionally result in excavation, but also in risk reduction measures such as covering soils and sediments with a soil or sediment layer that prevents access to the pollution, or simply doing nothing. The decision for a particular remediation approach is based on the expected risk reduction in which availability plays an important role. Pollution to be removed by *in-situ* remediation also requires adequate insight into availability, because it also determines remediation efficiencies as only the available fractions are expected to be microbiologically degraded, as well as the risks of any leftover pollution. Another role for SPME and POM-SPE in future laboratory research may be the determination of model parameters necessary to predict environmental and effect concentrations. The determination of bioaccumulation factors is often troubled by experimental artefacts such as sorption to dissolved organic material, resulting in an underestimation of bioaccumulation (*27*). One solution may be to use actual exposure concentrations instead of extracting the water phase, which includes organic carbon-bound organics (Chapter 2 and reference (*27*)). POM-SPE and SPME may furthermore reduce undesirable animal testing required for the authorization of new and existing chemicals as they are able to predict bioaccumulation and related risks. At last, SPME and POM are applicable for hazard identification and risk assessment because they can exclude the contribution of HOCs from the effects observed in organisms in sediments exposed to multiple biotic and abiotic stress factors. In conclusion, passive sampling can contribute substantially to an improved risk assessment procedure of organic contaminants. Some practical and regulatory challenges are, however, still lying ahead, but at least the road to implementation of the technique may be considered paved.

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Appendix I

Temperature-dependent bioaccumulation of Polycyclic Aromatic Hydrocarbons

additional information

Table S1. PAH fibre-water partition coefficients (K_f) measured at different temperatures for SPME fibres coated with 30µm PDMS. See experimental section for PAH abbreviations.

PAH	Temperature (°C)	Log K_f (± SD)	Slope (± SE)	Intercept (± SE)	r^2	*p*
Phe	4.7	3.93 ± 0.0365	-0.00987 ± 0.00105	3.98 ± 0.0199	0.854	< 0.0001
	13.1	3.86 ± 0.0301				
	22.2	3.73 ± 0.0686				
	30	3.69 ± 0.0416				
Ant	4.7	4.03 ± 0.0252	-0.0102 ± 0.000943	4.08 ± 0.0178	0.887	< 0.0001
	13.1	3.95 ± 0.0212				
	22.2	3.83 ± 0.0695				
	30	3.78 ± 0.0401				
Flu	4.7	4.43 ± 0.0230	-0.0131 ± 0.000854	4.49 ± 0.0161	0.940	< 0.0001
	13.1	4.32 ± 0.0199				
	22.2	4.20 ± 0.0691				
	30	4.10 ± 0.0356				
Pyr	4.7	4.53 ± 0.0243	-0.0139 ± 0.000865	4.59 ± 0.0163	0.945	< 0.0001
	13.1	4.41 ± 0.0192				
	22.2	4.28 ± 0.0699				
	30	4.18 ± 0.0357				
BaA	4.7	5.04 ± 0.0191	-0.0145 ± 0.000843	5.11 ± 0.0164	0.955	< 0.0001
	13.1	4.92 ± 0.0196				
	22.2	4.78 ± 0.0648				
	30	4.68 ± 0.0318				
Chr	4.7	4.98 ± 0.0209	-0.0155 ± 0.000863	5.06 ± 0.0163	0.956	< 0.0001
	13.1	4.85 ± 0.0243				
	22.2	4.70 ± 0.0702				
	30	4.59 ± 0.0340				
BeP	4.7	5.44 ± 0.0075	-0.0181 ± 0.000932	5.53 ± 0.0181	0.964	< 0.0001
	13.1	5.30 ± 0.0207				
	22.2	5.12 ± 0.0774				
	30	4.99 ± 0.0295				
BbF	4.7	5.49 ± 0.0223	-0.0175 ± 0.00101	5.58 ± 0.0190	0.953	< 0.0001
	13.1	5.37 ± 0.0251				
	22.2	5.18 ± 0.0827				
	30	5.05 ± 0.0305				
BkF	4.7	5.52 ± 0.0197	-0.0169 ± 0.00104	5.61 ± 0.0196	0.946	< 0.0001
	13.1	5.41 ± 0.0339				
	22.2	5.23 ± 0.0823				
	30	5.11 ± 0.0325				
BaP	4.7	5.54 ± 0.0124	-0.0183 ± 0.000941	5.64 ± 0.0178	0.962	< 0.0001
	13.1	5.41 ± 0.0220				
	22.2	5.22 ± 0.0842				
	30	5.09 ± 0.0308				

PAH	Temperature (°C)	Log K_f (± SD)	Slope (± SE)	Intercept (± SE)	r^2	*p*
BghiP	4.7	5.67 ± 0.148	-0.0194 ± 0.00242	6.05 ± 0.0538	0.866	<0.0001
	13.1	5.80 ± 0.0498				
	22.2	5.61 ± 0.107				
	30	5.47 ± 0.0380				
DahA	4.7	5.27 ± 0.201	-0.0114 ± 0.00547	5.82 ± 0.122	0.301	0.0646
	13.1	5.69 ± 0.165				
	22.2	5.52 ± 0.187				
	30	5.50 ± 0.0398				
InP	4.7	5.86 ± 0.0607	-0.0209 ± 0.00282	6.12 ± 0.0627	0.846	<0.0001
	13.1	5.86 ± 0.0637				
	22.2	5.63 ± 0.116				
	30	5.51 ± 0.0403				

Table S2: Values for the Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (TΔS) changes of partitioning of PAHs into 30µm PDMS-coated SPME fibres and lipids of *Lumbriculus variegates* at 293 K.

PAH	Fiber				*L. variegatus*			
	ΔG (kJ/mol)[1]	ΔH (kJ/mol)	TΔS (kJ/mol)	r^2	ΔG (kJ/mol)	ΔH (kJ/mol)	TΔS (kJ/mol)	r^2
Phe	-24.8±1.67	-15.7±1.67	9.06±1.70	0.855	-33.5±9.82	-46.3 ± 9.50	-12.8±9.78	0.725
Ant	-25.3±1.50	-16.2±1.50	9.06±1.53	0.886	-34.5±8.96	-42.2 ± 8.68	-7.68±8.93	0.724
Flu	-27.3±1.36	-20.8±1.36	6.52±1.39	0.939	-39.9±5.16	-41.9 ± 5.07	-1.98±5.15	0.932
Pyr	-27.8±1.38	-22.1±1.38	5.63±1.40	0.945	-40.8±4.89	-40.1 ± 4.82	0.77±4.90	0.933
BaA	-30.6±1.36	-23.0±1.37	7.54±1.38	0.953	-45.9±8.31	-39.6 ± 8.20	6.32±8.34	0.823
Chr	-30.2±1.38	-24.7±1.38	5.48±1.40	0.955	-45.7±6.73	-42.2 ± 6.63	3.53±6.74	0.890
BeP	-32.5±1.56	-28.8±1.55	3.77±1.57	0.961	-49.0±7.02	-30.6 ± 6.97	18.3±7.08	0.794
BbF	-32.9±1.69	-27.8±1.68	5.11±1.70	0.948	-49.1±7.27	-23.1±7.21	26.0±7.36	0.721
BkF	-33.1±1.73	-26.7±1.72	6.40±1.75	0.941	-49.3±6.48	-21.4 ± 6.47	27.9±6.58	0.685
BaP	-33.1±1.57	-29.1±1.56	4.04±1.58	0.959	-	-	-	-
BghiP	-35.3±3.97	-32.2±4.00	3.10±3.98	0.867	-	-	-	-
DahA	-[2]	-	-	-	-	-	-	-
InP	-35.6±4.62	-34.7±4.64	0.89±4.62	0.848	-	-	-	-

[1] Values are average ± SE

[2] Slope is not significantly different from zero

Table S3. PAH bioaccumulation factors (BAFs) for *L. variegatus* at different temperatures. See experimental section for PAH abbreviations.

PAH	Temperature (°C)	Log BAF (± SD)	Slope (± SE)	Intercept (± SE)	r^2	*p*
Phe	5.3	4.80 ± 0.178	-0.0292 ± 0.00602	4.94 ± 0.0872	0.723	0.0009
	12.9	4.51 ± 0.001				
	24.6	4.24 ± 0.197				
Ant	5.3	4.94 ± 0.166	-0.0266 ± 0.00551	5.07 ± 0.0798	0.721	0.0009
	12.9	4.65 ± 0.000				
	24.6	4.44 ± 0.158				
Flu	5.3	5.90 ± 0.117	-0.0275 ± 0.00342	6.04 ± 0.0495	0.878	< 0.0001
	12.9	5.65 ± 0.014				
	24.6	5.37 ± 0.060				
Pyr	5.3	6.06 ± 0.117	-0.0265 ± 0.00339	6.19 ± 0.0492	0.871	< 0.0001
	12.9	5.80 ± 0.011				
	24.6	5.55 ± 0.043				
BaA	5.3	6.96 ± 0.208	-0.0248 ± 0.00521	7.06 ± 0.0923	0.82	0.005
	12.9	6.70 ± 0.092				
	24.6	6.47 ± 0.052				
Chr	5.3	6.93 ± 0.169	-0.0264 ± 0.00422	7.05 ± 0.0747	0.887	0.0015
	12.9	6.68 ± 0.087				
	24.6	6.41 ± 0.036				
BeP	5.3	7.35 ± 0.117	-0.0193 ± 0.00430	7.49 ± 0.0761	0.802	0.0064
	12.9	7.31 ± 0.117				
	24.6	7.00 ± 0.038				
BbF	5.3	7.32 ± 0.086	-0.0147 ± 0.00446	7.43 ± 0.0727	0.73	0.0303
	12.9	7.30 ± 0.116				
	24.6	7.05 ± 0.001				
BkF	5.3	7.33 ± 0.086	-0.0135 ± 0.00401	7.44 ± 0.0710	0.695	0.0198
	12.9	7.34 ± 0.118				
	24.6	7.09 ± 0.036				
BaP	5.3	7.44 ± 0.117	-0.0127 ± 0.00507	7.54 ± 0.0899	0.558	0.0537
	12.9	7.42 ± 0.115				
	24.6	7.21 ± 0.113				
BghiP	5.3	7.23 ± 0.050	0.0180 ± 0.00809	7.23 ± 0.143	0.497	0.077
	12.9	7.61 ± 0.278				
	24.6	7.63 ± 0.044				
DahA	5.3	6.79 ± 0.060	0.0333 ± 0.00739	6.69 ± 0.131	0.803	0.0063
	12.9	7.25 ± 0.248				
	24.6	7.47 ± 0.068				
InP	5.3	7.31 ± 0.053	0.0109 ± 0.00747	7.34 ± 0.132	0.298	0.2051
	12.9	7.62 ± 0.248				
	24.6	7.57 ± 0.058				

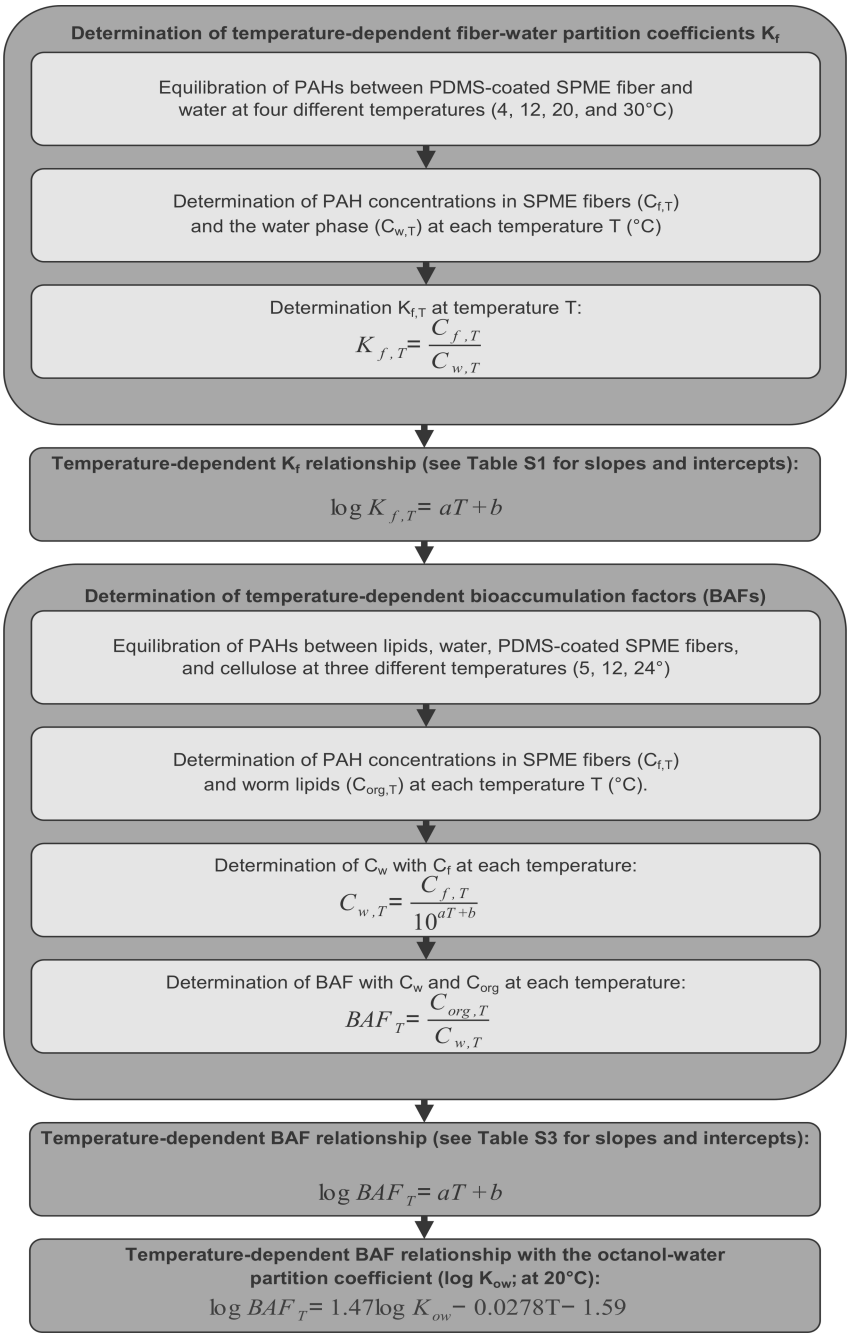


Figure S1. Schematic overview of the experiments and results.

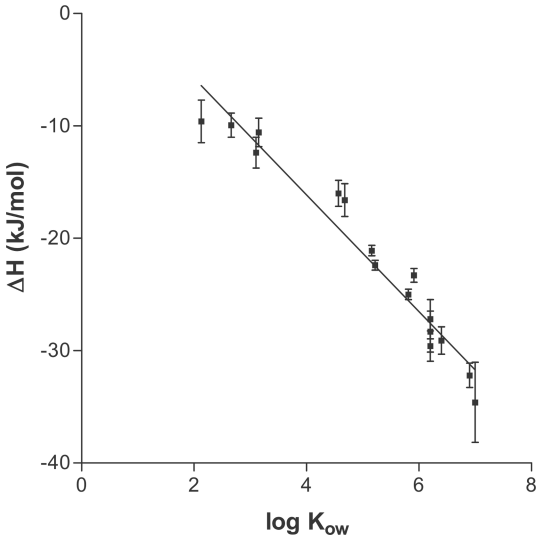


Figure S2. Relationship between sorption enthalpy (ΔH) and octanol-water partition coefficient (logKow) for (alkylated) mono-aromatic hydrocarbons (BTEX) and polycyclic aromatic hydrocarbons (PAHs). Error bars indicate standard errors. The solid line represents the linear regression relationship between enthalpy and hydrophobicity as given by (± standard errors): ΔH=-5.134 (± 0.345) logKow + 4.563 (± 1.828); r^2=0.941; p<0.001.

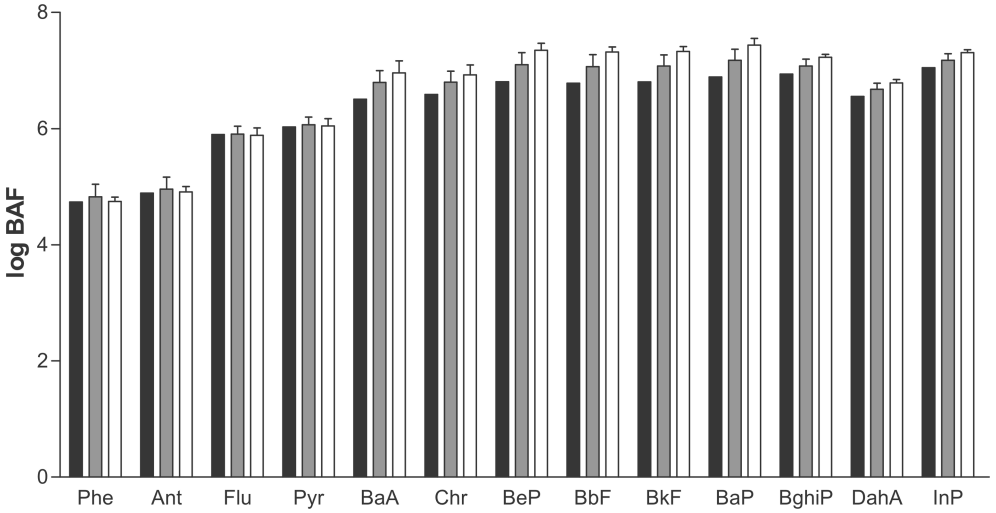


Figure S3. Bioaccumulation of PAHs in Lumbriculus variegatus at 5°C after 33 (black), 40 (grey), and 54 days (white). Error bars indicate standard deviations (n=4 for 40 days and n=2 for 54 days). See experimental section for PAH abbreviations.

Derivation of equation 1

The fiber-water partition coefficient K_f is calculated according to:

$$K_f = \frac{C_f}{C_w} \tag{S1}$$

where C_f represents the PAH concentration in the fiber and C_w the concentration in the aqueous phase.

The temperature-dependent fiber-water partition coefficient is calculated by:

$$\log K_f = aT + b \tag{S2}$$

where T is the temperature in °C and *a* and *b* are the slope and the intercept of the temperature correction, respectively (see Table S1).

The bioaccumulation factor (BAF) is given by:

$$BAF = \frac{C_{org}}{C_w} \tag{S3}$$

where C_{org} is the PAH concentration in the worms' lipid fraction.

Combining equations S1, S2, and S3, yields:

$$BAF = C_{org} \frac{10^{aT+b}}{C_f} \tag{S4}$$

Appendix II

Evaluation of clean-up agents for total petroleum hydrocarbon analysis in biota and sediments

additional information

Table S1. Initial clean-up experiments[1].

column	eluent (ml)	sample	colour after clean-up	chromatogram	Clean-up results (%)					
					parameter	C_{10}-C_{16}	C_{16}-C_{22}	C_{22}-C_{28}	C_{28}-C_{34}	C_{34}-C_{40}	tot
Al_2O_3 (10% deact)	30	worm	yellow	several peaks in C_{10}-C_{16} range, hump around C_{26}	lipid recovery	25	0	3	3	0	3
		oil	-	normal	oil recovery	85	100	99	95	0	85
		worm+oil	-	several peaks in C_{10}-C_{16} range, hump around C_{26}							
Al_2O_3 (100% act)	30	worm	clear	hump around C_{26}	lipid recovery	0	0	3	1	2	2
		oil	-	normal	oil recovery	54	66	69	62	0	60
		worm+oil	-	normal, but somewhat higher response, especially around C_{26}							
SiO_2 (100% act)	30	worm	light yellow	clear hump in C_{22}-C_{40} range	lipid recovery	0	24	52	26	43	32
		oil	-	normal	oil recovery	65	75	81	78	0	69
		worm+oil	-	clear hump in C_{22}-C_{40} range							
Al_2O_3 (10% deact, top) and SiO_2 (bottom)	30	worm	clear	some small peaks in the C_{10}-C_{40} range (around detection limit)	lipid recovery	4	5	2	0	0	1
		oil	-	normal	oil recovery	58	69	67	65	-	62
		worm+oil	-	normal, but somewhat higher response							
	55	worm	clear	some small peaks in the C_{10}-C_{40} range (around detection limit)	lipid recovery	0	0	0	0	0	0
		oil	-	normal	oil recovery	88	93	109	138	86	99
		worm+oil	-	normal							

Table S1 (continued). Initial clean-up experiments[1].

column	eluent (ml)	sample	colour after clean-up	chromatogram	Clean-up results (%)					
Florisil® (10% deact)	30	worm	clear	small hump in C_{28}-C_{40} range	lipid recovery	0	0	0	0	0	0
		oil	-	normal	oil recovery	80	95	96	91	-	85
		worm+oil	-	normal							
	55	worm	clear	hump from C_{28} to >C_{40}	lipid recovery	0	0	0	22	26	18
		oil	-	hump from C_{28} to >C_{40}	oil recovery	79	93	109	84	22	77
		worm+oil	-	normal							
Florisil® (100% act)	30	worm	light yellow	hump between C_{22}-C_{34} with small peaks in the C_{28}-C_{34} range	lipid recovery	16	22	19	8	10	11
		oil	-	normal	oil recovery	66	70	75	71	-	67
Florisil® (5% deact)	30	worm	light yellow	lower hump compared to Florisil 100% act, but rougher surface and higher peaks	lipid recovery	-	7	9	4	4	5
		oil	-	normal	oil recovery	103	101	99	90	-	96
Florisil® (10% deact)	30	worm	light yellow	hump as Florisil 5% deact., but higher peaks	lipid recovery	-	6	10	5	10	11
		oil	-	normal	oil recovery	136	130	126	116	-	130
SiO_2-H^+ (22%, top) SiO_2-OH^- (bottom)	30	worm	clear	IMC peak around C_{26} moved to the C_{22}-C_{26} range	lipid recovery	0	0	9	1	2	2
		oil	-	normal	oil recovery	67	77	74	71	0	70
		worm+oil	-	additional peaks in the C_{22}-C_{26} range							
SiO_2-H^+ (44%, top) SiO_2-OH^- (bottom)	30	worm	clear	hump in C_{22}-C_{26} range	lipid recovery	0	0	2	0	0	0
		oil	-	normal	oil recovery	78	78	80	79	65	78
		worm+oil	-	additional hump in C_{22}-C_{26} range							

Table S1 (continued). Initial clean-up experiments[1].

column	eluent (ml)	sample	colour after clean-up	chromatogram	Clean-up results (%)					
SiO_2-OH^- (top) SiO_2-H^+ (22%, bottom)	30	worm	clear	no peaks or hump visible	lipid recovery	0	0	0	0	0	0
		oil	-	normal	oil recovery	64	72	74	71	40	68
		worm+oil	-	normal, but somewhat higher response							
	55	worm	clear	some small peaks in C_{10}-C_{16} range. Due to carry over effect, somewhat higher in C_{24}-C_{40} range	lipid recovery	0	0	0	0	2	0
		oil		normal	oil recovery	67	85	86	74	0	67
		worm+oil		normal							
SiO_2-OH^-	30	worm	light yellow	big hump between C_{22}-C_{26}	lipid recovery	0	0	18	43	68	41
		oil	-	big hump between C_{22}-C_{26}	oil recovery	95	100	96	84	18	84
		worm+oil	-	normal							

[1] Initial experiments were performed singularly with 'bilge oil' (see Table S6)

Table S2. Effects of deactivation percentage of aluminium oxide (Al_2O_3) and Florisil on TPH recovery and lipid clean-up efficiency. Values are average ± SD.

Clean-up agent	deactivation (% w/w)	colour after purification	TPH recovery (%)	lipid clean-up efficiency (%)
Al_2O_3 (4 g, 30 mL eluent)	5	yellow	101 ± 0.49	92.8 ± 0.51
	7.5	yellow	102 ± 7.72	89.3 ± 1.00
	10	yellow	96.2 ± 3.13	95.6 ± 1.54
Florisil (2 g, 30 mL eluent)	0	white/yellow	74.8 ± 3.65	92.7 ± 0.89
	2.5	white/yellow	101 ± 3.26	93.4 ± 1.18
	5	yellow	100 ± 1.70	95.4 ± 0.78

Table S3. Effects of eluent volume on TPH recovery and lipid clean-up efficiency of aluminium oxide (Al_2O_3) and Florisil. Values are average ± SD.

Clean-up agent	eluent (mL)	colour after purification	TPH recovery (%)	lipid clean-up efficiency (%)
Al_2O_3 (4 g, 10% deactivated)	20	yellow	97.5 ± 2.57	95.6 ± 0.96
	25	yellow	101 ± 3.80	95.9 ± 0.70
	30	yellow	96.2 ± 3.13	95.6 ± 1.54
Florisil (2 g, 5 % deactivated)	20	white/yellow	95.7 ± 4.53	96.5 ± 1.00
	25	yellow	96.5 ± 4.95	94.6 ± 1.40
	30	yellow	100 ± 1.70	95.4 ± 0.78

Table S4. Effects of sulfuric acid percentage in silica gel on TPH recovery and lipid clean-up efficiency of a column filled with basic silica gel on top (1g 33% 1M NaOH) and 1.7 g acidified silica gel at the bottom. Values are average ± SD.

H_2SO_4 (% w/w)	colour after purification	TPH recovery (%)	lipid clean-up efficiency (%)
28	clear	86.2 ± 3.21	99.5 ± 0.94
14	clear	90.4 ± 1.85	99.9 ± 0.39
7	clear	90.9 ± 3.81	101 ± 0.30

Table S5. Effects of the amount of acidified (7% w/w H_2SO_4, bottom) and basic (33% w/w 1M NaOH, top) silica gel on lipid clean-up efficiency and TPH recovery after purification of 500 mg dw worms. Values are average ± SD.

Lipid clean-up efficiency (%)				
basic SiO_2 (g)	acidified SiO_2 (g)			
	1.7	2.2	2.5	2.7
1	95.3 ± 2.30	99.6 ± 0.07		99.5 ± 0.05
1.5			99.6 ± 0.13	
2	93.7 ± 4.97			
3	96.6 ± 1.43			
TPH recovery (%)				
basic SiO_2 (g)	acidified SiO_2 (g)			
	1.7	2.2	2.5	2.7
1	88.4 ± 1.55	89.0 ± 1.78		84.9 ± 1.03
1.5			85.7 ± 2.40	
2	89.4 ± 0.50			
3	89.8 ± 1.54			

Table S6. TPH recoveries of different oils after elution through 7% BAS columns. Values are average ± SD (*n*=3).

oil	description	TPH recovery (%)
RIVM standard oil	Mixture of a fuel and a lubricant oil	89.0 ± 1.78
'Bilge oil'	Waste oil collected from a bilge water collection depot (mixture of fuel and lubricant oils)	84.1 ± 3.44
Car lubricant oil	Commercial car lubricant (Visco 2000; BP)	90.4 ± 4.83
Nautic gasoil (DMA)	Fuel oil used in midsized to larger ships	75.2 ± 3.14

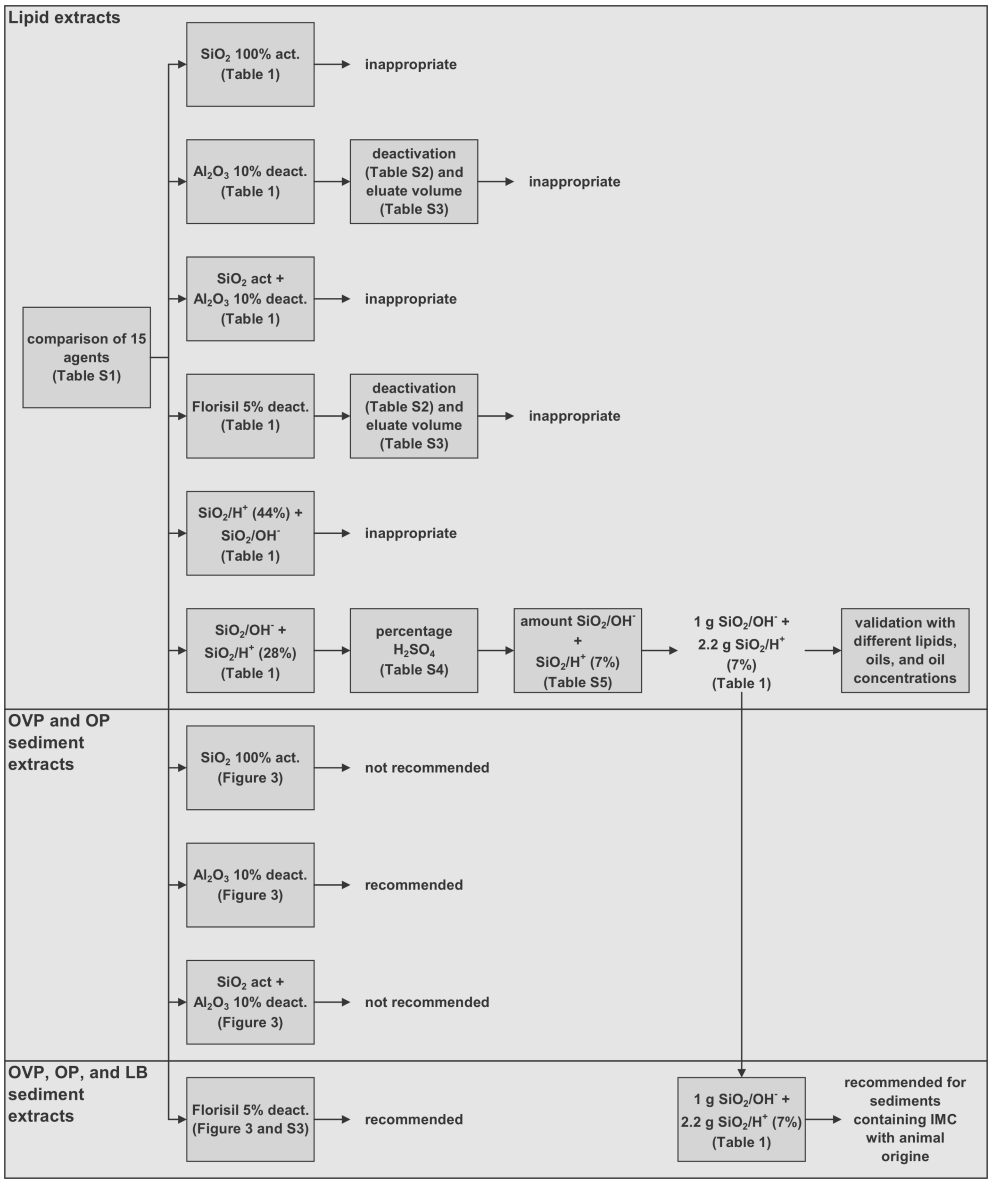


Figure S1. Schematic overview of the experiments, results, and conclusions.

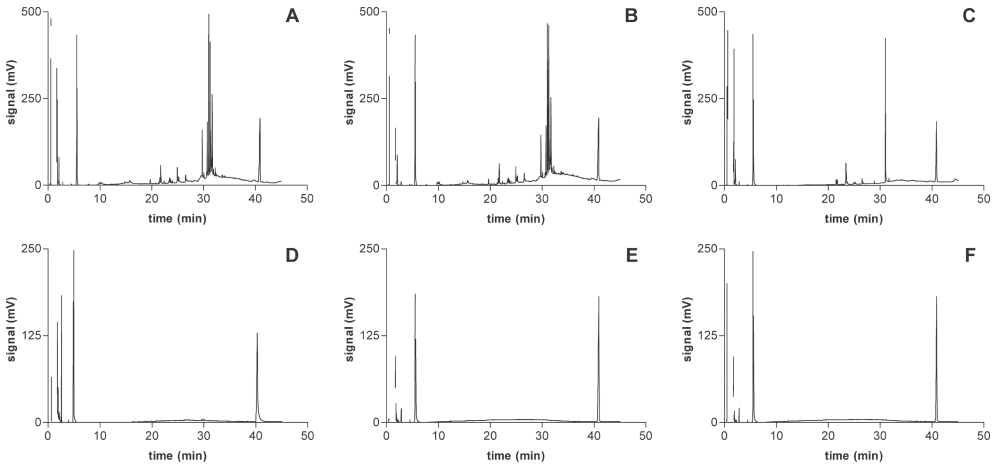


Figure S2. GC-FID chromatograms of worm, mussel, and fish extracts before (A, B, C, respectively) and after (D, E, F, respectively) purification through NaOH (top, 33% w/w) and H_2SO_4 (7% w/w)-impregnated silica gel.

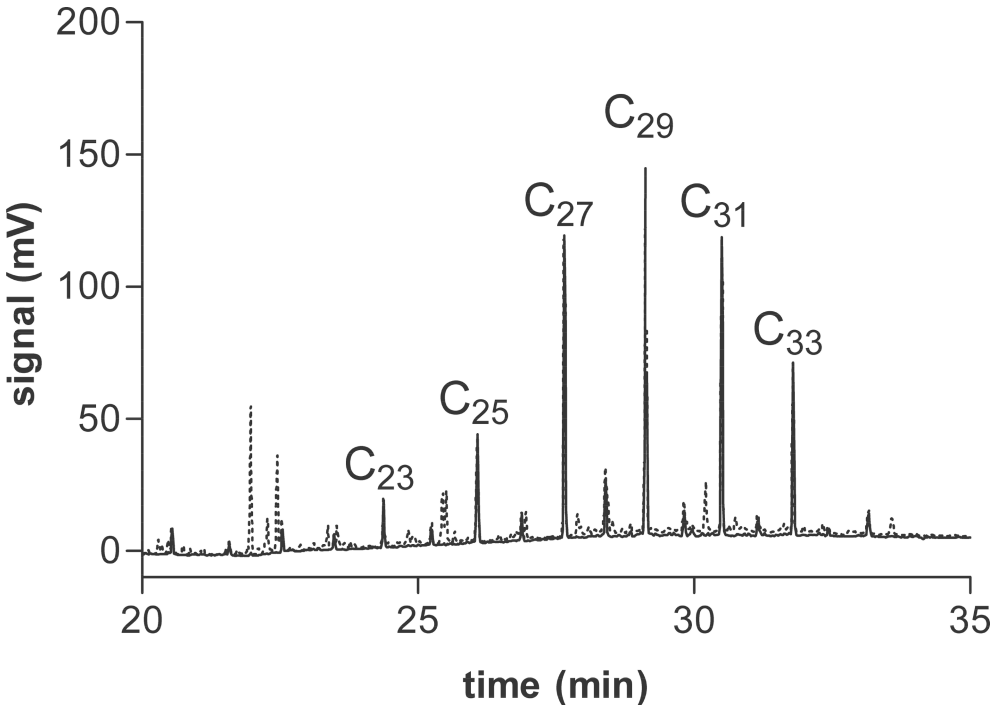


Figure S3. GC-FID chromatograms of LB sediment extracts cleaned-up through Florisil (dashed line) and NaOH (top, 33% w/w) and H_2SO_4 (7% w/w)-impregnated silica gel (solid line).

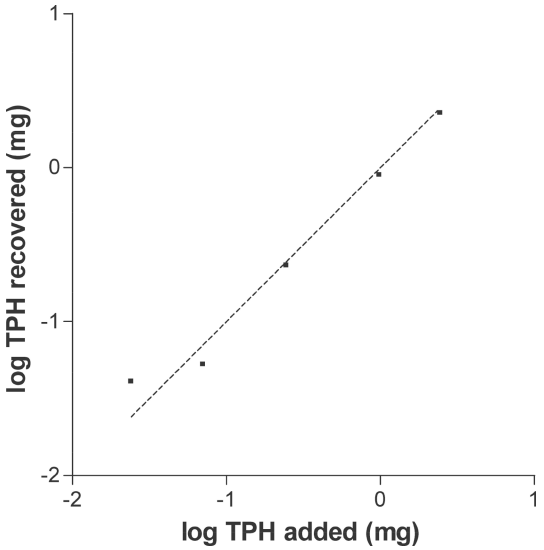


Figure S4. TPH concentrations recovered upon eluting worm extracts mixed with different concentrations of RIVM standard oil through 7% BAS columns, versus nominal (added) TPH concentrations. Values are averages ± standard deviations.

Appendix III

A closer look at bioaccumulation of petroleum hydrocarbon mixtures in aquatic worms

additional information

Table S1. TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
ADPH	C_{11}-C_{12}	50,6 ± 8,36	5,44 ± 0,104	0,295 ± 0,00402	260	n.a.	n.a.	n.a.
	C_{12}-C_{14}	415 ± 26,3				n.a.		n.a.
	C_{14}-C_{16}	997 ± 61,4				n.a.		n.a.
	C_{16}-C_{18}	1235 ± 70,0				n.a.		n.a.
	C_{18}-C_{20}	1473 ± 68,0				n.a.		n.a.
	C_{20}-C_{22}	1496 ± 72,0				n.a.		n.a.
	C_{22}-C_{24}	1009 ± 52,1				n.a.		n.a.
	C_{24}-C_{26}	1108 ± 55,4				n.a.		n.a.
	C_{26}-C_{28}	838 ± 42,1				n.a.		n.a.
	C_{28}-C_{30}	1122 ± 56,1				n.a.		n.a.
	C_{30}-C_{32}	1007 ± 74,3				n.a.		n.a.
	C_{32}-C_{34}	992 ± 35,7				n.a.		n.a.
	C_{34}-C_{36}	1056 ± 39,5				n.a.		n.a.
	C_{36}-C_{40}	1127 ± 70,3				n.a.		n.a.
	total	14163 ± 715				n.a.		n.a.
	C_{11}-C_{20}	4364 ± 241				n.a.		n.a.
	C_{11}-C_{34}	11992 ± 603				n.a.		n.a.

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
HIJG	C_{11}-C_{12}	<7,91	5,88 ± 0,101	0,357 ± 0,00428	39	<65,8	12,7 ± 1,46	n.d.
	C_{12}-C_{14}	36,3 ± 1,60				<101		n.d.
	C_{14}-C_{16}	69,9 ± 2,72				229 ± 193		0,193 ± 0,163
	C_{16}-C_{18}	112 ± 0,88				728 ± 429		0,382 ± 0,225
	C_{18}-C_{20}	138 ± 1,70				973 ± 174		0,414 ± 0,0744
	C_{20}-C_{22}	161 ± 2,86				782 ± 106		0,285 ± 0,0391
	C_{22}-C_{24}	161 ± 2,74				503 ± 88,3		0,184 ± 0,0324
	C_{24}-C_{26}	201 ± 4,04				361 ± 49,5		0,105 ± 0,0146
	C_{26}-C_{28}	190 ± 4,26				264 ± 24,8		0,0817 ± 0,00788
	C_{28}-C_{30}	239 ± 7,04				198 ± 17,6		0,0488 ± 0,00457
	C_{30}-C_{32}	245 ± 7,86				140 ± 14,6		0,0335 ± 0,00367
	C_{32}-C_{34}	226 ± 13,2				120 ± 17,5		0,0313 ± 0,00491
	C_{34}-C_{36}	251 ± 23,7				90,0 ± 19,7		0,0211 ± 0,00504
	C_{36}-C_{40}	228 ± 26,3				<287		n.d.
	total	2272 ± 87,0				4681 ± 966		0,121 ± 0,0254
	C_{11}-C_{20}	382 ± 3,78				2238 ± 842		0,344 ± 0,130
	C_{11}-C_{34}	1815 ± 40,8				4395 ± 905		0,142 ± 0,0295

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
JHOZ	C_{11}-C_{12}	<22,2	25,9 ± 2,30	1,44 ± 0,143	6	<69,6	12,0 ± 0,0992	n.d.
	C_{12}-C_{14}	16,1 ± 6,34				<100		n.d.
	C_{14}-C_{16}	14,9 ± 6,42				74,2 ± 17,2		1,29 ± 0,633
	C_{16}-C_{18}	<14,8				170 ± 41,0		n.d.
	C_{18}-C_{20}	31,7 ± 2,51				220 ± 25,9		1,79 ± 0,255
	C_{20}-C_{22}	82,1 ± 1,05				219 ± 18,9		0,692 ± 0,0603
	C_{22}-C_{24}	67,9 ± 2,96				182 ± 17,6		0,694 ± 0,0738
	C_{24}-C_{26}	127 ± 3,75				137 ± 6,68		0,280 ± 0,0159
	C_{26}-C_{28}	125 ± 3,76				87,7 ± 7,30		0,182 ± 0,0161
	C_{28}-C_{30}	229 ± 5,10				75,0 ± 3,60		0,0848 ± 0,00449
	C_{30}-C_{32}	221 ± 4,68				58,1 ± 10,2		0,0683 ± 0,0121
	C_{32}-C_{34}	237 ± 4,55				61,4 ± 21,3		0,0672 ± 0,0233
	C_{34}-C_{36}	215 ± 13,1				71,8 ± 11,3		0,0864 ± 0,0145
	C_{36}-C_{40}	184 ± 19,6				<285		n.d.
	total	1527 ± 36,8				1562 ± 381		0,265 ± 0,0650
	C_{11}-C_{20}	78,3 ± 28,0				650 ± 105		2,15 ± 0,843
	C_{11}-C_{34}	1161 ± 37,4				1405 ± 155		0,314 ± 0,0361

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
JHWE	C_{11}-C_{12}	<35,0	35,6 ± 1,10	2,21 ± 0,0868	8	<68,0	11,5 ± 0,348	n.d.
	C_{12}-C_{14}	16,6 ± 2,26				<104		n.d.
	C_{14}-C_{16}	<15,8				46,6 ± 2,07		n.d.
	C_{16}-C_{18}	<23,3				84,4 ± 31,9		n.d.
	C_{18}-C_{20}	<26,6				121 ± 70,5		n.d.
	C_{20}-C_{22}	<32,8				139 ± 104		n.d.
	C_{22}-C_{24}	54,5 ± 8,66				127 ± 79,8		0,826 ± 0,537
	C_{24}-C_{26}	155 ± 11,3				101 ± 34,2		0,232 ± 0,0803
	C_{26}-C_{28}	464 ± 13,5				77,2 ± 19,6		0,0593 ± 0,0151
	C_{28}-C_{30}	633 ± 13,0				53,9 ± 19,6		0,0303 ± 0,0110
	C_{30}-C_{32}	453 ± 4,21				39,7 ± 14,3		0,0311 ± 0,0112
	C_{32}-C_{34}	369 ± 12,9				46,0 ± 2,88		0,0444 ± 0,00319
	C_{34}-C_{36}	337 ± 20,2				<64,5		n.d.
	C_{36}-C_{40}	263 ± 32,8				<296		n.d.
	total	2732 ± 140				955 ± 357		0,124 ± 0,0470
	C_{11}-C_{20}	<96,5				380 ± 92,3		n.d.
	C_{11}-C_{34}	2182 ± 114				896 ± 391		0,146 ± 0,0643

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
KVGS	C_{11}-C_{12}	<9,24	3,60 ± 0,402	0,237 ± 0,00945	0	<67,8	11,6 ± 0,537	n.d.
	C_{12}-C_{14}	4,70 ± 1,22				<104		n.d.
	C_{14}-C_{16}	<4,18				90,4 ± 13,6		n.d.
	C_{16}-C_{18}	<6,14				208 ± 50,0		n.d.
	C_{18}-C_{20}	11,6 ± 1,00				269 ± 23,1		0,834 ± 0,101
	C_{20}-C_{22}	27,8 ± 1,49				270 ± 12,0		0,349 ± 0,0243
	C_{22}-C_{24}	27,8 ± 0,73				285 ± 13,1		0,368 ± 0,0195
	C_{24}-C_{26}	45,7 ± 2,02				337 ± 27,1		0,266 ± 0,0244
	C_{26}-C_{28}	44,6 ± 2,43				274 ± 25,7		0,221 ± 0,0239
	C_{28}-C_{30}	72,2 ± 4,96				211 ± 23,5		0,105 ± 0,0137
	C_{30}-C_{32}	74,1 ± 7,31				146 ± 18,1		0,0710 ± 0,0112
	C_{32}-C_{34}	73,0 ± 10,5				132 ± 18,3		0,0652 ± 0,0130
	C_{34}-C_{36}	74,1 ± 22,3				96,3 ± 15,1		0,0467 ± 0,0159
	C_{36}-C_{40}	67,3 ± 39,8				<295		n.d.
	total	516 ± 86,7				2633 ± 229		0,183 ± 0,0347
	C_{11}-C_{20}	<25,9				746 ± 53,3		n.d.
	C_{11}-C_{34}	387 ± 25,8				2330 ± 177		0,216 ± 0,0219

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
KVHB	C_{11}-C_{12}	<14,4	6,73 ± 0,0977	0,581 ± 0,00767	17	<67,0	11,7 ± 0,611	n.d.
	C_{12}-C_{14}	8,45 ± 1,08				<103		n.d.
	C_{14}-C_{16}	8,52 ± 0,96				93,6 ± 15,3		0,739 ± 0,147
	C_{16}-C_{18}	<11,3				235 ± 103		n.d.
	C_{18}-C_{20}	21,7 ± 8,49				312 ± 97,0		0,967 ± 0,483
	C_{20}-C_{22}	47,5 ± 9,48				318 ± 72,5		0,450 ± 0,136
	C_{22}-C_{24}	64,3 ± 7,07				290 ± 47,6		0,303 ± 0,0599
	C_{24}-C_{26}	104 ± 5,19				278 ± 40,0		0,181 ± 0,0275
	C_{26}-C_{28}	112 ± 2,40				233 ± 51,2		0,140 ± 0,0308
	C_{28}-C_{30}	160 ± 0,83				196 ± 45,0		0,0827 ± 0,0190
	C_{30}-C_{32}	169 ± 4,35				146 ± 32,6		0,0582 ± 0,0130
	C_{32}-C_{34}	159 ± 2,97				132 ± 23,6		0,0558 ± 0,0100
	C_{34}-C_{36}	174 ± 7,59				88,4 ± 14,6		0,0341 ± 0,00583
	C_{36}-C_{40}	158 ± 6,00				<292		n.d.
	total	1167 ± 42,8				2620 ± 256		0,151 ± 0,0158
	C_{11}-C_{20}	55,4 ± 13,7				865 ± 193		1,05 ± 0,349
	C_{11}-C_{34}	860 ± 49,7				2377 ± 348		0,186 ± 0,0292

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
LERI	C_{11}-C_{12}	<12,0	9,03 ± 1,00	0,460 ± 0,00311	45	<66,7	12,3 ± 0,523	n.d.
	C_{12}-C_{14}	59,7 ± 8,78				122 ± 30,7		0,185 ± 0,0538
	C_{14}-C_{16}	120 ± 15,4				237 ± 91,5		0,178 ± 0,0724
	C_{16}-C_{18}	171 ± 15,6				670 ± 154		0,353 ± 0,0872
	C_{18}-C_{20}	254 ± 25,9				951 ± 59,4		0,339 ± 0,0406
	C_{20}-C_{22}	319 ± 24,2				782 ± 48,0		0,222 ± 0,0217
	C_{22}-C_{24}	288 ± 15,6				556 ± 54,4		0,174 ± 0,0195
	C_{24}-C_{26}	367 ± 27,2				489 ± 107		0,120 ± 0,0277
	C_{26}-C_{28}	318 ± 20,0				362 ± 94,4		0,103 ± 0,0276
	C_{28}-C_{30}	431 ± 33,4				275 ± 60,5		0,0575 ± 0,0134
	C_{30}-C_{32}	402 ± 22,3				181 ± 21,4		0,0407 ± 0,00533
	C_{32}-C_{34}	435 ± 27,4				141 ± 11,1		0,0293 ± 0,00294
	C_{34}-C_{36}	487 ± 23,5				87,2 ± 29,4		0,0162 ± 0,00550
	C_{36}-C_{40}	400 ± 56,3				<281		n.d.
	total	4072 ± 321				5045 ± 140		0,112 ± 0,00936
	C_{11}-C_{20}	652 ± 73,8				2243 ± 298		0,311 ± 0,0542
	C_{11}-C_{34}	3234 ± 244				4817 ± 75,4		0,135 ± 0,0104

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
ROSL	C_{11}-C_{12}	50,8 ± 9,72	10,5 ± 0,222	0,397 ± 0,0105	139	n.a.	n.a.	n.a.
	C_{12}-C_{14}	248 ± 12,6				n.a.		n.a.
	C_{14}-C_{16}	263 ± 5,11				n.a.		n.a.
	C_{16}-C_{18}	359 ± 17,9				n.a.		n.a.
	C_{18}-C_{20}	464 ± 21,0				n.a.		n.a.
	C_{20}-C_{22}	675 ± 28,8				n.a.		n.a.
	C_{22}-C_{24}	908 ± 36,0				n.a.		n.a.
	C_{24}-C_{26}	1426 ± 50,9				n.a.		n.a.
	C_{26}-C_{28}	1480 ± 53,4				n.a.		n.a.
	C_{28}-C_{30}	1809 ± 68,1				n.a.		n.a.
	C_{30}-C_{32}	1787 ± 73,0				n.a.		n.a.
	C_{32}-C_{34}	1576 ± 73,4				n.a.		n.a.
	C_{34}-C_{36}	1764 ± 103				n.a.		n.a.
	C_{36}-C_{40}	1853 ± 156				n.a.		n.a.
	total	14677 ± 666				n.a.		n.a.
	C_{11}-C_{20}	1458 ± 33,1				n.a.		n.a.
	C_{11}-C_{34}	11199 ± 417				n.a.		n.a.

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
S120	C_{11}-C_{12}	<8,41	3,55 ± 0,0745	0,238 ± 0,00622	13	<69,4	11,5 ± 0,725	n.d.
	C_{12}-C_{14}	6,64 ± 2,88				<106		n.d.
	C_{14}-C_{16}	6,42 ± 2,54				92,1 ± 54,0		0,509 ± 0,360
	C_{16}-C_{18}	<6,29				304 ± 142		n.d.
	C_{18}-C_{20}	12,7 ± 2,98				424 ± 131		1,18 ± 0,457
	C_{20}-C_{22}	23,0 ± 3,80				370 ± 109		0,571 ± 0,193
	C_{22}-C_{24}	25,3 ± 4,26				293 ± 79,5		0,410 ± 0,131
	C_{24}-C_{26}	38,2 ± 5,57				254 ± 47,3		0,236 ± 0,0558
	C_{26}-C_{28}	38,9 ± 5,12				197 ± 35,7		0,179 ± 0,0401
	C_{28}-C_{30}	55,4 ± 6,39				149 ± 29,3		0,0956 ± 0,0217
	C_{30}-C_{32}	57,2 ± 6,62				97,5 ± 20,5		0,0604 ± 0,0145
	C_{32}-C_{34}	58,4 ± 8,67				76,0 ± 17,9		0,0462 ± 0,0129
	C_{34}-C_{36}	67,1 ± 11,2				<65,8		n.d.
	C_{36}-C_{40}	72,0 ± 18,4				<303		n.d.
	total	461 ± 75,3				2372 ± 660		0,182 ± 0,0589
	C_{11}-C_{20}	34,9 ± 12,7				1029 ± 369		1,04 ± 0,533
	C_{11}-C_{34}	332 ± 49,2				2368 ± 625		0,253 ± 0,0766

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
S130	C_{11}-C_{12}	<22,7	23,0 ± 0,516	1,81 ± 0,0460	4	<73,8	10,6 ± 0,366	n.d.
	C_{12}-C_{14}	12,2 ± 2,56				<113		n.d.
	C_{14}-C_{16}	<10,3				90,2 ± 24,5		n.d.
	C_{16}-C_{18}	<15,1				160 ± 34,3		n.d.
	C_{18}-C_{20}	<17,3				176 ± 20,1		n.d.
	C_{20}-C_{22}	<21,1				191 ± 5,60		n.d.
	C_{22}-C_{24}	17,6 ± 2,01				179 ± 0,61		2,34 ± 0,266
	C_{24}-C_{26}	50,4 ± 2,42				118 ± 14,3		0,539 ± 0,0702
	C_{26}-C_{28}	117 ± 2,40				88,7 ± 25,6		0,175 ± 0,0506
	C_{28}-C_{30}	185 ± 7,40				80,1 ± 31,5		0,0993 ± 0,0392
	C_{30}-C_{32}	192 ± 9,51				56,8 ± 29,2		0,0681 ± 0,0352
	C_{32}-C_{34}	150 ± 13,9				62,4 ± 21,7		0,0955 ± 0,0344
	C_{34}-C_{36}	128 ± 10,2				73,5 ± 7,62		0,132 ± 0,0172
	C_{36}-C_{40}	102 ± 26,2				<322		n.d.
	total	886 ± 64,8				1572 ± 425		0,408 ± 0,114
	C_{11}-C_{20}	<62,7				641 ± 135		n.d.
	C_{11}-C_{34}	681 ± 29,8				1349 ± 261		0,455 ± 0,0904

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
S150	C_{11}-C_{12}	<11,6	3,58 ± 0,190	0,267 ± 0,0101	16	<59,3	13,3 ± 0,194	n.d.
	C_{12}-C_{14}	5,39 ± 0,58				<90,9		n.d.
	C_{14}-C_{16}	<5,24				86,5 ± 19,3		n.d.
	C_{16}-C_{18}	<8,51				276 ± 49,3		n.d.
	C_{18}-C_{20}	23,7 ± 13,0				399 ± 13,9		0,602 ± 0,329
	C_{20}-C_{22}	52,9 ± 36,2				334 ± 39,7		0,226 ± 0,157
	C_{22}-C_{24}	58,7 ± 48,6				272 ± 36,6		0,166 ± 0,139
	C_{24}-C_{26}	55,1 ± 22,9				287 ± 14,1		0,186 ± 0,0779
	C_{26}-C_{28}	49,4 ± 3,41				213 ± 28,9		0,154 ± 0,0235
	C_{28}-C_{30}	69,4 ± 1,75				147 ± 21,3		0,0756 ± 0,0111
	C_{30}-C_{32}	71,8 ± 2,82				85,4 ± 14,5		0,0425 ± 0,00742
	C_{32}-C_{34}	66,4 ± 4,05				61,8 ± 12,7		0,0333 ± 0,00711
	C_{34}-C_{36}	70,0 ± 8,75				<56,3		n.d.
	C_{36}-C_{40}	68,2 ± 3,54				<259		n.d.
	total	600 ± 119				2305 ± 180		0,137 ± 0,0294
	C_{11}-C_{20}	47,1 ± 13,8				938 ± 91,8		0,713 ± 0,221
	C_{11}-C_{34}	472 ± 127				2250 ± 113		0,170 ± 0,0466

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
S160	C_{11}-C_{12}	<11,1	5,82 ± 0,120	0,344 ± 0,00984	53	<70,6	11,9 ± 0,299	n.d.
	C_{12}-C_{14}	45,8 ± 7,78				<124		n.d.
	C_{14}-C_{16}	95,9 ± 8,77				373 ± 238		0,226 ± 0,146
	C_{16}-C_{18}	160 ± 6,71				995 ± 508		0,361 ± 0,185
	C_{18}-C_{20}	197 ± 4,56				1162 ± 298		0,342 ± 0,0881
	C_{20}-C_{22}	229 ± 4,62				946 ± 247		0,240 ± 0,0630
	C_{22}-C_{24}	228 ± 3,70				583 ± 220		0,149 ± 0,0563
	C_{24}-C_{26}	283 ± 4,90				290 ± 47,8		0,0596 ± 0,00988
	C_{26}-C_{28}	262 ± 3,18				169 ± 12,2		0,0376 ± 0,00274
	C_{28}-C_{30}	322 ± 5,14				124 ± 9,98		0,0225 ± 0,00184
	C_{30}-C_{32}	326 ± 5,69				85,9 ± 11,2		0,0153 ± 0,00202
	C_{32}-C_{34}	292 ± 7,51				66,6 ± 6,84		0,0133 ± 0,00141
	C_{34}-C_{36}	321 ± 6,98				<62,9		n.d.
	C_{36}-C_{40}	301 ± 10,0				<289		n.d.
	total	3093 ± 76,6				4965 ± 1353		0,0934 ± 0,0256
	C_{11}-C_{20}	537 ± 30,5				2924 ± 1083		0,317 ± 0,119
	C_{11}-C_{34}	2496 ± 62,3				4913 ± 1430		0,115 ± 0,0334

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
SCSL	C_{11}-C_{12}	11,7 ± 3,02	5,81 ± 0,307	0,449 ± 0,00164	33	<76,4	12,0 ± 0,774	n.d.
	C_{12}-C_{14}	30,6 ± 3,52				<115		n.d.
	C_{14}-C_{16}	37,6 ± 3,51				161 ± 69,6		0,248 ± 0,110
	C_{16}-C_{18}	50,3 ± 7,38				412 ± 148		0,476 ± 0,184
	C_{18}-C_{20}	68,4 ± 7,33				526 ± 79,8		0,446 ± 0,0829
	C_{20}-C_{22}	112 ± 8,82				478 ± 88,7		0,247 ± 0,0497
	C_{22}-C_{24}	111 ± 5,92				434 ± 103		0,228 ± 0,0554
	C_{24}-C_{26}	167 ± 6,99				597 ± 225		0,207 ± 0,0787
	C_{26}-C_{28}	174 ± 4,91				508 ± 214		0,169 ± 0,0713
	C_{28}-C_{30}	240 ± 6,07				391 ± 168		0,0947 ± 0,0407
	C_{30}-C_{32}	233 ± 6,33				251 ± 110		0,0625 ± 0,0273
	C_{32}-C_{34}	228 ± 9,64				187 ± 86,3		0,0475 ± 0,0220
	C_{34}-C_{36}	244 ± 10,7				114 ± 61,9		0,0271 ± 0,0148
	C_{36}-C_{40}	215 ± 22,9				<304		n.d.
	total	1914 ± 102				4432 ± 1178		0,134 ± 0,0364
	C_{11}-C_{20}	211 ± 25,8				1402 ± 267		0,385 ± 0,0871
	C_{11}-C_{34}	1483 ± 70,0				4115 ± 989		0,161 ± 0,0394

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
UTNB	C_{11}-C_{12}	<10,1	5,24 ± 0,363	0,431 ± 0,0189	18	<79,1	11,5 ± 0,435	n.d.
	C_{12}-C_{14}	7,51 ± 2,82				<110		n.d.
	C_{14}-C_{16}	7,29 ± 2,57				142 ± 50,4		1,02 ± 0,512
	C_{16}-C_{18}	8,86 ± 2,10				347 ± 56,9		2,06 ± 0,592
	C_{18}-C_{20}	25,4 ± 3,83				435 ± 89,5		0,900 ± 0,230
	C_{20}-C_{22}	49,5 ± 5,14				462 ± 161		0,489 ± 0,178
	C_{22}-C_{24}	60,6 ± 5,08				461 ± 114		0,398 ± 0,104
	C_{24}-C_{26}	92,3 ± 6,91				524 ± 93,6		0,298 ± 0,0577
	C_{26}-C_{28}	94,9 ± 6,53				449 ± 91,5		0,248 ± 0,0533
	C_{28}-C_{30}	132 ± 8,69				367 ± 71,6		0,146 ± 0,0301
	C_{30}-C_{32}	133 ± 9,42				271 ± 52,9		0,107 ± 0,0222
	C_{32}-C_{34}	122 ± 7,81				240 ± 53,1		0,103 ± 0,0237
	C_{34}-C_{36}	127 ± 7,77				163 ± 43,9		0,0672 ± 0,0186
	C_{36}-C_{40}	108 ± 2,62				351 ± 61,5		-0,1682 ± -0,0298
	total	961 ± 70,1				4330 ± 624		0,236 ± 0,0382
	C_{11}-C_{20}	54,8 ± 14,4				1191 ± 232		1,14 ± 0,373
	C_{11}-C_{34}	744 ± 63,7				3851 ± 546		0,272 ± 0,0450

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
VK16	C_{11}-C_{12}	<9,29	3,98 ± 0,105	0,278 ± 0,00321	24	<62,2	12,5 ± 0,556	n.d.
	C_{12}-C_{14}	6,65 ± 2,16				<95,4		n.d.
	C_{14}-C_{16}	16,1 ± 1,68				89,7 ± 40,7		0,222 ± 0,103
	C_{16}-C_{18}	34,3 ± 1,30				247 ± 91,0		0,287 ± 0,106
	C_{18}-C_{20}	50,7 ± 1,75				339 ± 60,0		0,266 ± 0,0480
	C_{20}-C_{22}	64,8 ± 1,86				281 ± 44,8		0,172 ± 0,0279
	C_{22}-C_{24}	71,3 ± 1,51				177 ± 40,1		0,0988 ± 0,0225
	C_{24}-C_{26}	91,9 ± 2,16				102 ± 20,1		0,0441 ± 0,00876
	C_{26}-C_{28}	88,8 ± 1,88				64,5 ± 12,1		0,0289 ± 0,00546
	C_{28}-C_{30}	115 ± 3,70				50,3 ± 13,7		0,0174 ± 0,00476
	C_{30}-C_{32}	121 ± 8,29				33,6 ± 8,59		0,0111 ± 0,00294
	C_{32}-C_{34}	104 ± 8,18				<40,8		n.d.
	C_{34}-C_{36}	113 ± 17,2				<59,0		n.d.
	C_{36}-C_{40}	102 ± 31,5				<271		n.d.
	total	981 ± 47,3				1533 ± 456		0,0622 ± 0,0188
	C_{11}-C_{20}	117 ± 9,15				875 ± 220		0,297 ± 0,0782
	C_{11}-C_{34}	779 ± 20,0				1518 ± 349		0,0776 ± 0,0179

n.a. not analysed
n.d. not detected

Table S1 (continued). TPH concentrations in sediments (C_s) and *Lumbriculus variegatus* (C_b), organic carbon (f_{oc}) and nitrogen contents, saturation percentage of the organic carbon phase with TPH, lipid contents of worms, and biota-to-sediment accumulation factors (BSAFs). Values are averages ± standard deviation.

sediment	fraction	C_s (mg/kg dw)	f_{oc} (%)	Nitrogen (%)	Saturation (%)	C_b (mg/kg lipid)	Lipids (%)	BSAF
WOGR	C_{11}-C_{12}	<8,89	6,01 ± 0,0578	0,423 ± 0,0343	60	<60,1	12,8 ± 0,859	n.d.
	C_{12}-C_{14}	35,0 ± 1,28				<92,5		n.d.
	C_{14}-C_{16}	60,5 ± 1,54				151 ± 42,9		0,150 ± 0,0427
	C_{16}-C_{18}	108 ± 6,55				487 ± 143		0,271 ± 0,0812
	C_{18}-C_{20}	193 ± 25,1				686 ± 162		0,214 ± 0,0576
	C_{20}-C_{22}	313 ± 47,5				760 ± 176		0,146 ± 0,0404
	C_{22}-C_{24}	298 ± 21,9				452 ± 150		0,0911 ± 0,0309
	C_{24}-C_{26}	365 ± 35,5				246 ± 82,9		0,0405 ± 0,0142
	C_{26}-C_{28}	302 ± 18,3				125 ± 57,8		0,0248 ± 0,0116
	C_{28}-C_{30}	409 ± 46,2				88,1 ± 49,2		0,0129 ± 0,00736
	C_{30}-C_{32}	386 ± 28,4				64,3 ± 39,2		0,0100 ± 0,00615
	C_{32}-C_{34}	361 ± 45,0				59,8 ± 29,8		0,00994 ± 0,00511
	C_{34}-C_{36}	370 ± 39,0				<60,1		n.d.
	C_{36}-C_{40}	356 ± 63,8				<262		n.d.
	total	3608 ± 384				3379 ± 833		0,0563 ± 0,0151
	C_{11}-C_{20}	432 ± 35,0				1577 ± 349		0,219 ± 0,0516
	C_{11}-C_{34}	2906 ± 281				3222 ± 867		0,0666 ± 0,0190

n.a. not analysed
n.d. not detected

Figure S1. GC-FID chromatograms of extracts from field-contaminated sediments and *Lumbriculus variegatus,* exposed to these sediments for 28 days.

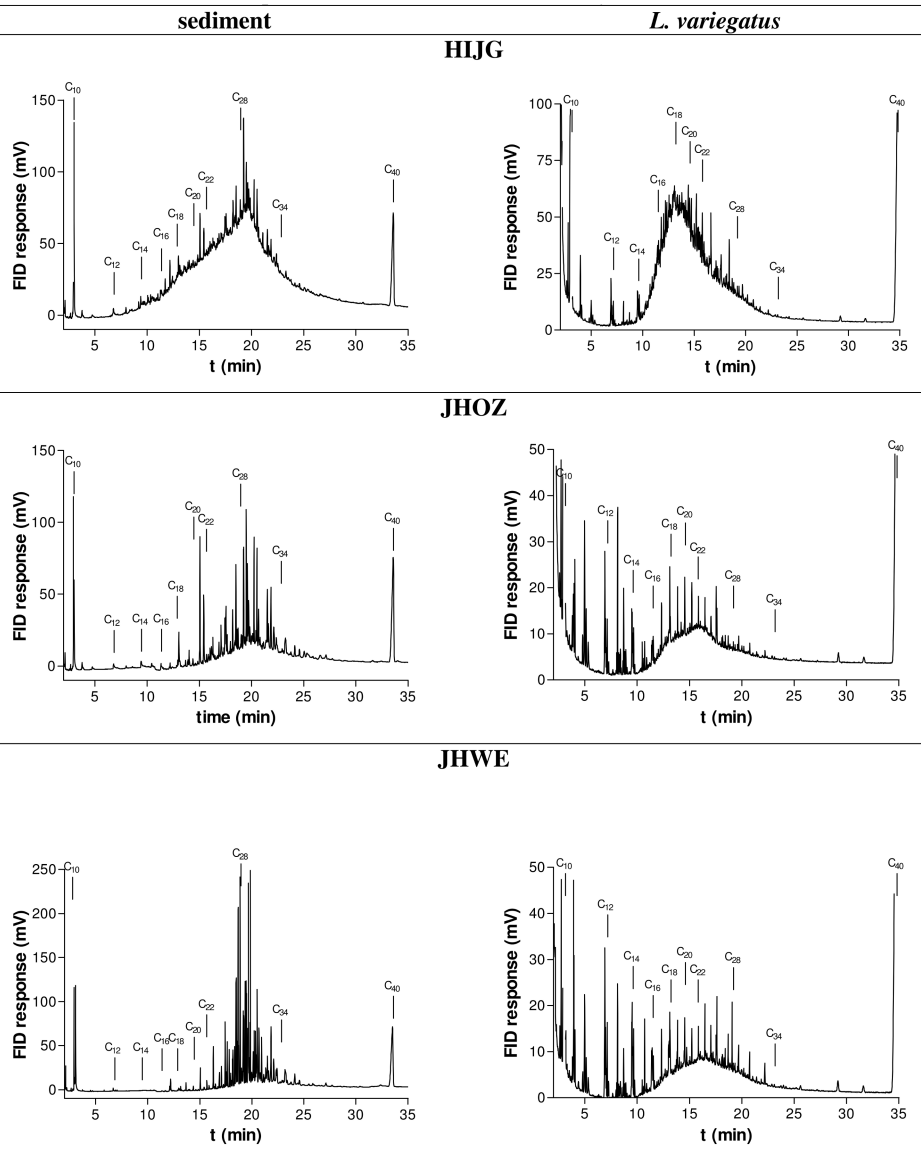


Figure S1 (continued). GC-FID chromatograms of extracts from field-contaminated sediments and *Lumbriculus variegatus,* exposed to these sediments for 28 days.

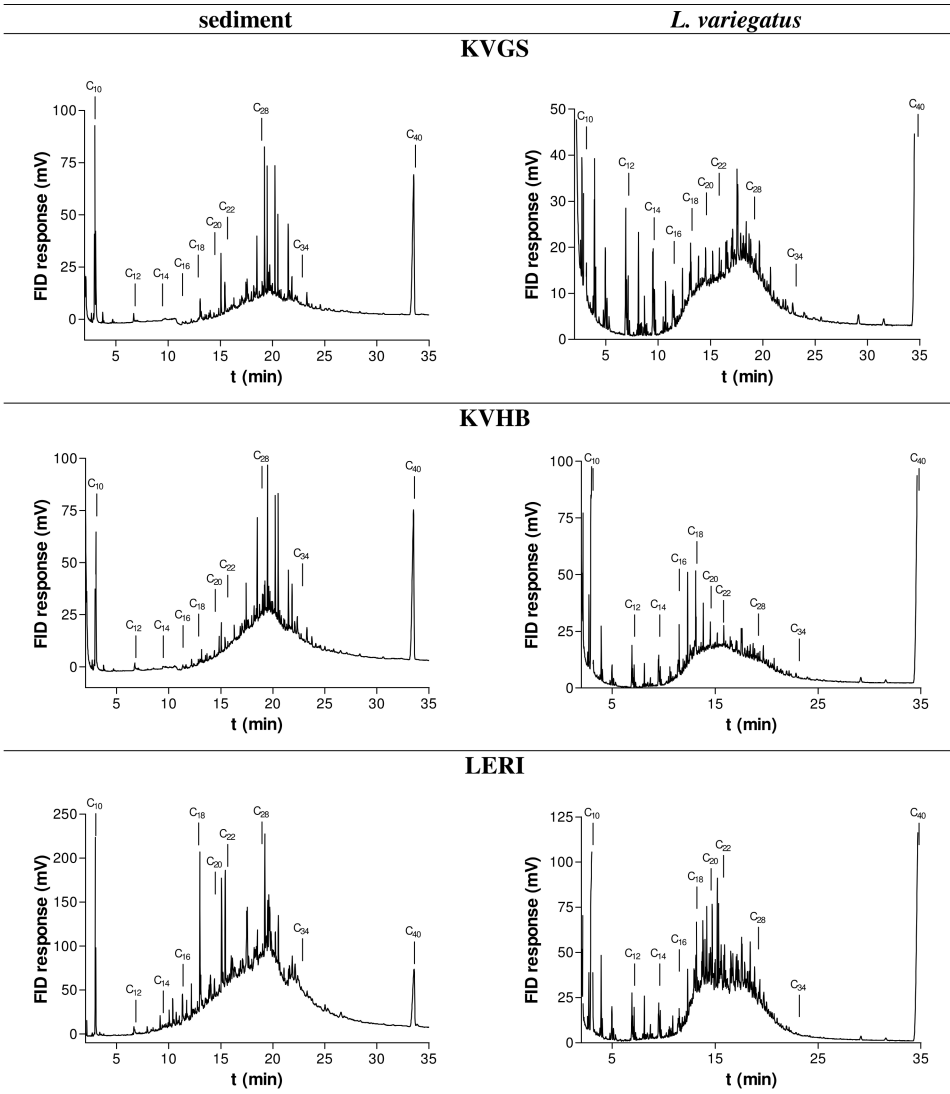


Figure S1 (continued). GC-FID chromatograms of extracts from field-contaminated sediments and *Lumbriculus variegatus,* exposed to these sediments for 28 days.

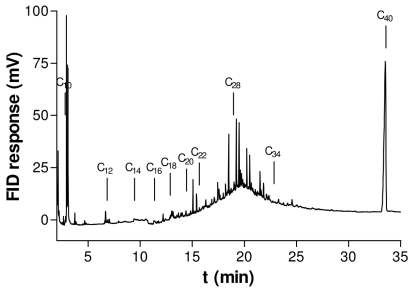
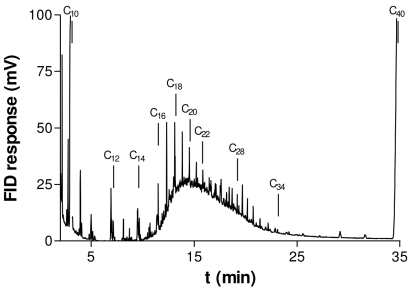
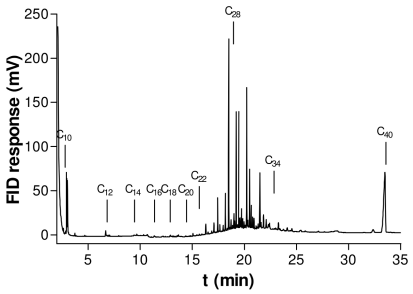
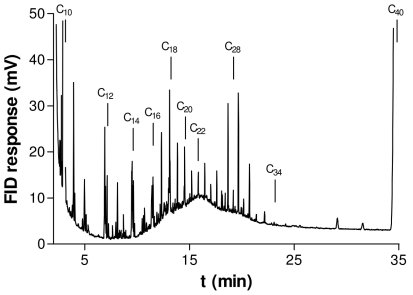
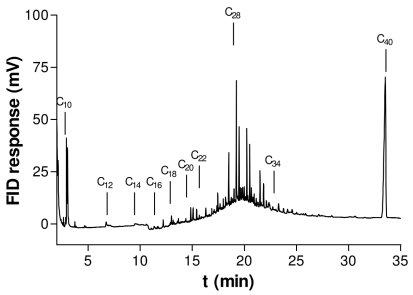
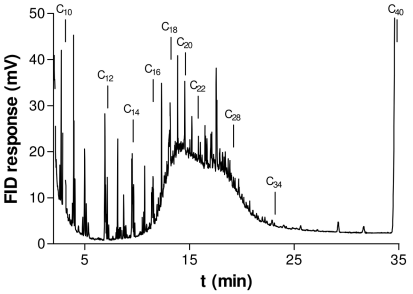
sediment	*L. variegatus*
S12O	
	
S13O	
	
S15O	
	

Figure S1 (continued). GC-FID chromatograms of extracts from field-contaminated sediments and *Lumbriculus variegatus,* exposed to these sediments for 28 days.

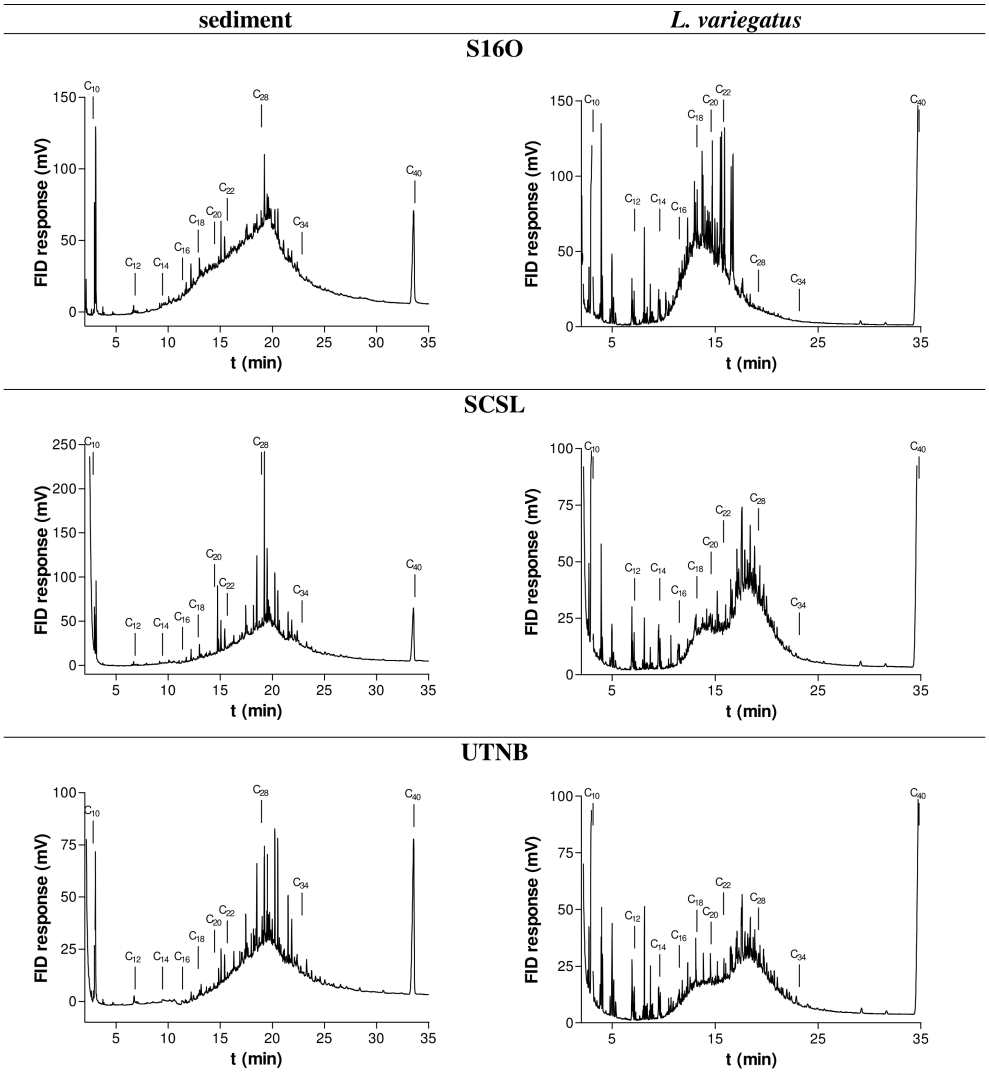
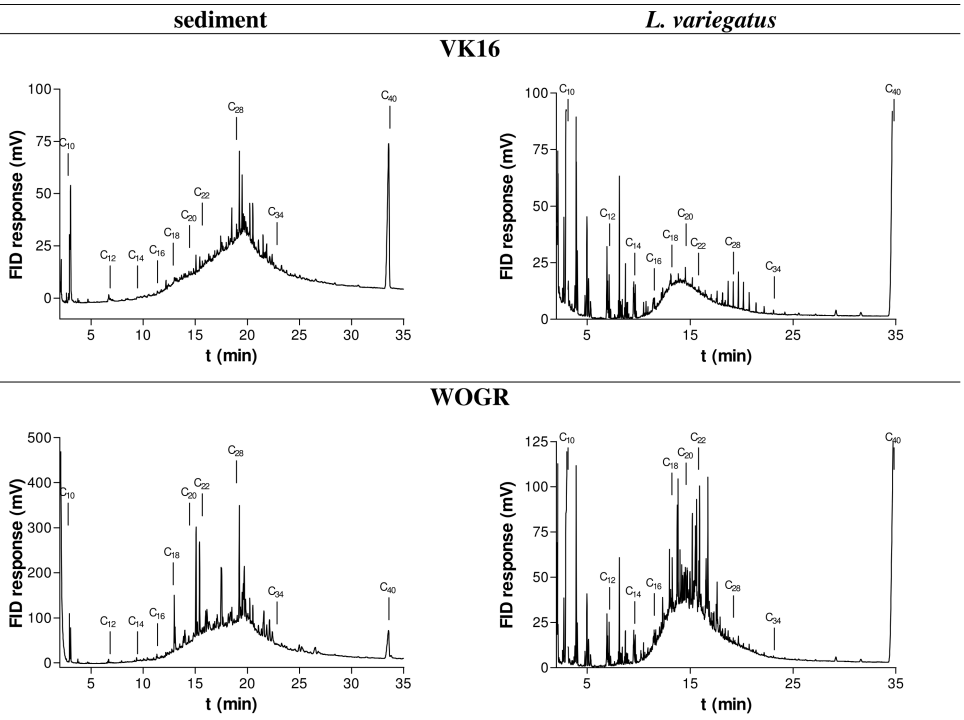


Figure S1 (continued). GC-FID chromatograms of extracts from field-contaminated sediments and *Lumbriculus variegatus,* exposed to these sediments for 28 days.



Appendix IV

Assessing the bioavailability of complex petroleum hydrocarbon mixtures in sediments

additional information

Table S1. Oil-contaminated field sediments used in the present study: codes, sampling location descriptions, probable oil sources, Total Petroleum Hydrocarbon (TPH; C_{10}-C_{40}) concentrations, and organic carbon contents (f_{oc}).

code	location	probable oil source	f_{oc} (%)	TPH (mg/kg dw)
ADPH	harbour	storage and transportation of fuel and tar, accidents, destruction of the oil storage capacity during the 2nd world war.	5.44 ± 0.104	14,163 ± 715
HIJG	outlet of a pumping station	diesel-powered pumping station, ships, diffuse sources	5.88 ± 0.101	2,272 ± 87.0
JHOZ	yacht harbour	fuel pomp	25.9 ± 2.30	1,527 ± 36.8
JHWE	yacht harbour	fuel pomp	35.6 ± 1.10	2,732 ± 140
KVGS	canal behind a gas station	gas station, rail emplacement, ships	3.60 ± 0.402	516 ± 86.7
KVHB	canal under a lift bridge	lubricants from the bridge, ships	6.73 ± 0.098	1,167 ± 42.8
ROSL	ditch in a heavily industrialized area	adjacent industrial activities, including breaker's yard, and a rail road	10.5 ± 0.222	14,677 ± 666
LERI	city canal	adjacent industrial activities, including breaker's yard and fuel storage	9.03 ± 1.00	4,072 ± 321
S12O	polluted river	shipping, adjacent industrial activities, waste dumps	3.55 ± 0.074	461 ± 75.3
S13O	ditch around a breaker's yard	adjacent breaker's yard activities	23.0 ± 0.516	886 ± 64.8
S15O	ditch behind a gas station	gas station	3.58 ± 0.190	600 ± 119
S16O	outlet of a pumping station	diesel-powered pumping station, ships, diffuse sources	5.82 ± 0.120	3,093 ± 76.6
SCSL	polluted ditch	unknown	5.81 ± 0.307	1,914 ± 102
UTNB	city canal	ships, traffic, adjacent activities	5.24 ± 0.363	961 ± 70.1
VK16	harbour	ships, adjacent activities	3.98 ± 0.105	981 ± 47.4
WOGR	city canal	ships, adjacent activities	6.01 ± 0.058	3,608 ± 384

Table S2. Boiling point fraction-dependent correction factors required to match polyoxymethylene solid phase extraction (POM-SPE)-derived BPH concentrations with concentrations measured in *Lumbriculus variegatus*.

sediment	C_{11}-C_{12}	C_{12}-C_{14}	C_{14}-C_{16}	C_{16}-C_{18}	C_{18}-C_{20}	C_{20}-C_{22}	C_{22}-C_{24}	C_{24}-C_{26}	C_{26}-C_{28}	C_{28}-C_{30}	C_{30}-C_{32}	C_{32}-C_{34}	C_{11}-C_{34}
HIJG	7.95	4.37	7.07	15.14	15.46	10.03	6.20	5.47	6.24	5.18	5.97	6.57	8.41
JHOZ	45.59	57.37	18.87	24.35	19.02	13.32	16.88	17.78	20.45	16.54	20.29	23.18	18.74
JHWE	78.29	74.83	29.79	26.49	19.85	13.99	14.08	14.60	9.58	5.46	8.27	11.04	13.61
KVGS	96.62	40.33		51.05	35.65	20.30	23.09	29.06	33.03	37.97			32.69
KVHB	74.12	75.07	30.42	59.92	52.90	31.13	29.16	26.87	30.79	31.06	48.06	54.58	36.63
LERI	4.87	3.43	3.39	7.01	6.56	6.18	5.16	6.93	10.59	8.72	13.11	10.36	6.35
S120	98.98	65.81	19.57	30.76	27.40	17.02	14.02	13.83	15.15	11.44	10.05	9.87	17.48
S130	105.20	99.77								33.46	32.77	50.47	
S150	84.57	34.93	18.71	28.03	30.86	17.88	19.98	24.69	27.04	18.97	21.59	20.24	23.25
S160	5.99	3.83	8.82	15.03	13.05	8.67	4.97	2.99	2.46	1.98	1.92	1.83	6.32
SCSL	9.64	6.82	10.56	22.30	21.51	9.76	14.52	18.66	26.98	16.27	27.33	22.04	16.17
UTNB	58.55	27.34	26.73	43.84	34.85	19.51	24.01	28.10	32.59	24.99	31.46	41.00	28.41
VK16	81.32	76.30	20.98	27.91	28.49	20.29	13.01	8.00	6.99	6.40	7.80	11.97	16.50
WOGR	9.65	5.41	7.50	17.01	13.30	7.10	4.09	2.86	2.76	1.68	3.17	3.55	5.73
average	54.38	41.12	16.87	28.37	24.53	15.01	14.55	15.37	17.28	15.72	17.83	20.52	17.71
SD	39.22	33.61	9.29	15.14	12.33	6.98	8.02	9.67	11.66	12.09	13.90	17.55	10.16

Table S3. Linear regression parameters (r2 values and slopes with accompanying 95% confidence intervals) of the relationships presented in figure 1.

	sediment	sediment (Organic carbon-normalised)	SPME	hs-SPME	POM	cyclo-dextrine	Tenax	model (70% aliphatics)
slope	0.468	1.27	2.93	1.59	1.49	1.97	2.52	1.10
r^2	0.110	0.652	0.769	0.749	0.646	0.674	0.787	0.637
95% confidence interval	-0.369-1.31	0.685-1.86	1.91-3.95	1.00-2.17	0.745-2.23	1.10-2.84	1.69-3.35	0.607-1.60

Table S4. Sediment-averaged (n = 14) squared values of the difference between the actually measured (in worms) and predicted (by the indicated experimental methods and the model) percentage of the total BPH, occupied by the respective hydrocarbon block (or, in other words, the squared values of the difference between the bars presented in Figure 2 for worms and those for the different methods; calculated for each separate bar). The last column presents the sum of squares value, with the bold value indicating the method (POM-SPE) having the lowest value, thus the smallest deviation from the pattern observed for worms. Bold values in the other columns indicate the method with the smallest deviation from the worm pattern for that particular boiling point fraction (number of fractions closest to the worm pattern: POMSPE: 4, hs-SPME: 3, cyclodextrin: 2, model and Tenax: 1, SPME and total extraction: 0).

	C_{11}-C_{14}	C_{14}-C_{16}	C_{16}-C_{18}	C_{18}-C_{20}	C_{20}-C_{22}	C_{22}-C_{24}	C_{24}-C_{26}	C_{26}-C_{28}	C_{28}-C_{30}	C_{30}-C_{32}	C_{32}-C_{34}	sum of squares
model	33.9	7.9	27.1	**32.1**	30.8	26.4	35.4	34.6	36.1	5.6	9.6	279
SPME	43.8	43.7	26.1	61.8	50.2	24.8	12.4	11.5	16.0	35.7	78.9	405
hs-SPME	83.2	43.8	**13.5**	39.2	22.3	**3.3**	14.4	31.6	11.6	**4.2**	7.3	274
POM-SPE	**28.8**	**2.9**	33.7	41.9	**20.7**	14.7	15.6	12.4	24.1	9.1	**4.2**	**208**
cyclodextrin	132.0	21.4	30.5	44.2	28.7	8.4	**7.8**	**10.6**	15.9	26.1	21.2	347
Tenax	78.3	21.7	15.1	39.9	34.4	12.7	13.9	11.6	**9.3**	7.9	7.8	253
total extraction	36.6	9.1	91.7	144.4	75.5	28.3	9.3	33.3	143.7	170.1	145.2	887

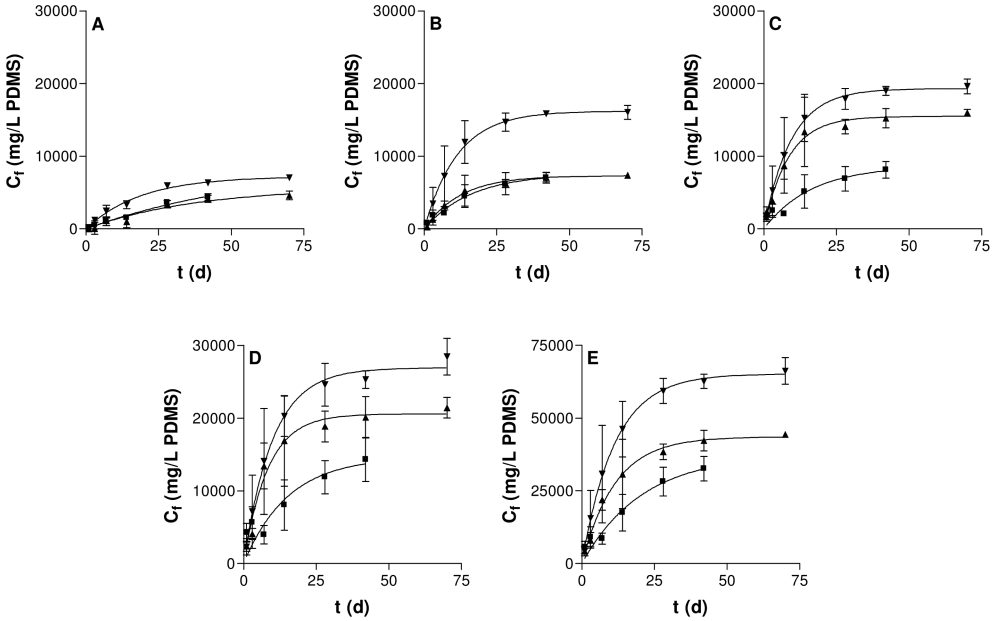


Figure S1. Uptake kinetics of petroleum hydrocarbons in 30 µm PDMS-coated SPME fibers during direct exposure to sediment slurries from (■) HIJG, (▼) LERI, and (▲) ROSL sediment. Graphs are shown for the fractions (A) C_{11}-C_{16}, (B) C_{16}-C_{22}, (C), C_{22}-C_{28}, (D) C_{28}-C_{40}, and (E) (C_{11}-C_{40}). Values are averages ± standard deviations (n=4).

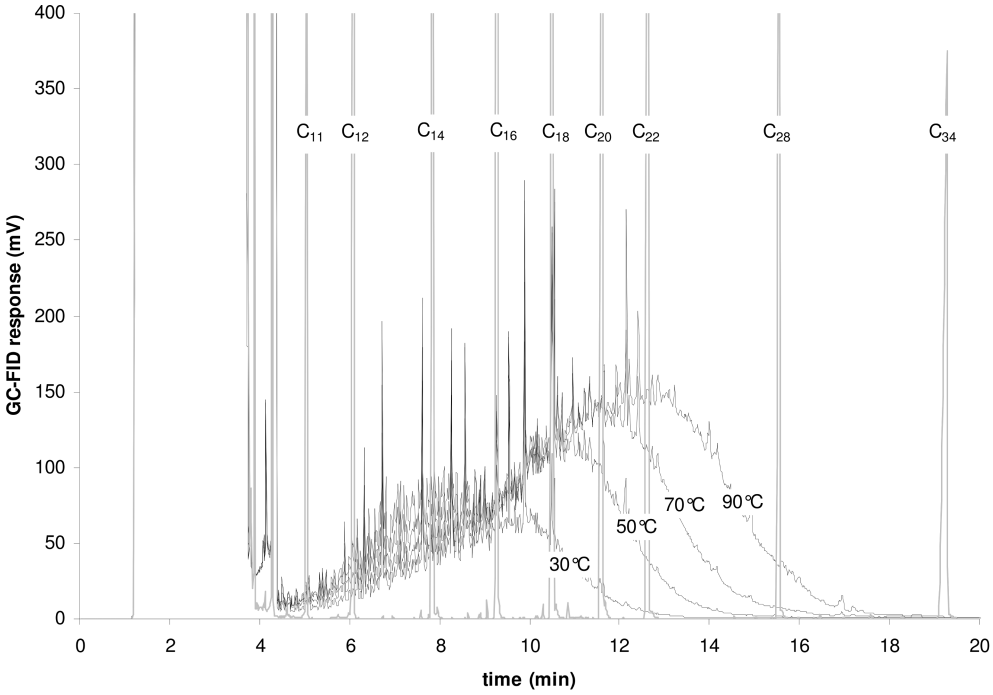


Figure S2. GC-FID chromatograms of petroleum hydrocarbons extracted from LERI sediment using 30 µm PDMS-coated SPME fibers. The fibers were placed in the headspace of the sediment slurries that were heated to different temperatures (indicated).

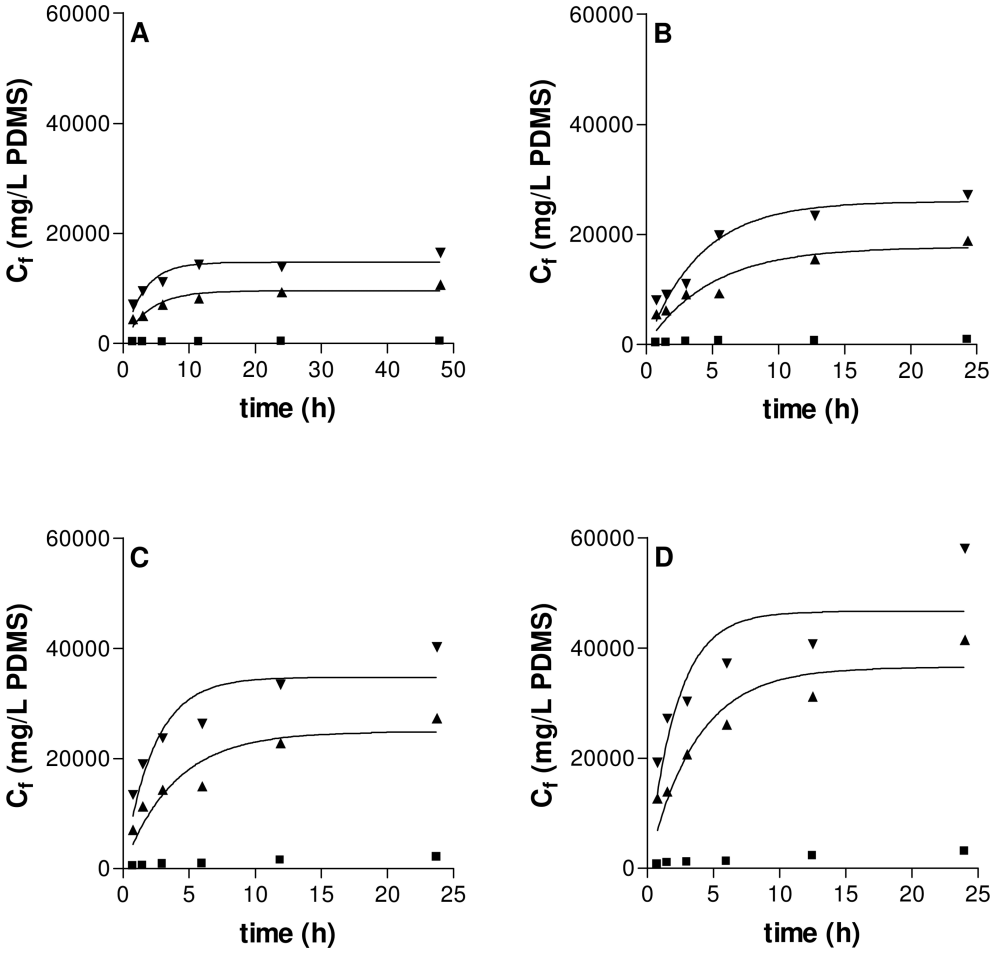


Figure S3. Uptake kinetics of BPH (C_{11}-C_{34}) extracted with 30 µm PDMS-coated SPME fibers exposed in the headspace above (■) JHWE, (▼) S160, and (▲) LERI sediment; at (A) 30°C, (B) 50°C, (C), 70°C, and (D) 90°C. Data points represent singular measurements.

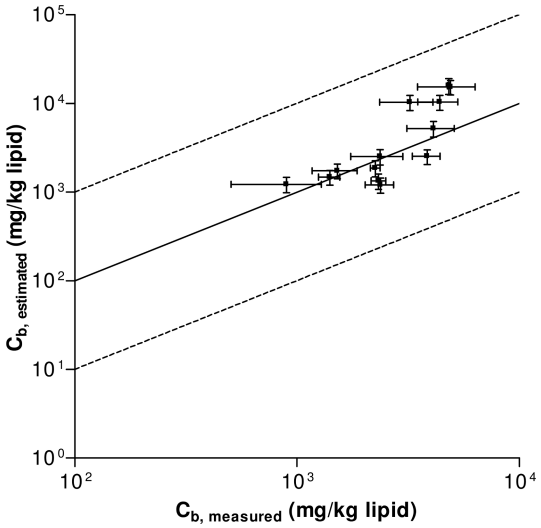


Figure S4. Relationship between BPH concentrations in *Lumbriculus variegatus* exposed to oil-contaminated sediments ($C_{b,\ measured}$) and BPH concentrations predicted by extractions using polyoxymethylene (POM) ($C_{b,\ estimated}$) and the application of hydrocarbon block-dependent correction factors (see Table S2). Values are averages ± standard deviations. The solid line represents the 1:1 relationship; the dotted lines delimit the one order of magnitude deviation interval.

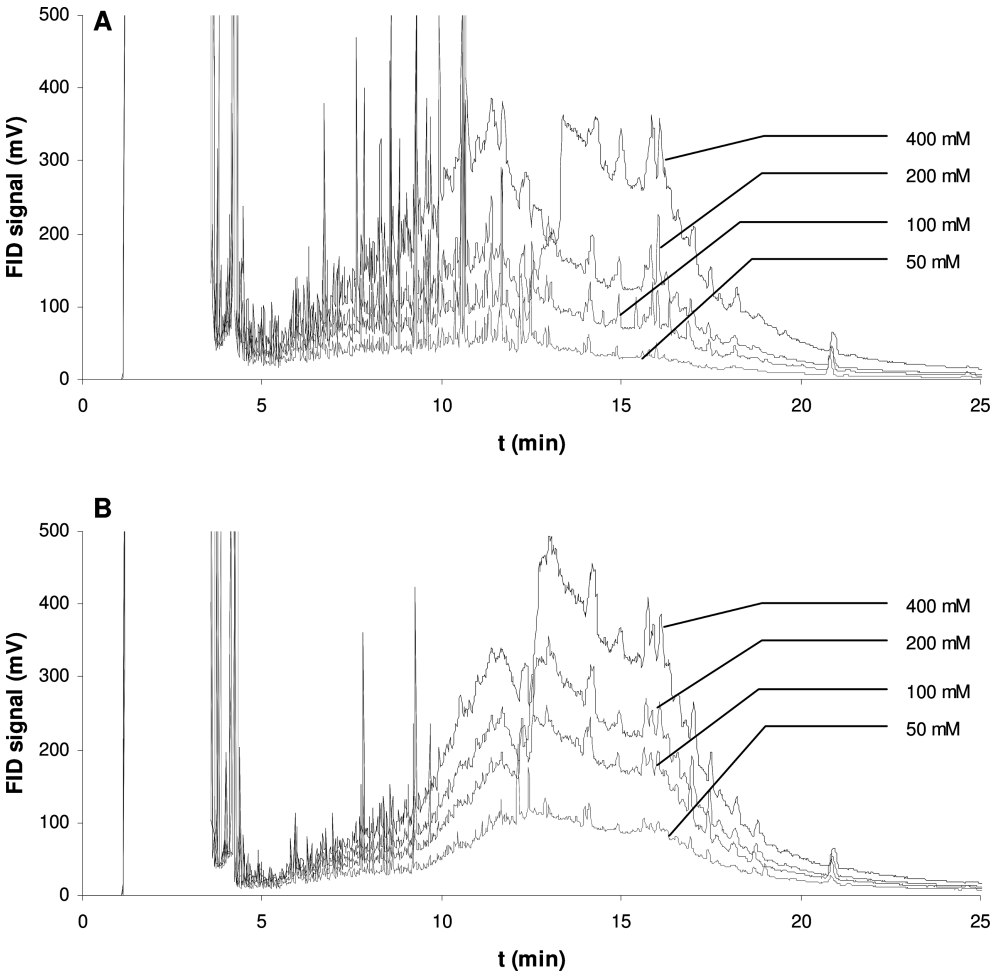


Figure S5. GC-FID chromatograms of BPH (C_{11}-C_{34}) extracted from (A) LERI and (B) WOGR sediment using different concentrations of 2-propyl-β-cyclodextrin (indicated).

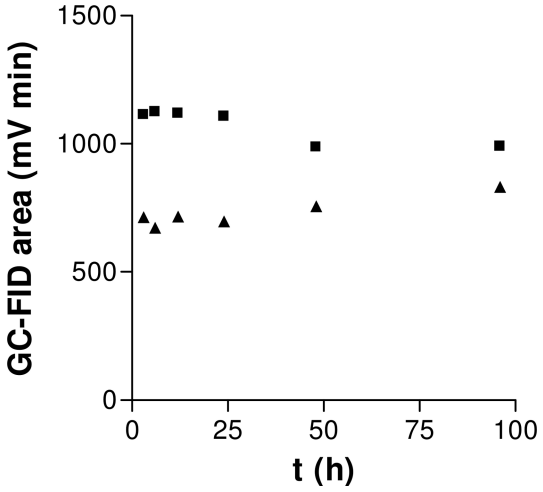


Figure S6. Uptake kinetics of BPH (C_{11}-C_{34}) extracted with 2-propyl-*β*-cyclodextrin from (■)WOGR and (▲) LERI sediment.

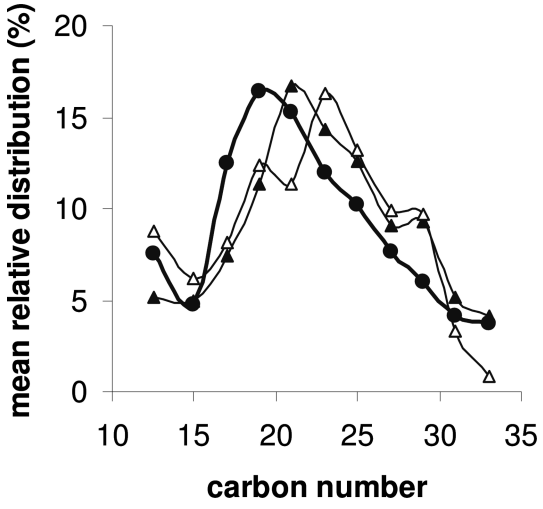


Figure S7. Comparison of the averaged (n = 14 sediments) relative BPH boiling point fraction distribution (indicated by carbon number) as observed in *L. variegatus* (●) with that predicted by POM-SPE experiments (▲) and the model from Verbruggen (△) (*1*).

References

(1) Verbruggen, E. M. J.; Beek, M.; Pijnenburg, J.; Traas, T. P. Ecotoxicological environmental risk limits for total petroleum hydrocarbons on the basis of internal lipid concentrations. *Environmental Toxicology and Chemistry* 2008, *27*, 2436-2448.

Appendix V

Testing the potential of passive samplers to assess actual in situ bioaccumulation of PAHs and petroleum hydrocarbon mixtures

additional information

Table S1: Overview of PAH field locations, sediment characteristics, lipid contents of worms, PAH concentrations in sediments (C_s), PAH concentrations in *Lumbriculus variegatus* (C_b) and PDMS-coated SPME fibres (C_f), SPME-derived logarithmic organic carbon-water partition coefficients (K_{oc}) and bioaccumulation factors (BAF), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	PAH	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	log K_{oc} (L/kg)	log BAF (L/kg)	BSAF
2P	Organic-rich (plant material and roots) sediment in a fringe of reeds adjacent to a bunker ship. Canal known to be PAH-polluted.	0,860±0,058	10,8±0,480	n.a.[1]	Phe	1,76	9,92	0,23	5,65	5,43	0,61
					Ant	0,38	4,10	0,06	5,63	5,70	1,17
					Flu	3,96	39,7	1,15	5,75	5,79	1,09
					Pyr	3,10	33,5	0,88	5,85	5,92	1,17
					BaA	1,39	12,8	0,19	6,68	6,68	0,99
					Chr	1,51	8,64	0,22	6,56	6,36	0,62
					BeP	1,46	7,04	0,14	7,19	6,90	0,52
					BbF	2,11	10,3	0,21	7,22	6,94	0,53
					BkF	1,03	3,86	0,08	7,35	6,96	0,41
					BaP	1,94	3,64	0,11	7,52	6,83	0,20
					BghiP	1,37	1,94	0,06	8,01	7,20	0,15
					DahA	n.d.	0,40	0,04	n.a.	6,61	n.a.
					InP	1,34	1,53	0,06	8,04	7,13	0,12
					total	21,3	137	3,44	n.a.		0,70

[1] not analysed due to insufficient sample. Lipid-normalized PAH concentrations were based on the average value of the other locations (14.4%)
[2] below detection limit of 0.095 mg carbon. Detection limits were used do calculate normalized PAHs concentrations in sediments and BSAFs.
n.a. not analysed
n.d. not detected

Table S1 (continued). Overview of PAH field locations, sediment characteristics, lipid contents of worms, PAH concentrations in sediments (C_s), PAH concentrations in *Lumbriculus variegatus* (C_b) and PDMS-coated SPME fibres (C_f), SPME-derived logarithmic organic carbon-water partition coefficients (K_{oc}) and bioaccumulation factors (BAF), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	PAH	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	log K_{oc} (L/kg)	log BAF (L/kg)	BSAF
3P	Muddy and dirty (waste dump) sediment in a canal known to be PAH-polluted.	1,15±0,030	16,6±0,582	15,6	Phe	1,31	n.a.	0,19	5,41		n.a.
					Ant	0,69	n.a.	0,06	5,75		n.a.
					Flu	2,40	n.a.	0,79	5,51		n.a.
					Pyr	1,80	n.a.	0,67	5,54		n.a.
					BaA	0,62	n.a.	0,16	6,21		n.a.
					Chr	0,59	n.a.	0,25	5,93		n.a.
					BeP	0,51	n.a.	n.d.	n.a.		n.a.
					BbF	0,75	n.a.	0,11	6,89		n.a.
					BkF	0,34	n.a.	0,04	6,97		n.a.
					BaP	0,55	n.a.	0,03	7,28		n.a.
					BghiP	0,44	n.a.	n.d.	n.a.		n.a.
					DahA	n.d.	n.a.	n.d.	n.a.		n.a.
					InP	0,42	n.a.	n.d.	n.a.		n.a.
					total	10,4	n.a.	2,30	n.a.		n.a.

[1] not analysed due to insufficient sample. Lipid-normalized PAH concentrations were based on the average value of the other locations (14.4%)
[2] below detection limit of 0.095 mg carbon. Detection limits were used do calculate normalized PAHs concentrations in sediments and BSAFs.
n.a. not analysed
n.d. not detected

Table S1 (continued). Overview of PAH field locations, sediment characteristics, lipid contents of worms, PAH concentrations in sediments (C_s), PAH concentrations in *Lumbriculus variegatus* (C_b) and PDMS-coated SPME fibres (C_f), SPME-derived logarithmic organic carbon-water partition coefficients (K_{oc}) and bioaccumulation factors (BAF), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	PAH	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	log K_{oc} (L/kg)	log BAF (L/kg)	BSAF
6P	Sandy sediment in a canal receiving road run-off. Canal is known to be PAH-polluted.	n.d.	n.d.[2]	14,1	Phe	1,21	2,28	0,13	6,98	5,04	0,01
					Ant	0,56	3,05	0,50	6,17	4,69	0,03
					Flu	6,05	90,1	7,50	6,39	5,35	0,09
					Pyr	5,03	83,5	5,04	6,57	5,57	0,10
					BaA	4,10	22,2	0,68	7,85	6,37	0,03
					Chr	3,00	10,5	0,37	7,91	6,24	0,02
					BeP	2,65	8,07	0,12	8,80	7,06	0,02
					BbF	3,58	8,87	0,17	8,82	7,00	0,02
					BkF	1,98	4,91	0,08	8,94	7,12	0,02
					BaP	4,60	9,13	0,13	9,08	7,16	0,01
					BghiP	2,43	2,54	0,05	9,65	7,45	0,01
					DahA	n.d.	0,30	n.d.	n.a.		n.a.
					InP	2,57	2,16	0,05	9,68	7,39	0,01
					total	37,8	248	14,8	n.a.		0,04

[1] not analysed due to insufficient sample. Lipid-normalized PAH concentrations were based on the average value of the other locations (14.4%)
[2] below detection limit of 0.095 mg carbon. Detection limits were used do calculate normalized PAHs concentrations in sediments and BSAFs.
n.a. not analysed
n.d. not detected

Table S1 (continued). Overview of PAH field locations, sediment characteristics, lipid contents of worms, PAH concentrations in sediments (C_s), PAH concentrations in *Lumbriculus variegatus* (C_b) and PDMS-coated SPME fibres (C_f), SPME-derived logarithmic organic carbon-water partition coefficients (K_{oc}) and bioaccumulation factors (BAF), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	PAH	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	log K_{oc} (L/kg)	log BAF (L/kg)	BSAF
7P	Same canal as location 6, but about 2 km downstream. Opposite of the outlet of a pumping station.	n.d.	1,15±0,272	16,7	Phe	0,27	1,46	0,09	6,20	4,99	0,06
					Ant	0,09	1,29	n.d.	n.a.		0,17
					Flu	0,73	8,85	0,26	6,63	5,77	0,14
					Pyr	0,62	16,2	0,36	6,51	5,98	0,30
					BaA	0,45	3,65	n.d.	n.a.		0,09
					Chr	0,31	1,08	0,18	6,95		0,04
					BeP	0,27	1,91	n.d.	n.a.		0,08
					BbF	0,39	2,24	0,05	8,04	6,86	0,07
					BkF	0,19	0,83	n.d.	n.a.		0,05
					BaP	0,40	1,12	n.d.	n.a.		0,03
					BghiP	0,24	0,52	n.d.	n.a.		0,03
					DahA	n.d.	0,06	n.d.	n.a.		n.a.
					InP	0,23	0,40	n.d.	n.a.		0,02
					total	4,20	39,7	0,95	n.a.		0,11

[1] not analysed due to insufficient sample. Lipid-normalized PAH concentrations were based on the average value of the other locations (14.4%)
[2] below detection limit of 0.095 mg carbon. Detection limits were used do calculate normalized PAHs concentrations in sediments and BSAFs.
n.a. not analysed
n.d. not detected

Table S1 (continued). Overview of PAH field locations, sediment characteristics, lipid contents of worms, PAH concentrations in sediments (C_s), PAH concentrations in *Lumbriculus variegatus* (C_b) and PDMS-coated SPME fibres (C_f), SPME-derived logarithmic organic carbon-water partition coefficients (K_{oc}) and bioaccumulation factors (BAF), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	PAH	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	log K_{oc} (L/kg)	log BAF (L/kg)	BSAF
8P	Ditch adjacent to a road. Ditch is known to be PAH-polluted.	0,501±0,163	8,54±2,12	n.a.	Phe	6,75	n.a.	6,05	4,94		n.a.
					Ant	1,01	n.a.	0,82	5,08		n.a.
					Flu	10,7	n.a.	6,08	5,61		n.a.
					Pyr	7,38	n.a.	3,30	5,80		n.a.
					BaA	3,82	n.a.	0,46	6,88		n.a.
					Chr	3,56	n.a.	0,45	6,78		n.a.
					BeP	3,69	n.a.	0,24	7,51		n.a.
					BbF	4,91	n.a.	0,32	7,57		n.a.
					BkF	2,22	n.a.	0,11	7,71		n.a.
					BaP	4,30	n.a.	0,15	7,89		n.a.
					BghiP	5,74	n.a.	0,20	8,29		n.a.
					DahA	n.d.	n.a.	0,03	n.a.		n.a.
					InP	4,75	n.a.	0,15	8,36		n.a.
					total	58,8	n.a.	18,4	n.a.		n.a.

[1] not analysed due to insufficient sample. Lipid-normalized PAH concentrations were based on the average value of the other locations (14.4%)
[2] below detection limit of 0.095 mg carbon. Detection limits were used do calculate normalized PAHs concentrations in sediments and BSAFs.
n.a. not analysed
n.d. not detected

Table S1 (continued). Overview of PAH field locations, sediment characteristics, lipid contents of worms, PAH concentrations in sediments (C_s), PAH concentrations in *Lumbriculus variegatus* (C_b) and PDMS-coated SPME fibres (C_f), SPME-derived logarithmic organic carbon-water partition coefficients (K_{oc}) and bioaccumulation factors (BAF), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	PAH	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	log K_{oc} (L/kg)	log BAF (L/kg)	BSAF
12P	Clayey sediment on the bank of a heavy polluted river due to shipping and adjacent industries.	0,036±0,016	2,84±0,354	13,2	Phe	0,59	n.a.	0,10	6,10		n.a.
					Ant	0,27	n.a.	n.d.	n.a.		n.a.
					Flu	1,25	n.a.	0,29	6,43		n.a.
					Pyr	0,99	n.a.	0,45	6,22		n.a.
					BaA	0,90	n.a.	0,10	7,32		n.a.
					Chr	0,99	n.a.	0,21	6,98		n.a.
					BeP	0,74	n.a.	0,06	7,84		n.a.
					BbF	1,05	n.a.	0,09	7,89		n.a.
					BkF	0,50	n.a.	n.d.	n.a.		n.a.
					BaP	1,06	n.a.	0,04	8,31		n.a.
					BghiP	0,80	n.a.	n.d.	n.a.		n.a.
					DahA	n.d.	n.a.	n.d.	n.a.		n.a.
					InP	0,82	n.a.	n.d.	n.a.		n.a.
					total	9,95	n.a.	1,32	n.a.		n.a.

[1] not analysed due to insufficient sample. Lipid-normalized PAH concentrations were based on the average value of the other locations (14.4%)
[2] below detection limit of 0.095 mg carbon. Detection limits were used do calculate normalized PAHs concentrations in sediments and BSAFs.
n.a. not analysed
n.d. not detected

Table S1 (continued). Overview of PAH field locations, sediment characteristics, lipid contents of worms, PAH concentrations in sediments (C_s), PAH concentrations in *Lumbriculus variegatus* (C_b) and PDMS-coated SPME fibres (C_f), SPME-derived logarithmic organic carbon-water partition coefficients (K_{oc}) and bioaccumulation factors (BAF), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	PAH	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	log K_{oc} (L/kg)	log BAF (L/kg)	BSAF
14P	Sandy sediment receiving road run-off from an adjacent highway.	n.d.	n.d.[2]	13,5	Phe	0,03	2,10	0,08	5,62	5,24	0,42
					Ant	0,00	0,92	n.d.	n.a.		2,68
					Flu	0,06	7,85	0,20	6,05	5,91	0,72
					Pyr	0,05	6,73	0,19	6,12	5,96	0,69
					BaA	0,01	1,56	n.d.	n.a.		0,60
					Chr	0,02	2,63	0,14	6,22	6,13	0,80
					BeP	0,00	8,46	0,20	n.a.	6,91	n.a.
					BbF	0,03	2,40	n.d.	n.a.		0,44
					BkF	0,01	0,76	n.d.	n.a.		0,33
					BaP	0,02	0,72	n.d.	n.a.		0,21
					BghiP	0,02	0,55	n.d.	n.a.		0,13
					DahA	n.d.	0,04	n.d.	n.a.		n.a.
					InP	0,02	0,33	n.d.	n.a.		0,12
					total	0,28	35,1	0,81	n.a.		0,70

[1] not analysed due to insufficient sample. Lipid-normalized PAH concentrations were based on the average value of the other locations (14.4%)
[2] below detection limit of 0.095 mg carbon. Detection limits were used do calculate normalized PAHs concentrations in sediments and BSAFs.
n.a. not analysed
n.d. not detected

Table S1 (continued). Overview of PAH field locations, sediment characteristics, lipid contents of worms, PAH concentrations in sediments (C_s), PAH concentrations in *Lumbriculus variegatus* (C_b) and PDMS-coated SPME fibres (C_f), SPME-derived logarithmic organic carbon-water partition coefficients (K_{oc}) and bioaccumulation factors (BAF), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	PAH	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	log K_{oc} (L/kg)	log BAF (L/kg)	BSAF
15P	Sandy sediment in a ditch behind a gas station.	0,464±0,213	7,42±2,88	16,7	Phe	0,49	10,1	0,09	5,68	5,86	1,53
					Ant	0,07	4,60	n.d.	n.a.		4,58
					Flu	2,60	25,2	0,65	6,02	5,88	0,72
					Pyr	1,99	21,3	0,52	6,09	5,99	0,80
					BaA	0,94	10,8	0,18	6,74	6,67	0,85
					Chr	0,96	9,99	0,27	6,49	6,38	0,78
					BeP	1,19	8,82	0,13	7,34	7,08	0,55
					BbF	1,57	11,2	0,18	7,39	7,11	0,53
					BkF	0,70	3,41	0,05	7,61	7,16	0,36
					BaP	1,17	2,57	0,06	7,79	7,00	0,16
					BghiP	1,24	2,44	0,03	8,51	7,68	0,15
					DahA	n.d.	0,48	n.d.	n.a.		n.a.
					InP	1,05	1,03	n.d.	n.a.		0,07
					total	14,0	112	2,16	n.a.		0,60

[1] not analysed due to insufficient sample. Lipid-normalized PAH concentrations were based on the average value of the other locations (14.4%)
[2] below detection limit of 0.095 mg carbon. Detection limits were used do calculate normalized PAHs concentrations in sediments and BSAFs.
n.a. not analysed
n.d. not detected

Table S1 (continued). Overview of PAH field locations, sediment characteristics, lipid contents of worms, PAH concentrations in sediments (C_s), PAH concentrations in *Lumbriculus variegatus* (C_b) and PDMS-coated SPME fibres (C_f), SPME-derived logarithmic organic carbon-water partition coefficients (K_{oc}) and bioaccumulation factors (BAF), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	PAH	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	log K_{oc} (L/kg)	log BAF (L/kg)	BSAF
16P	Clayey sediment on the bank of a heavy polluted river due to shipping, adjacent industries, and the outlet of a nearby diesel-powered pumping station.	0,231±0,010	4,67±0,199	10,8	Phe	0,82	1,57	0,17	5,81	4,77	0,09
					Ant	0,48	1,10	0,06	6,08	5,12	0,11
					Flu	1,90	12,4	0,29	6,39	5,87	0,30
					Pyr	1,57	15,6	0,38	6,27	5,94	0,46
					BaA	1,04	7,82	0,12	7,10	6,64	0,35
					Chr	0,91	6,81	0,21	6,73	6,28	0,35
					BeP	0,79	7,22	0,08	7,49	7,12	0,43
					BbF	1,17	9,11	0,12	7,58	7,14	0,36
					BkF	0,55	3,79	0,04	7,74	7,25	0,32
					BaP	1,04	4,19	0,05	7,92	7,19	0,19
					BghiP	0,72	2,62	0,05	8,14	7,37	0,17
					DahA	n.d.	0,30	n.d.	n.a.		n.a.
					InP	0,71	1,85	0,04	8,27	7,36	0,12
					total	11,7	74,3	1,62	n.a.		0,30

[1] not analysed due to insufficient sample. Lipid-normalized PAH concentrations were based on the average value of the other locations (14.4%)
[2] below detection limit of 0.095 mg carbon. Detection limits were used do calculate normalized PAHs concentrations in sediments and BSAFs.
n.a. not analysed
n.d. not detected

Table S2. Overview of oil field locations, sediment characteristics, worm lipid contents, TPH concentrations in sediments (C_s), BPH concentrations in *Lumbriculus variegatus* (C_b), PDMS-coated SPME fibres (C_f), and POM (C_{POM}), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	fraction	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	C_{POM} (mg/kg POM)	BSAF
20	Organic-rich (plant material and roots) sediment in a fringe of reeds adjacent to a bunker ship. Canal known to be PAH-polluted.	0,752±0,084	10,1±1,10	n.a.[1]	C_{11}-C_{12}	n.d.	198	2,23	n.d.	n.d.
					C_{12}-C_{14}	n.d.	198	41,4	n.d.	n.d.
					C_{14}-C_{16}	8,22	104	51,6	1,30	1,28
					C_{16}-C_{18}	24,0	156	32,8	1,65	0,65
					C_{18}-C_{20}	48,0	260	45,7	2,57	0,55
					C_{20}-C_{22}	69,6	436	55,7	4,84	0,63
					C_{22}-C_{24}	73,3	399	65,3	4,24	0,55
					C_{24}-C_{26}	91,8	225	87,5	4,03	0,25
					C_{26}-C_{28}	89,4	123	70,1	2,19	0,14
					C_{28}-C_{30}	128	87,5	78,6	2,37	0,07
					C_{30}-C_{32}	128	n.d.	n.d.	0,688	n.d.
					C_{32}-C_{34}	127	n.d.	54,0	0,529	n.d.
					C_{34}-C_{36}	116	n.d.	58,1	n.d.	n.d.
					C_{36}-C_{40}	100,0	n.d.	109	0,322	n.d.
					total	998	2690	719	18,4	0,27
					C_{11}-C_{20}	86,1	825	474	n.d.	0,97
					C_{11}-C_{34}	798	2950	584	18,5	0,37

[1] not analysed due to insufficient sample. Lipid-normalized BPH concentrations were based on the average value of the other locations (14.4%)
n.a. not analysed
n.d. not detected

Table S2 (continued). Overview of oil field locations, sediment characteristics, worm lipid contents, TPH concentrations in sediments (C_s), BPH concentrations in *Lumbriculus variegatus* (C_b), PDMS-coated SPME fibres (C_f), and POM (C_{POM}), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	fraction	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	C_{POM} (mg/kg POM)	BSAF
30	Muddy and dirty (waste dump) sediment in a canal known to be PAH-polluted.	0,105±0,012	5,02±0,904	15.6	C_{11}-C_{12}	n.d.	n.d.	n.d.	n.d.	n.d.
					C_{12}-C_{14}	n.d.	n.d.	63,5	n.d.	n.d.
					C_{14}-C_{16}	n.d.	32,0	37,2	n.d.	n.d.
					C_{16}-C_{18}	n.d.	55,1	n.d.	n.d.	n.d.
					C_{18}-C_{20}	17,5	74,2	19,0	n.d.	0,21
					C_{20}-C_{22}	62,0	97,8	30,4	0,847	0,08
					C_{22}-C_{24}	67,7	70,5	51,8	0,940	0,05
					C_{24}-C_{26}	33,9	43,9	85,3	n.d.	0,06
					C_{26}-C_{28}	13,8	37,7	67,9	n.d.	0,14
					C_{28}-C_{30}	20,5	34,3	79,3	n.d.	0,08
					C_{30}-C_{32}	38,5	29,1	34,6	n.d.	0,04
					C_{32}-C_{34}	55,1	n.d.	48,7	0,390	n.d.
					C_{34}-C_{36}	49,7	n.d.	45,9	n.d.	n.d.
					C_{36}-C_{40}	42,4	n.d.	81,2	0,326	n.d.
					total	396	625	642	n.d.	0,08
					C_{11}-C_{20}	n.d.	148	377	n.d.	n.d.
					C_{11}-C_{34}	314	649	555	n.d.	0,10

[1] not analysed due to insufficient sample. Lipid-normalized BPH concentrations were based on the average value of the other locations (14.4%)
n.a. not analysed
n.d. not detected

Table S2 (continued). Overview of oil field locations, sediment characteristics, worm lipid contents, TPH concentrations in sediments (C_s), BPH concentrations in *Lumbriculus variegatus* (C_b), PDMS-coated SPME fibres (C_f), and POM (C_{POM}), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	fraction	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	C_{POM} (mg/kg POM)	BSAF
70	Same location as location 6 (PAH). Opposite of the outlet of a pumping station.	0,226±0,055	4,14±0,777	16.7	C_{11}-C_{12}	n.d.	n.d.	4,18	n.d.	n.d.
					C_{12}-C_{14}	5,98	n.d.	55,2	n.d.	n.d.
					C_{14}-C_{16}	12,0	30,9	81,3	26,3	0,11
					C_{16}-C_{18}	n.d.	53,5	51,5	4,27	n.d.
					C_{18}-C_{20}	n.d.	71,6	43,8	5,62	n.d.
					C_{20}-C_{22}	10,8	91,3	25,9	8,71	0,35
					C_{22}-C_{24}	14,0	73,7	29,5	6,28	0,22
					C_{24}-C_{26}	20,2	56,6	33,0	6,06	0,12
					C_{26}-C_{28}	21,4	43,6	n.d.	5,28	0,08
					C_{28}-C_{30}	46,8	31,3	29,7	5,62	0,03
					C_{30}-C_{32}	44,7	22,3	n.d.	2,81	0,02
					C_{32}-C_{34}	39,7	n.d.	21,4	1,48	n.d.
					C_{34}-C_{36}	33,1	n.d.	n.d.	0,660	n.d.
					C_{36}-C_{40}	25,0	n.d.	n.d.	0,809	n.d.
					total	271	601	419	70,8	0,09
					C_{11}-C_{20}	n.d.	143	369	33,1	n.d.
					C_{11}-C_{34}	220	643	380	69,3	0,12

[1] not analysed due to insufficient sample. Lipid-normalized BPH concentrations were based on the average value of the other locations (14.4%)
n.a. not analysed
n.d. not detected

Table S2 (continued). Overview of oil field locations, sediment characteristics, worm lipid contents, TPH concentrations in sediments (C_s), BPH concentrations in *Lumbriculus variegatus* (C_b), PDMS-coated SPME fibres (C_f), and POM (C_{POM}), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	fraction	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	C_{POM} (mg/kg POM)	BSAF
80	Ditch adjacent to a road. Ditch is known to be PAH-polluted.	0,548±0,156	9,02±2,05	n.a.	C_{11}-C_{12}	n.d.	n.a.	62,6	n.a.	n.a.
					C_{12}-C_{14}	n.d.	n.a.	158	n.a.	n.a.
					C_{14}-C_{16}	4,75	n.a.	69,8	n.a.	n.a.
					C_{16}-C_{18}	n.d.	n.a.	26,0	n.a.	n.a.
					C_{18}-C_{20}	8,10	n.a.	30,7	n.a.	n.a.
					C_{20}-C_{22}	21,7	n.a.	44,9	n.a.	n.a.
					C_{22}-C_{24}	20,5	n.a.	75,5	n.a.	n.a.
					C_{24}-C_{26}	47,3	n.a.	104	n.a.	n.a.
					C_{26}-C_{28}	58,8	n.a.	78,7	n.a.	n.a.
					C_{28}-C_{30}	115	n.a.	105	n.a.	n.a.
					C_{30}-C_{32}	132	n.a.	76,2	n.a.	n.a.
					C_{32}-C_{34}	130	n.a.	101	n.a.	n.a.
					C_{34}-C_{36}	137	n.a.	88,0	n.a.	n.a.
					C_{36}-C_{40}	168	n.a.	156	n.a.	n.a.
					total	819	n.a.	1180	n.a.	n.a.
					C_{11}-C_{20}	n.d.	n.a.	672	n.a.	n.a.
					C_{11}-C_{34}	529	n.a.	969	n.a.	n.a.

[1] not analysed due to insufficient sample. Lipid-normalized BPH concentrations were based on the average value of the other locations (14.4%)
n.a. not analysed
n.d. not detected

Table S2 (continued). Overview of oil field locations, sediment characteristics, worm lipid contents, TPH concentrations in sediments (C_s), BPH concentrations in *Lumbriculus variegatus* (C_b), PDMS-coated SPME fibres (C_f), and POM (C_{POM}), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	fraction	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	C_{POM} (mg/kg POM)	BSAF
100	Ditch with sandy sediment adjacent to local road. Ditch is known to be oil-polluted.	n.d.	1,24±0,376	19.2	C_{11}-C_{12}	n.d.	27,6	n.d.	n.d.	n.d.
					C_{12}-C_{14}	n.d.	29,2	25,4	n.d.	n.d.
					C_{14}-C_{16}	n.d.	25,1	24,6	n.d.	n.d.
					C_{16}-C_{18}	n.d.	36,2	n.d.	n.d.	n.d.
					C_{18}-C_{20}	n.d.	56,9	5,95	n.d.	n.d.
					C_{20}-C_{22}	n.d.	78,1	9,57	1,37	n.d.
					C_{22}-C_{24}	n.d.	62,8	33,3	0,767	n.d.
					C_{24}-C_{26}	4,10	38,7	38,4	1,18	0,12
					C_{26}-C_{28}	9,32	27,4	34,9	3,39	0,04
					C_{28}-C_{30}	16,4	26,5	45,1	n.d.	0,02
					C_{30}-C_{32}	18,1	19,5	13,5	n.d.	0,01
					C_{32}-C_{34}	22,0	n.d.	26,4	0,117	n.d.
					C_{34}-C_{36}	29,5	n.d.	29,9	n.d.	n.d.
					C_{36}-C_{40}	40,3	n.d.	n.d.	n.d.	n.d.
					total	114	523	344	3,08	0,06
					C_{11}-C_{20}	n.d.	160	189	n.d.	n.d.
					C_{11}-C_{34}	50,5	558	289	4,17	0,14

[1] not analysed due to insufficient sample. Lipid-normalized BPH concentrations were based on the average value of the other locations (14.4%)
n.a. not analysed
n.d. not detected

Table S2 (continued). Overview of oil field locations, sediment characteristics, worm lipid contents, TPH concentrations in sediments (C_s), BPH concentrations in *Lumbriculus variegatus* (C_b), PDMS-coated SPME fibres (C_f), and POM (C_{POM}), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	fraction	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	C_{POM} (mg/kg POM)	BSAF
120	Clayey sediments on a bank of a heavy polluted river due to shipping and adjacent industries.	0,046±0,012	2,69±0,166	13.2	C_{11}-C_{12}	n.d.	27,6	n.d.	n.d.	n.d.
					C_{12}-C_{14}	n.d.	165	58,0	2,29	n.d.
					C_{14}-C_{16}	n.d.	75,4	70,2	6,06	n.d.
					C_{16}-C_{18}	n.d.	94,9	59,3	8,19	n.d.
					C_{18}-C_{20}	9,49	151	81,6	12,0	0,43
					C_{20}-C_{22}	19,4	265	79,4	18,6	0,37
					C_{22}-C_{24}	21,6	259	137	17,2	0,32
					C_{24}-C_{26}	34,8	222	107	15,8	0,17
					C_{26}-C_{28}	35,2	192	90,5	10,2	0,15
					C_{28}-C_{30}	52,0	166	175	9,58	0,09
					C_{30}-C_{32}	51,3	160	125	4,81	0,08
					C_{32}-C_{34}	49,3	131	130	3,61	0,07
					C_{34}-C_{36}	54,1	n.d.	210	1,48	n.d.
					C_{36}-C_{40}	48,9	n.d.	388	2,95	n.d.
					total	375	2510	1720	111	0,18
					C_{11}-C_{20}	n.d.	585	706	26,8	n.d.
					C_{11}-C_{34}	281	2440	1150	107	0,23

[1] not analysed due to insufficient sample. Lipid-normalized BPH concentrations were based on the average value of the other locations (14.4%)
n.a. not analysed
n.d. not detected

Table S2 (continued). Overview of oil field locations, sediment characteristics, worm lipid contents, TPH concentrations in sediments (C_s), BPH concentrations in *Lumbriculus variegatus* (C_b), PDMS-coated SPME fibres (C_f), and POM (C_{POM}), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	fraction	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	C_{POM} (mg/kg POM)	BSAF
130	Ditch with sandy sediment containing plant residues (branches, leafs, roots) adjacent to a breaker's yard.	1,69±0,135	21,2±1,67	16.7	C_{11}-C_{12}	n.d.	n.d.	28,0	n.d.	n.d.
					C_{12}-C_{14}	n.d.	n.d.	80,6	n.d.	n.d.
					C_{14}-C_{16}	n.d.	20,0	40,1	n.d.	n.d.
					C_{16}-C_{18}	n.d.	37,9	n.d.	n.d.	n.d.
					C_{18}-C_{20}	n.d.	49,4	6,44	n.d.	n.d.
					C_{20}-C_{22}	14,4	62,6	12,6	1,33	0,92
					C_{22}-C_{24}	20,6	47,7	33,9	1,21	0,49
					C_{24}-C_{26}	49,2	32,4	67,5	0,898	0,14
					C_{26}-C_{28}	93,1	26,5	61,2	n.d.	0,06
					C_{28}-C_{30}	151	22,4	103	n.d.	0,03
					C_{30}-C_{32}	139	n.d.	53,1	n.d.	n.d.
					C_{32}-C_{34}	104	n.d.	78,2	0,274	n.d.
					C_{34}-C_{36}	78,4	n.d.	117	n.d.	n.d.
					C_{36}-C_{40}	46,0	n.d.	274	n.d.	n.d.
					total	680	n.d.	963	n.d.	n.d.
					C_{11}-C_{20}	n.d.	74,9	355	n.d.	n.d.
					C_{11}-C_{34}	569	443	605	n.d.	0,16

[1] not analysed due to insufficient sample. Lipid-normalized BPH concentrations were based on the average value of the other locations (14.4%)
n.a. not analysed
n.d. not detected

Table S2 (continued). Overview of oil field locations, sediment characteristics, worm lipid contents, TPH concentrations in sediments (C_s), BPH concentrations in *Lumbriculus variegatus* (C_b), PDMS-coated SPME fibres (C_f), and POM (C_{POM}), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	fraction	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	C_{POM} (mg/kg POM)	BSAF
150	Sandy sediment in a ditch opposite of a gas station.	0,325±0,075	5,63±1,09	16.7	C_{11}-C_{12}	n.d.	42,8	13,8	n.d.	n.d.
					C_{12}-C_{14}	6,69	41,3	38,3	n.d.	0,35
					C_{14}-C_{16}	9,33	34,2	26,2	6,29	0,21
					C_{16}-C_{18}	18,2	70,1	n.d.	9,90	0,22
					C_{18}-C_{20}	37,8	192	n.d.	11,8	0,29
					C_{20}-C_{22}	56,1	305	n.d.	16,7	0,31
					C_{22}-C_{24}	57,9	217	15,0	12,8	0,21
					C_{24}-C_{26}	84,0	87,6	40,4	11,5	0,06
					C_{26}-C_{28}	95,9	40,7	32,1	7,41	0,02
					C_{28}-C_{30}	130	26,9	47,0	6,92	0,01
					C_{30}-C_{32}	143	17,7	30,4	2,70	0,01
					C_{32}-C_{34}	114	n.d.	35,8	2,02	n.d.
					C_{34}-C_{36}	105	n.d.	39,8	n.d.	n.d.
					C_{36}-C_{40}	75,6	n.d.	114	0,611	n.d.
					total	934	1160	440	85,8	0,07
					C_{11}-C_{20}	84,1	365	184	25,1	0,24
					C_{11}-C_{34}	767	1240	313	85,1	0,09

[1] not analysed due to insufficient sample. Lipid-normalized BPH concentrations were based on the average value of the other locations (14.4%)
n.a. not analysed
n.d. not detected

Table S2 (continued). Overview of oil field locations, sediment characteristics, worm lipid contents, TPH concentrations in sediments (C_s), BPH concentrations in *Lumbriculus variegatus* (C_b), PDMS-coated SPME fibres (C_f), and POM (C_{POM}), and biota-to-sediment accumulation factors (BSAFs).

location	description	nitrogen (%)	organic carbon (%)	lipid (%)	fraction	C_s (mg/kg dw)	C_b (mg/kg lipid)	C_f (mg/L PDMS)	C_{POM} (mg/kg POM)	BSAF
160	Clayey sediments on a bank of a heavy polluted river due to shipping, adjacent industries, and the outlet of a nearby diesel-powered pumping station.	0,234±0,008	4,37±0,085	12.1	C_{11}-C_{12}	n.d.	n.d.	n.d.	n.d.	n.d.
					C_{12}-C_{14}	9,42	n.d.	39,5	4,92	n.d.
					C_{14}-C_{16}	11,9	82,7	76,6	8,41	0,30
					C_{16}-C_{18}	16,9	290	73,3	9,16	0,75
					C_{18}-C_{20}	28,6	367	71,8	13,1	0,56
					C_{20}-C_{22}	41,8	309	49,4	19,5	0,32
					C_{22}-C_{24}	50,3	242	55,8	18,8	0,21
					C_{24}-C_{26}	73,7	244	46,6	17,2	0,14
					C_{26}-C_{28}	81,0	233	33,3	11,5	0,13
					C_{28}-C_{30}	106	159	52,5	10,3	0,07
					C_{30}-C_{32}	110	129	n.d.	5,11	0,05
					C_{32}-C_{34}	99,2	80,1	33,8	3,77	0,04
					C_{34}-C_{36}	101	n.d.	36,1	1,57	n.d.
					C_{36}-C_{40}	78,3	n.d.	75,0	3,52	n.d.
					total	805	2380	591	126	0,13
					C_{11}-C_{20}	76,3	740	466	34,3	0,42
					C_{11}-C_{34}	640	2440	511	120	0,17

[1] not analysed due to insufficient sample. Lipid-normalized BPH concentrations were based on the average value of the other locations (14.4%)
n.a. not analysed
n.d. not detected

Table S3: Location characteristics

location	compound	exposure (d)	temperature (°C)	depth (m)	Worm recovery (g FW)
2O	oil	28	18,5	0,5	0,37
2P	PAH	28	18,5	0,5	0,26
3P	PAH	28	18,7	0,4	-
3O	oil	28	19,1	0,6	7,77
6P	PAH	28	17,2	0,47	6,77
7O	oil	28	18,8	0,4	4,21
7P	PAH	28	18,8	0,4	4,39
8O	oil	28	15,2	destroyed	-
8P	PAH	28	15,2	destroyed	-
10O	oil	28	15,4	0,25	2,09
12O	oil	28	18,9	destroyed	0,77[1]
12P	PAH	28	18,9	destroyed	
13O	oil	28	14,7	0,1	4,99
14P	PAH	27	13,6	0,35	5,35
	PAH	46[2]	13,6	0,35	
15O	oil	28	15,4	0,4	1,48
15P	PAH	28	15,4	0,4	0,45
16O	oil	28	19,2	0,7[3]	9,04
16P	PAH	28	19,2	0,7[3]	10,56

[1] Although enclosures were flushed away for about 1 m, fibres and some worms were recovered. Worms were used for oil analysis only.

[2] Enclosure was destroyed due to maintenance works after three weeks, but replaced. Fibres from the first exposure were found back and placed back in the enclosure, together with new ones.

[3] Enclosures were placed in a tidal river. Sampling took place at low tide. Water level at high tide was about 1.5 m higher.

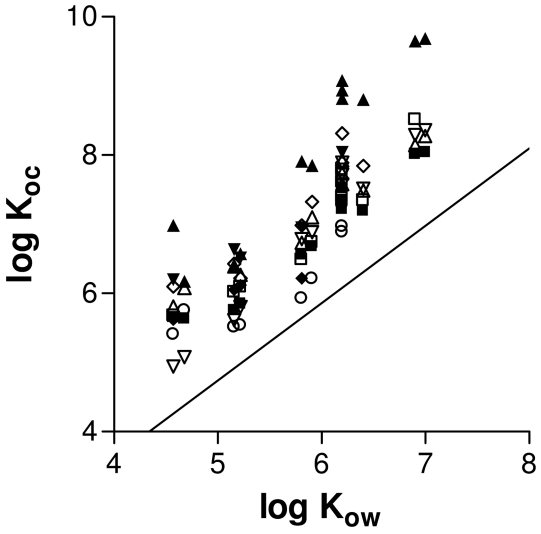


Figure S1. Relationship between in situ SPME-derived organic carbon-water partition coefficients (log K_{oc}) for PAHs and log K_{ow}. The solid line represent log K_{oc}'s derived by linear regression from Nguyen *(1)*. Different symbols represent different locations (■ 2P, ○ 3P, ▲ 6P, ▼ 7P, ▽ 8P, ◇ 12P, ◆ 14P, □ 15P, and △ 16P).

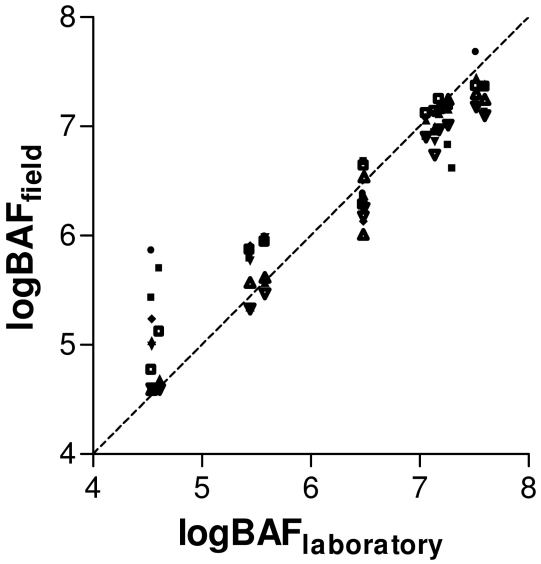


Figure S2. Relationship between laboratory- and field-derived PAH BAF values for Lumbriculus variegatus. Different symbols represent different locations.

(1) Nguyen, T. H.; Goss, K. U.; Ball, W. P. Polyparameter linear free energy relationships for estimating the equilibrium partition of organic compounds between water and the natural organic matter in soils and sediments. Environmental Science and Technology 2005, 39, 913-924.

Samenvatting

Ons consumptiegedrag heeft geleid tot de aanwezigheid van organische verbindingen in het aquatische milieu in concentraties die hoger zijn dan wat acceptabel wordt geacht. Om onaanvaardbare effecten te voorkomen is een gedegen risicobeoordeling van groot belang. Risicobeoordeling bestaat uit het vergelijken van blootstellingsconcentraties met effectconcentraties, waarbij risico's worden verwacht wanneer de eerstgenoemde concentraties hoger zijn dan de tweede. Risico's wordt echter niet bepaald door enkel en alleen de aanwezigheid van verontreinigingen, maar door hoe deze aanwezig zijn. Alleen dat deel dat is opgelost in het water, inclusief het bodemvocht, is beschikbaar voor opname door organismen en vormt daarmee de actuele blootstellingsconcentratie. Onze huidige risicobeoordelingsmethodiek is echter gebaseerd op de totale aanwezigheid van stoffen en richt zich verder alleen op individuele stoffen, terwijl organismen in het milieu worden blootgesteld aan een cocktail van stoffen afkomstig uit meerdere bronnen en/of geloosd als een complex mengsel. Het effect van blootstelling aan mengsels is vaak anders dan dat kan worden verwacht op basis van de effecten van de afzonderlijke stoffen, omdat stoffen elkaars werking kunnen beïnvloeden. Veel organische verbindingen worden echter gekarakteriseerd door een aspecifiek toxiciteitsmechanisme, waarbij het totale effect van een mengsel wordt bepaald door het aantal opgenomen moleculen in het celmembraan. Wanneer dit een drempelwaarde overschrijdt, zal het membraan niet meer optimaal functioneren, wat leidt tot het disfunctioneren van de cel en uiteindelijk tot de dood van het organisme. Uit diverse experimenten is gebleken dat deze

drempelwaarde ligt op 50-200 mmol/kg membraanvet en dat de waarde bruikbaar kan zijn als effectconcentratie voor de risicobeoordeling van complexe mengsels.

Deze waarde is echter gebaseerd op de door organismen opgenomen verontreinigingen en het is dus noodzakelijk om blootstellingsconcentraties te baseren op daadwerkelijk opgenomen gehalten. Opname van stoffen geschiedt via een proces dat bioaccumulatie wordt genoemd. In de huidige systematiek wordt bioaccumulatie geschat op basis van evenwichtpartitieprocessen, waarbij wordt verondersteld dat verontreinigingen zich verdelen over het organisch materiaal in bodems en sedimenten, het (porie)water en vetten in organismen, volgens stofspecifieke verdelings- of partitiecoefficienten. Op basis van voorspelde of gemeten concentraties in bodems en sedimenten worden concentraties in het (porie)water berekend om vervolgens daaruit de door organismen opgenomen hoeveelheden in te schatten. Deze methode is echter niet optimaal, omdat is gebleken dat de parameter die de verdeling tussen organisch materiaal en het (porie)water beschrijft niet alleen stofspecifiek is, maar ook afhankelijk van de samenstelling van het organische materiaal, de “ouderdom” van de verontreiniging en de aanwezigheid van overige matrices waaraan verontreinigingen zich kunnen binden. Omdat verontreinigingen vaak minder beschikbaar zijn voor transport naar het (porie)water dan op basis van evenwichtpartitie wordt aangenomen, kan bioaccumulatie en het daaraan gerelateerde risico worden overschat. De opvatting heerst dan ook dat het direct meten van de blootstellingsconcentraties zal leiden tot meer betrouwbare schattingen van bioaccumulatie en risico’s, omdat hiermee alle factoren worden meegenomen die de “beschikbaarheid” van verontreinigingen bepalen. Voor het inschatten van bioaccumulatie van polycyclische aromatische koolwaterstoffen (PAKs; stoffen die vrijkomen bij (onvolledige) verbrandingsprocessen) zijn inmiddels methoden voorhanden waarmee alleen de actueel beschikbare blootstelingsconcentratie in sedimenten en bodems kan worden bepaald. Anders is dit voor complexe mengsels die bestaan uit vele honderden tot duizenden stoffen zoals olie, die alleen met behulp van ingewikkelde analysemethoden individueel aangetoond en gekwantificeerd kunnen worden. Voor dergelijke mengsels lijkt het eenvoudiger om de risicobeoordeling te baseren op de totale hoeveelheid organische moleculen die beschikbaar is. Deze fractie kan dan vervolgens worden gerelateerd aan bioaccumulatie en worden getoetst aan de eerdergenoemde 50-200 mmol/kg membraanvet. Op dit moment zijn echter geen analytische methoden voorhanden die dit kunnen. Doel van dit proefschrift is dan ook om een bijdrage te leveren aan de verbetering van de risicobeoordeling van complexe mengsels in het aquatische milieu door het verbeteren van bestaande methoden en het ontwikkelen van nieuwe methoden om interne blootstellingsconcentraties (bioaccumulatie) aan respectievelijk PAKs en olie in te schatten.

Hoewel bioaccumulatie een belangrijke rol speelt in de risicobeoordeling, ontbreken vaak betrouwbare inschattingsparameters (zogenaamde “bioaccumulatiefactoren”), omdat deze moeilijk experimenteel zijn te bepalen. Bovendien zijn bioaccumulatiefactoren doorgaans bepaald in het laboratorium, waarin omgevingsfactoren gestandaardiseerd, maar niet representatief voor het veld zijn. In dit proefschrift zijn daarom de effecten van temperatuur, wat wordt gezien als één van

de meest sturende omgevingsfactoren, op de bioaccumulatie van een dertiental PAKs door de aquatische worm *Lumbriculus variegatus* onderzocht (hoofdstuk 2). Tijdens de testen werden de blootstellingsconcentraties bepaald middels "solid phase micro-extraction" (SPME), omdat is bewezen dat deze benadering leidt tot de meest betrouwbare bioaccumulatiefactoren. Omdat temperatuur eveneens invloed heeft op de absorptie van PAKs in de PDMS (polydimethylsiloxane) coating van de gebruikte SPME fibers, is deze invloed eerst gekwantificeerd, zodat het mogelijk was bij elke temperatuur de PAK concentratie in de waterfase nauwkeurig te bepalen. Een verlaging van de temperatuur bleek vervolgens te leiden tot een significante toename van bioaccumulatie van PAKs. Dit effect heeft mogelijk ecotoxicologische consequenties. Uit hoofdstuk 2 is eveneens gebleken dat SPME bruikbaar is als meetmethode om bioaccumulatie van PAKs in aquatische wormen te voorspelen, maar niet middels een directie vergelijking waarbij de SPME fiber wordt beschouwd als een surrogaat voor organismen. Dit omdat het absorptiegedrag bij temperatuurverandering voor organisme en PDMS verschillend is. Daarom is het noodzakelijk om eerst de blootstellingsconcentratie met behulp van SPME te berekenen en vervolgens de concentratie in de organismen. Omdat de effecten van temperatuur op zowel de partitie van PAKs naar PDMS als de bioaccumulatiefactor bekend zijn, is dit nu mogelijk voor een brede temperatuurrange.

Olieconcentraties in sedimenten en organismen worden bepaald door de monsters met een geschikt oplosmiddel te extraheren en de concentraties in het extract te bepalen met behulp van gaschromatografie. Het extract bevat echter ook organische verbindingen zoals humuszuren en vetten, die eveneens worden gedetecteerd op de gaschromatograaf en ten onrechte worden aangemerkt als olie. Omdat het niet mogelijk is om na de analyse de gemeten olieconcentraties te corrigeren voor deze storende componenten, is het noodzakelijk de extracten zodanig op te zuiveren dat deze componenten volledig worden verwijderd. Een veel gebruikte techniek is open kolom chromatografie, waarbij het extract door een kolom met een geschikt adsorptiemiddel wordt geleid om storende componenten en olie te scheiden. Hoewel olieanalyses op sedimenten dagelijkse routine is en in het verleden meermaals bioaccumulatie van olie in aquatische organismen is onderzocht, bestaat er geen consensus over welk adsorptiemateriaal het beste is. Daarom is een studie uitgevoerd (hoofdstuk 3) waarin diverse materialen voor open kolom chromatografie zijn vergeleken. Niet alle storende componenten bleken te worden verwijderd wanneer extracten van sedimenten met het veelgebruikte silica gel, Florisil en aluminium oxide werden opgezuiverd, maar het aandeel hiervan was echter zelfs bij lage olieconcentraties verwaarloosbaar. Zowel Florisil als aluminium oxide, waaraan respectievelijk 5 en 10 % water is toegevoegd, bleken de preferente adsorptiematerialen, omdat minder olie verloren ging (5%) dan bij silica gel. Vet (afkomstig uit organismen) werd echter niet verwijderd met deze materialen, waardoor ze ongeschikt zijn voor het opzuiveren van extracten uit biota. Extracten die werden opgezuiverd met silica gel waaraan 44% zwavelzuur is toegevoegd en vervolgens met silica gel dat basisch is gemaakt door het toevoegen van een 1M natriumhydroxide oplossing (33%) waren daarentegen vrijwel vrij van vet. Volledig vetvrije extracten werden echter verkregen wanneer de extracten eerst door de basische en vervolgens

door de zure silica gel werden geleid. Bovendien werd door het onconventioneel omdraaien van de twee materialen het verlies aan olie gereduceerd tot 10%, omdat kon worden volstaan met minder zwavelzuur (7%). Dit onderzoek heeft dan ook geleid tot een analysemethode, op basis waarvan met zekerheid kan worden gesteld dat de olieconcentraties die in sedimenten en organismen worden gemeten ook daadwerkelijk olie betreffen en niet (deels) storende organische verbindingen.

Hoewel olie een vaak voorkomende verontreiniging is in het aquatische milieu, is er weinig bekend over bioaccumulatie van dit complexe mengsel. Daarom is de opname van olie in *L. variegatus* uit olieverontreinigde sedimenten onderzocht (Hoofdstuk 4), waarbij is gekeken naar opnamesnelheden, welk deel van de aanwezige olie wordt opgenomen en de effecten van sedimentsamenstelling. Oliefracties die worden gekenmerkt door een hoog kookpunt bleken trager te worden opgenomen dan fracties met een laag kookpunt, maar bereikten wel 70-90 % van de evenwichtsconcentratie na 28 dagen. Oliefracties met een hoog kookpunt werden ook moeilijker uit het sediment opgenomen. Dit kan deels worden verklaard doordat bioaccumulatie van hogere kookpuntsfracties niet in evenwicht is met de olieconcentraties in het sediment, maar ook doordat deze fracties mogelijk minder beschikbaar zijn voor opname (bijvoorbeeld door sterke binding aan sediment). Eveneens werd geconstateerd dat wormen in sterk verontreinigde sedimenten verhoudingsgewijs minder olie opnamen. Dit kon worden verklaard doordat niet alle olie kan worden gebonden aan het organisch materiaal in het sediment of kan oplossen in het poriewater. Het resterende deel is aanwezig als oliedruppels of –films, die mogelijk worden vermeden door wormen, zodat de olie daarin niet wordt opgenomen. Het bleek verder niet mogelijk bioaccumulatie te voorspellen vanuit de gemeten concentratie in het sediment, zelfs niet wanneer er werd gecorrigeerd voor de hoeveelheid organisch materiaal. Andere factoren, zoals de samenstelling van het organisch materiaal, de verschijningsvorm van olie, de ouderdom van de olieverontreiniging en de aanwezigheid van additionele substanties waaraan olie zich kan binden, hebben waarschijnlijk eveneens invloed op de beschikbaarheid.

Omdat bioaccumulatie en dus het risico van olie niet kan worden beoordeeld op basis van de gemeten concentraties in het sediment en de hoeveelheid organisch materiaal, is in hoofdstuk 5 gezocht naar een meer nauwkeurig alternatief. Uitgangspunt was het extraheren van de beschikbare fractie. Hoewel diverse methoden voor dit doel voorhanden zijn, is geen van deze methoden eerder toegepast op complexe mengsels, waarin stoffen niet individueel te bepalen zijn. Daarom werden de beschikbare technieken met elkaar vergeleken. De sedimenten zoals gebruikt in hoofdstuk 4 werden hiertoe geëxtraheerd met SPME, polyoxymethylene (POM), Tenax en cyclodextrine. De geëxtraheerde hoeveelheden werden vervolgens vergeleken met de in hoofdstuk 4 gemeten bioaccumulatie. De resultaten lieten zien dat de geëxtraheerde hoeveelheden niet overeen kwamen met de concentraties in wormen en daarom waren correctiefactoren nodig om bioaccumulatie te kunnen voorspelen. Voor zowel SPME, Tenax als cyclodextrine waren deze correctiefactoren afhankelijk van de concentratie in het sediment, hetgeen suggereert dat in sterk verontreinigde sedimenten verhoudingsgewijs grotere hoeveelheden olie beschikbaar zijn. Dit kan worden verklaard doordat deze methoden in staat zijn olie te extraheren uit

oliedruppels en -films die ontstaan bij hogere olieconcentraties, terwijl deze niet beschikbaar zijn voor bioaccumulatie, of omdat de materialen worden besmeurd met olie. Besmeuring treedt niet op wanneer SPME wordt uitgevoerd in head-space modus, waarbij de fibers boven de sediment-waterslurrie worden gehangen. Omdat deze methode echter alleen in staat is vluchtige oliefracties te extraheren, is deze methode niet representatief voor bioaccumulatie van olie door wormen die ook zwaardere componenten opnemen. Alleen in het extract dat met POM was verkregen, kwam het olieprofiel overeen met dat van wormen. Bovendien leek besmeuring de resultaten niet negatief te beïnvloeden, omdat olie zich moeilijk hecht aan het harde en gladde materiaal en eventueel aanwezige oliedruppels en -films makkelijk te verwijderen zijn. Ook voor POM was echter een correctiefactor noodzakelijk om bioaccumulatie in wormen nauwkeurig te voorspellen. Met behulp van de afgeleide factor kon bioaccumulatie binnen een aanvaardbare marge worden geschat. Extractie met POM lijkt daarmee een geschikte methode om bioaccumulatie van olie in te schatten.

Hoewel de resultaten uit hoofdstuk 5 bewijzen dat met analytisch-chemische methoden een betere inschatting van opname van olie in organismen kan worden verkregen dan op basis van concentraties gemeten in het sediment, zijn geen veldstudies voorhanden waarmee de waarden gevalideerd kunnen worden. Daarom is een veldstudie uitgevoerd (hoofdstuk 6) waarin is onderzocht of de in hoofdstuk 5 beschreven POM techniek ook geschikt is om bioaccumulatie van olie in wormen blootgesteld onder veldomstandigheden te kunnen schatten. Tevens is in dit veldonderzoek onderzocht of de in hoofdstuk 2 beschreven SPME methode leidt tot een verbetering van het inschatten van 'in-situ' (in het veld) bioaccumulatie van PAKs, wanneer de gevonden temperatuursafhankelijke correctiefactoren voor partitie naar PDMS en opname door wormen worden toegepast. Hiervoor zijn wormen (*L. variegatus*) in het veld blootgesteld aan een negental PAK-verontreinigde en een negental olieverontreinigde sedimenten verspreid over diverse locaties in midden Nederland. In zowel de PAK- als olieverontreinigde sedimenten werd in-situ de biobeschikbare blootstellingsconcentratie gemeten met behulp van SPME, gelijktijdig met de blootstelling van de wormen. In monsters afkomstig van de olieverontreinigde locaties werd bioaccumulatie eveneens ingeschat middels extracties met POM. Voor PAKs bleken de op basis van SPME voorspelde concentraties in wormen maximaal een factor 4 af te wijken van de gemeten concentraties in wormen, wanneer de in hoofdstuk 2 gevonden temperatuursafhankelijke correctiefactoren werden toegepast. De in-situ toepassing van SPME leidde niet tot een goede schatting van de door wormen opgenomen olieconcentratie, maar bioaccumulatie werd daarentegen binnen een factor 3 voorspeld in geval van POM extracties in het laboratorium. Ook kwam wederom het olieprofiel in POM sterk overeen met dat van wormen. De in hoofdstuk 5 als beste geteste methode blijkt daarmee ook in staat te zijn om bioaccumulatie van olie in het veld adequaat in te schatten.

Dit onderzoek heeft geleid tot een verbetering van de bestaande methode voor het beoordelen van bioaccumulatie van PAKs in wormen door het vaststellen van de relatie tussen temperatuur en opname, in zowel PDMS als wormen. De methode is nu onder meer bruikbaar om interne blootstellingsconcentraties van PAKs in wormen blootgesteld in het veld in te schatten. Met betrekking tot olie heeft het onderzoek

laten zien dat bioaccumulatie sterk afhankelijk is van de biobeschikbaarheid, welke beter bepaald kan worden door het extraheren met POM, dan deze in te schatten op basis van de gemeten concentratie in het sediment. Aannemende dat PAKs en olie hoofdzakelijk een aspecifiek toxiciteitsmechanisme hebben, is het mogelijk om risico's van PAK- en olieverontreinigde sedimenten te voorspellen door de op basis van SPME en POM geschatte interne blootstellingsconcentraties te vergelijken met de eerder genoemde effectconcentratie van 50-200 mmol/kg membraanvet. Tijdens het veldexperiment zijn op een aantal PAK-verontreinigde locaties geen wormen teruggevonden. Met SPME zijn echter geen interne concentraties boven deze drempelwaarde voorspeld (maximaal 1.8 mmol/kg), zodat de waargenomen sterfte eerder lijkt samen te hangen met de aanwezigheid van andere verontreinigingen of abiotische factoren. De maximaal voorspelde olieconcentratie in wormen blootgesteld onder veldomstandigheden was 75 mmol/kg, maar sterfte werd in deze sedimenten echter niet waargenomen. Hoewel dit onderzoek suggereert dat POM bruikbaar is als methode om risico's van olieverontreiniging in te schatten, is de techniek dus nog niet geverifieerd op sedimenten die wel toxisch zijn voor wormen. Samenvattend kan desalniettemin gesteld worden dat SPME en POM waardevol kunnen zijn bij onder meer risicobeoordelingen tijdens het saneren van verontreinigde sedimenten en bodems, het terugdringen van proefdiergebruik, het vaststellen van parameters die van belang zijn voor de risicobeoordeling en het vaststellen of en welke verontreinigingen verantwoordelijk zijn voor eventueel waargenomen effecten. De in dit proefschrift beschreven methoden kunnen daarmee naar verwachting een prominente rol gaan spelen in de toekomst, hoewel enkele obstakels nog zullen moeten worden overwonnen. De weg naar implementatie is echter ingeslagen.

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Barry

Publications

Publications

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Curriculum vitae

Barry Muijs was born on 10 November 1974 in Amsterdam, The Netherlands. He graduated from the Scholengemeenschap Het Baken in Almere, The Netherlands in 1993 and started studying Biology at Vrije Universiteit in Amsterdam, The Netherlands in the same year. As a part of his study, he did two research projects both focused on ecotoxicology: Applicability of bioassays to assess ecotoxicological risks at Vrije Universiteit supervised by Kees van Gestel, and degradation and toxicity of PAHs in dredged sediments disposed on land at the National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, supervised by Herman Eijsackers. After obtaining his Masters in Biology in 1998, Barry became an environmental consultant and specialist at Witteveen+Bos, Deventer, The Netherlands were he was involved in innovative and interdisciplinary projects about risk assessment and remediation of polluted soil and sediment. In 2004 he started his PhD-research that resulted in this thesis. Barry is currently employed as an environmental risk evaluator at the Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb) in Wageningen, The Netherlands.