Sequestration and bioavailability of hydrophobic chemicals in sediment

Sequestratie en biobeschikbaarheid van hydrofobe chemicaliën in sediment

(met een samenvatting in het Nederlands)

Proefschrift

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Voor mijn ouders

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[1]citaat huisgenoot John Raprager, Brisbane, overwoog een wetenschappelijke carriere, maar werd private investigator
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Chapter 1 Introduction

Soils of water bodies as estuaries, rivers and lakes, also called sediments, have been polluted with hydrophobic organic contaminants worldwide (e.g. [1]). Hydrophobic organic contaminants (HOC) are pollutants that are poorly soluble in water. As a result, HOC in water sorb to sediment particles. Due to this behaviour and to their persistence, HOC strongly accumulate in sediments. 'Hot spots', or highly polluted locations, are found in harbours and sedimentation areas of (formerly) heavily polluted rivers, such as the Ketelmeer and the Western Scheldt in the Netherlands.

In the past, sediments were regarded as waste depots in which pollutants were safely locked away. Nowadays, sediment pollution is recognized as a potential risk to ecosystems. Organisms living in or depending on the sediment, the so-called benthic organisms, may accumulate the HOC and life-functions may be threatened. In addition, HOC may be passed into food chains, for example the worms-flatfish or midge (larvae) - bats food chain (e.g. [2]).

Of all organisms that are dependent on sediment as a habitat, deposit-feeders, such as oligochaete or polychaete worms and amphipods (see Figure 1), are probably at highest risk. Deposit-feeders are organisms that feed on settled, deposited sediment particles. In addition to uptake from pore water, this type of organisms is exposed to pollution by ingestion of sediment particles and sediment-bound contaminants. Therefore, the life-style of deposit-feeding organisms represents a worst-case scenario in which organisms are in very close contact with their polluted surroundings. In addition, deposit-feeders are of interest as they form lower parts of food chains.

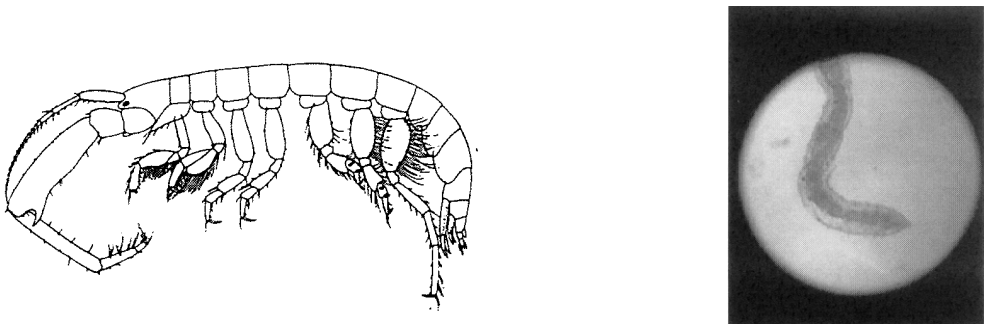

Fig. 1 Examples of deposit feeding organisms: Amphipod *Corophium volutator* (left) and oligochaete *Tubifex tubifex* Müller (right) (photograph: Demidov, Yaroslavl State University).

In order to assess the risk of HOC in sediment to deposit-feeders, we need to know how much HOC are accumulated. Measurements of the accumulation of HOC in benthic organisms have shown that the accumulation cannot be simply estimated from the concentration of HOC in the sediment. The accumulation of HOC in a sediment may be up to 1000 times higher or lower than the accumulation in another sediment with equal concentrations of HOC (e.g. [3]). These differences in accumulation at a given concentration in the sediment, also expressed at the biota to sediment accumulation factor or BSAF, are caused by differences in the so-called bioavailability (see Explanation of key words, page 2) of HOC to benthic organisms.

As it is very time-consuming and expensive to collect and analyze organisms from all sediments for which a risk-assessment is required, models are developed to predict the bioaccumulation. These models should be based on fundamental understanding of the biological, chemical and ecological processes that are involved in bioaccumulation. One model that is frequently used in risk-assessment is the equilibrium partitioning model (EqP) (see Explanation of key words). The EqP assumes constant bioavailability of HOC in sediments and predicts a constant BSAF-value. In practice however, BSAF-values are highly variable [3, 4].

One of the factors that initially seemed to cause these differences in BSAF, was the contact-time between chemical and sediment. Some studies indicated a decline in bioavailability in the period after addition of test-compounds to sediments or soils in the laboratory (e.g. [5], [6]). Other studies demonstrated that the bioavailability in field sediments or soils, with typical contact-times of years to decades, was lower than expected from laboratory experiments in which chemicals were freshly added to sediment (e.g. [7],[8]). Both results suggested that the risk of HOC in field sediments was lower than expected and would even continuously decrease in time. Therefore, these results raised a lot of attention from policy makers, industries and other parties involved.

In the research project that is presented in this thesis, we tried to gain deeper understanding of the interaction of processes that determine bioavailability of HOC to deposit-feeders. With these new insights, a refined model for the estimation of the BSAF was developed. The chemical parameters in the model should be experimentally accessible. Also, we required that the model be based on understanding of fundamental processes to ensure broad applicability. Literature suggested that the formation of slowly desorbing fractions, also called sequestration (see Explanation of key words), reduced the bioavailability and biodegradation of HOC in sediments (e.g. [9-12]). Some authors reported an increase of slowly desorbing fractions in time (e.g. [13]). We started with the hypothesis that a reduction of bioavailability in time is related to a decrease in rapidly desorbing fractions and that contact-time is a main determinant of bioavailability. In the first part of the project we investigated the effect of contact-time on the bioavailability of HOC to deposit-feeders (Chapter 2 and 3). Simultaneously, we studied the distribution of chemicals over rapidly, slowly and very slowly desorbing fractions in the sediment.

The results prompted us to abandon this hypothesis. It seemed that contact time was not a major determinant of bioavailability. Therefore, we focused on chemical desorption processes instead. We investigated if rapidly desorbing fractions were related to the bioavailability for deposit-feeders (Chapter 2, 3) and studied the causality between the two (Chapter 4). By doing so, we got closer to our original goal of understanding basic processes leading to bioaccumulation. In addition, we tried to unravel the relationship between rapidly desorbing fractions and bio-accumulation by investigating the distribution of HOC between rapidly desorbing sediment compartment, pore water and organism. A recently developed technique was applied to measure freely dissolved concentrations of HOC in the pore water (Chapter 5).

The results led us to modify the EqP concept. Finally, we propose practical approaches of assessing the bioaccumulation of HOC to benthic deposit-feeders.

Explanation of the key words sequestration, bioavailability and EqP

Sequestration

Research on the desorption of hydrophobic organic contaminants from soil or sediment has demonstrated that the kinetics of this process are complex. When soil or sediment is suspended in clean water, an initial stage of rapid desorption (half-time ~ hours) is usually followed by a stage of slower desorption (half-time ~ hours/days or longer) (e.g. [13, 14]). The formation of a slowly desorbing fraction, besides a rapidly desorbing fraction, has been called sequestration [15]. Various research groups tried to elucidate the nature of sequestration. The mechanistic explanations of sequestration that were developed were mainly derived from indirect experimental observations: Because of the small molecular scale of the interactions involved and the 'hidden' character in the sediment/soil particles, the phenomenon of sequestration is 'invisible'. Two mechanisms have been put forward to explain slow desorption [14]: activation energy at specific interaction sites and diffusional limitations. The first explanation is based on high activation energy of desorption at specific loci where contaminant molecules can intensively interact with the surrounding organic matter or pores. Some authors suggested anomalous sorption to small amounts of high-surface area carbonaceous material (HSACM) ([16]) or soot ([17, 18]). The second explanation, diffusional limitations, is also located in pores and/or in the organic matrix [14]. In pores, diffusion might be limited by chromatographic-like interactions with the pore-walls. In the matrix, desorption might be slow from regions where the organic matter has condensed and rigid properties with low diffusivity ('glassy phase'). Diffusion might also be limited as a consequence of long pathways at remote sites of the bulk organic matter.

In the present thesis, the distribution over complementary rapidly, slowly and very slowly desorbing fractions, following the approach of Cornelissen and co-authors *[13]*, is used as a measure for sequestration.

Bioavailability

In the past, risk assessment of HOC was based on total concentrations of HOC in the soil or sediment, i.e. concentrations obtained after a rigorous extraction. These concentrations were found to be poor predictors of bioaccumulation and toxicity. This prompted researchers to widen the focus of risk-assessment to the interaction between chemicals, soil or sediment and biota. The term bioavailability was introduced to address these interactions. A review on factors that influence bioavailability has been given by Belfroid et al. [19]. Generally, bioavailability has been defined in the literature as the total concentration of chemicals in soil or sediment that is or will potentially be taken up by an organism. The apparent clarity of this general definition is obscured by different definitions of uptake. Some authors define uptake as 'uptake in the target-tissue, others as uptake in the whole organism, while others explicitly exclude gut-contents. A more elaborate overview of the definitions of bioavailability can be found in Belfroid et al. [19].

The present thesis focuses on *differences* in bioavailability, e.g. differences in bioavailability between sediments, treatments and compounds. Differences in bioavailability can be measured by differences in bioaccumulation. Bioaccumulation is expressed in this thesis as the steady state accumulation into the organism divided by the concentration in the sediment, normalised to lipid and organic carbon content, or the ‘biota to sediment accumulation factor’ (BSAF). The BSAF can be described with the following equation:

$$BSAF = C_b/C_{sed} \qquad (1).$$

with BSAF is the biota to sediment accumulation factor (kg organic carbon/kg lipid organism), C_b is the concentration in the organism lipid at steady-state (µg/kg lipid) and C_{sed} is the concentration in the soil or sediment organic carbon (µg/kg organic carbon). In conclusion, the present thesis addresses differences in bioavailability, which will be measured by differences in BSAF.

The equilibrium partitioning theory (EqP)

The EqP has been put forward by Shea [20] and Di Toro et al [21] as a method to estimate bioaccumulation of nonionic organic chemicals in sediments. The EqP describes the distribution of these chemicals between sediment and biota with three compartments: biota, sediment organic carbon and pore water (Figure 1). The model assumes that the pore water and

the organic carbon are in equilibrium, and that the distribution over organic carbon and pore water can be described by a constant partition coefficient K_{oc} (L/kg organic carbon):

$$K_{oc} = C_{sed}/C_{pore\ water} \qquad (2)$$

with $C_{pore\ water}$ is the concentration in the pore water (µg/L).

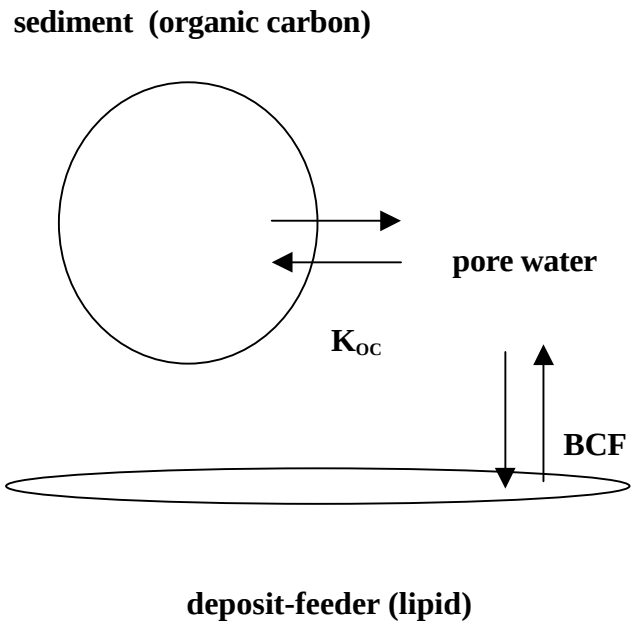

Fig. 1 Model of distribution of hydrophobic organic chemicals in sediments according to the equilibrium partitioning model (EqP). K_{oc} = partition coefficient between sediment and pore water (l/kg organic carbon); BCF = bioconcentration factor (L/kg lipid).

In addition, it is assumed that the fugacities in the pore water and the biota are equal. This means that at steady state, accumulation from the sediment in the organism would be equal to accumulation from pore water exposure only. If it is assumed that the partition coefficient between biota and pore water is constant, this assumption can be written as:

$$BCF = C_b/C_{pore\ water} \qquad (3)$$

with BCF is the constant bioconcentration factor (L/kg lipid).

Combination of equation (1), (2) and (3) results into:

$$BSAF = BCF/K_{oc} \qquad (4).$$

In conclusion, the EqP is a method to describe the bioaccumulation of non-ionic organic chemicals from sediments. The model assumes that partitioning between sediment organic carbon and pore water is at equilibrium, with constant partition coefficient K_{oc}, and that the BSAF can be calculated as the bioconcentration factor (BCF) divided by the $K_{oc.}$

Chapter 2. Bioavailability of lab-contaminated and native Polycyclic aromatic hydrocarbons to the amphipod Corophium volutator relates to chemical desorption.

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ABSTRACT - In the present study, the relationship between bioavailability of Polycyclic aromatic hydrocarbons (PAHs) to benthic amphipods and the PAH desorption kinetics was examined. To that end, field contaminated sediment was treated in three different ways. One subsample had no addition of PAHs and contained native PAHs only. To a second subsample, 6 PAHs (phenanthrene, fluoranthene, anthracene, pyrene, benzo[*b*]fluoranthene and benzo[*k*]fluoranthene) were added in the laboratory. Two of the PAHs were added at higher concentrations to a third subsample, serving as a control for concentration dependent uptake. Marine amphipods (*Corophium volutator*) were exposed to the three subsamples for a maximum of 25 days and were subsequently analysed. Desorption kinetics were determined for both the lab-contaminated and the native PAHs. The Biota to sediment accumulation factor (BSAF) values of the individual native and lab-contaminated PAHs correlated well with the rapidly desorbing fraction (R^2 = 0.76). Biota to sediment accumulation factors were 1.4 to 3.3 higher for the lab-contaminated PAHs compared with the native PAHs, while the difference between the rapidly desorbing fractions was a factor of 1.1 to 1.8. Biota to sediment accumulation factors of the lab-contaminated PAHs in the second and third subsample were equal indicating concentration independent accumulation. The results suggest that lab-contaminated PAHs are more available to amphipods than native PAHs and that differences in bioavailability of lab-contaminated and native PAHs to marine amphipods are related to differences in desorption behaviour.

INTRODUCTION

Measurements of total concentrations of hydrophobic organic compounds in soil or sediments are often inadequate to assess bioavailability and toxicity to organisms. It has been observed that bioavailability and toxicity are inversely related to contact time between

compound and soil or sediment even if total concentrations do not change (e.g. [5, 6, 22, 23]). This phenomenon has been attributed to sequestration. Sequestration was first postulated as an explaining mechanism for slow desorption and non-equilibrium partitioning behaviour over prolonged periods of time (months to years) (e.g. [24-26]). According to the proposed mechanism, hydrophobic chemicals become sequestered by interacting with high affinity pores of subnanometer dimension in the glassy polymer phase of the organic matter or with narrow pores [24]. The interaction is predominantly located in the organic matrix [26]. As the specific interactions might take place at less accessible sites in the organic matter matrix, the sequestration process might be far from instantaneous. For example, Landrum et al. [5] observed a continuous shift in the partition coefficient of two PAHs over a period of 6 months after lab-contamination. More recently, several authors related chemical sequestration to a decrease in bioavailability ([5, 11, 25, 27-29]).

The limited value of total concentration measurements for risk assessment has prompted researchers to develop efficient methods to assess site specific bioavailability. Several authors [5, 22, 30-32] showed a relationship between measured pore water concentrations and the accumulation of hydrophobic organic chemicals in organisms. Weston and Mayer [33] determined the extraction of PAHs by gut fluid that was collected from a polychaete worm and correlated the extraction efficiency with the accumulation. Kelsey et al. [34] showed that the extractability of freshly added phenanthrene and atrazine with selective mild solvents approximated the percentage uptake by earthworms or bacterial degradation. Lamoureux and Brownawell [9] demonstrated a correlation between the desorption kinetics of PAHs and linear alkylbenzenes and accumulation in a deposit feeding clam.

In this study, we investigated whether PAH accumulation is related to the rapidly desorbing fraction. The rapidly desorbing fraction is the fraction that desorbed with a relatively fast rate (0.33 to 3.10 hr^{-1}). The rapidly desorbing fraction was determined from measurements of the desorption kinetics [13]. We compared the accumulation of native PAHs and PAHs that were added in the laboratory (‘lab-contaminated PAHs’) in marine amphipods with experimentally determined rapidly desorbing fractions. By this approach, the relationship between PAH accumulation and the rapidly desorbing fraction was tested on 1) seven different PAHs and 2) lab-contaminated versus native PAHs.

EXPERIMENTAL APPROACH

Amphipods

Marine amphipods (*Corophium volutator*) were collected from an intertidal mudflat (Oesterput) located in the Oosterschelde, a relatively unpolluted estuary in the southwestern

part of The Netherlands, by sieving the upper layer of the sediment over a 500 µm sieve. The organisms were transported to the laboratory and transferred to 10 L jars containing a 3 cm layer of clean, sieved sediment (Oesterput) and sand filtered estuarine water. The organisms were acclimatised for two days to the same salinity (approx. 32 mg/L), temperature (15 °C) and light conditions (24 h light) as used in the experiments.

Chemicals

Fluoranthene (98 %), pyrene (99 %), benzo[*b*]fluoranthene (99 %), benzo[*k*]fluoranthene (98 %), 7-methylbenzo[*a*]pyrene (98 %) were purchased from Aldrich Chemical (Steinheim, Germany). Phenanthrene, anthracene (99+%) and 2-ethylanthracene (98 %) were obtained from Sigma (St Louis, MO, USA). Octadecyl (C-18) was purchased from JT Baker (Phillipsburg, NJ, USA). Tenax TA (60-80 mesh; 177-250 µm) was obtained from Chrompack (Bergen op Zoom, The Netherlands). Florisil was obtained from Merck (Darmstadt, Germany) and Alumina was purchased from ICN Biomedicals (Eschwege, Germany).

Sediment sampling and lab-contamination

Sediment was collected at an intertidal mudflat (Kappellebank), located in the Westerschelde (51° 27'N, 3°58' E), a polluted estuary in the south-west of the Netherlands. Sediment was homogenised on a rolling machine. The organic carbon content was determined to be 2.1 %, using an element analyzer (Carlo Erba NA 1500, Milan, Italy) after removal of carbonates with phosphoric acid. Prior to lab-contamination, the batch of sediment was split into three portions that were subsequently suspended with natural filtered seawater (ratio 0.5 L/kg wet sediment) in order to facilitate homogenisation. Each portion was lab-contaminated by dropwise addition of the acetone solution. During the lab-contamination procedure, the suspension was vigorously mixed by a concrete mixer. The portion named field + additive was spiked with a mixture of PAHs (phenanthrene, anthracene, fluoranthene, pyrene, benzo[*b*]fluoranthene and benzo[*k*]fluoranthene) in acetone (5 ml/ L slurry, final ratio 1 ml/ 60 g dry weight). The same test compounds were added to a second subsample (field + extra additive) but this subsample differed from the field + additive treatment by nominal increments in the concentrations of two of the PAHs (anthracene and benzo[*k*]fluoranthene). The subsample called field received a solvent additive only and contained only native PAHs. The suspensions were allowed to equilibrate at 4 °C for 6, 6 and 7 days for the field + additive, field + extra additive and field batch respectively.

Exposure

After the equilibration time, 36 3 L beakers were filled with 1 kg of the sediment suspensions with a sediment:water ratio of 2:1 by weight and 2 L of natural filtered seawater. Three days later, 300 amphipods were added to each beaker and exposed for 1, 4, 7, 12, 19 and 25 days (field and field + additive) or 1, 4, 7 and 13 days (field + extra additive). The experiments were carried out in duplicate, so there were two beakers for each time point for eacht treatment. Before the start of the accumulation experiment, 2 groups of 300 unexposed amphipods were sampled for duplicate analyses. Also, duplicate beakers without amphipods (field and field + additive) were examined 25 days after the start of the exposure period. Overlying water in all beakers was aerated mildly to prevent the oxygen content from dropping below the 70 % saturation level. Only beakers without any observed increase in mortality were analysed. All amphipods were sampled and counted to determine mortality, washed with demineralized water, dried on a filter paper and frozen for further analysis. At each sampling day, the dry weight was determined on two subsamples of 10 individuals each. At the beginning of the experiment and after 19 days, subsamples of amphipods were set aside for lipid determination according to Folch et al. [35]. At each sampling day, Total Suspended Solids (TSS) in overlying water of field and field + additive samples were determined gravimetrically after filtration of 100 ml. Pre-weighed and pre-ashed (500 °C) glass fibre filters (Type GF/C Whatman, 1 µm nominal pore size) were dried at 50 °C and weighed after 24 h. Total Suspended Solids is a measure of bioturbation and is as such indicative of the activity and stress of the amphipods [36].

Desorption experiment

At the beginning of the exposure period, desorption characteristics were studied in lab-contaminated and sediments that were not lab-contaminated according to [13]. An equivalent of 4 g dry weight sediment was shaken for 8 days with 100 mL milli-Q water and 0.8 g Tenax TA (60-80 mesh; 177-250 µm). The tenax effectively removes solutes from the aqueous phase. During the desorption experiment the Tenax beads were replaced at 10 predetermined time points. In order to exceed the analytical detection limit for the desorption study with the field sediment, three batches of sediment were desorbed and the extracts of the Tenax beads were pooled. All desorption experiments were carried out in duplicate with a sediment sample that was pooled from two beakers. The influence of amphipods on the desorption kinetics was investigated by desorption experiments performed with field + additive sediments at the end of the exposure time. One pooled sediment sample was populated with amhipods for 25 days, the other pooled sample was not. The rapidly desorbing fractions in these sediments were compared with the rapidly desorbing fraction at the start of the exposure period to check for changes during the exposure period.

Analyses

Amphipods

Amphipods were extracted using Matrix Solid Phase Dispersion [37]. Samples of amphipods were ground in a mortar. Internal standard, 2 g octadecyl (C-18) per 0.5 g wet weight tissue and 1 ml methanol were added. The mixture was homogenised with a pestle. A disposable 20 ml syringe (Becton Dickinson, Drogheda, Ireland)) was filled with cleaned glasswool and activated florisil (2 g florisil per 0.5 g wet weight tissue). The amphipod-C18-homogenate was carefully transferred to the syringe which was subsequently eluted with 18 ml acetonitrile per 0.5 g wet weight tissue. In a separate experiment, recovery of the individual PAHs in oligochaetes was 101-113 %. The recovery was determined by adding a known amount of PAHs in acetonitrile to the sample prior to the extraction and clean-up procedure and determination of the amount of PAHs recovered in the final extract. The eluate was concentrated to approximately 0.2 - 2 ml under a gentle flow of nitrogen. Recovery of the internal standard was 99.9 and 89.7 for 2-ethyl-anthracene and 7-methyl-benzo[*a*]pyrene respectively, with a coefficient of variation of 3.0 and 4.8 respectively (n=33). Concentrations of phenanthrene, anthracene, fluoranthene, pyrene and benz[*a*]anthracene were corrected with the recovery of 2-ethyl-anthracene and concentrations of benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene were corrected with the recovery of 7-methyl-benzo[*a*]pyrene.

Sediment and Tenax

The dry weight of the sediment was determined by drying for 24 h at 70°C to constant weight. Sediment samples (field and field + additive) were Soxhlet extracted with hexane and acetone (9/1 vv) for 6 hours. The extract was concentrated to 10 ml in a Kuderna Danish set-up, eluted over an alumina column (15 % deactivated) with petroleum ether and transferred to acetonitrile by concentration to approximately 1 ml, addition of 9 ml acetonitrile, and concentration. Results were corrected for the recovery of the internal standard PCB 103 (2,2',4,5',6-pentachlorobiphenyl) which exceeded 80 % in all analyses. Field + extra additive sediments were extracted for 4 hours with hexane and methanol (1/1) using a heated reflux system. The extract was shaken with Na_2SO_3 and 30 ml water to remove sulphur and enhance phase separation of the hexane. After centrifugation (10 min, 1000 rpm) the hexane layer was removed, concentrated to 1 ml under a gentle flux of nitrogen, transferred to a pre-rinsed 3 ml silica gel spe column (J.T. Baker, Phillipsburg, NJ, USA) and eluted with 8 ml hexane. The first ml of eluate was discarded and the remainder of the eluate was collected. Compounds were transferred to acetonitrile by concentration of the eluate to approximately 1 ml, addition of 9 ml acetonitrile, and concentration to approximately 1 ml. Each portion of Tenax was extracted by shaking with 20 ml hexane, and

compounds were transferred to acetontrile prior to analysis with HPLC. All extracts were analysed by reversed phase HPLC with fluorescence detection [38]. The PAH mixture was separated on a C18 column using an acetonitrile-water gradient. Chromatograms were recorded on a personal computer using data acquisition software. Polycyclic aromatic hydrocarbon identification was performed using retention times.

Data analysis

The accumulation in the amphipods was described using the model proposed by Landrum [39]:

$$\frac{dQ}{dt} = k_s C_s e^{-\lambda t} - k_d Q \qquad (1)$$

where Q = analyte concentration in amphipod (µg/kg wet weight), t = time (d), k_s = uptake clearance coefficient (kg * kg^{-1} * d^{-1}), C_s = concentration in sediment (µg/kg dry sediment), k_d= elimination rate constant (d^{-1}) and λ = rate of reduction in the bioavailable concentration (d^{-1}). This model describes first order kinetics of accumulation and incorporates a first order decline in the exposure conditions. It is assumed that the coefficients k_s, k_d and λ remain constant over the course of the experiment. Although the meaning of λ is unclear, it will be included as a fit parameter in our calculations. The differential equation was integrated, accounting for a non-negligible concentration of the analyte in the amphipods at time = 0:

$$Q(t) = \frac{k_s C_s^0}{k_d - \lambda} \cdot (e^{-\lambda t} - e^{-k_d t}) + Q(0) \cdot e^{-k_d t} \qquad (2)$$

The experimental data were fitted to Eq. 2 by non-linear regression, using Graphpad, version 2.01 (GraphPad Software, San Diego, Ca, USA) using k_s, k_d and λ as adjustable parameters. Because of some scatter in the data and the fact that three parameters had to be estimated in a fitting procedure with only 10 degrees of freedom, some parameter estimations had a high standard deviation. Therefore, we only used the parameter estimations to calculate a kinetic BSAF: the ratio of k_s and k_d, normalized to the lipid content of the amphipods and organic carbon content of the sediment:

$$BSAF = \frac{k_s}{k_d} \cdot \frac{f_{oc}}{lc} \qquad (3)$$

where BSAF = Biota to sediment accumulation factor (kg organic carbon/kg lipid), f_{oc} = organic carbon content of sediment (%) and lc = lipid content amphipods (% wet weight). It can be derived from Equation (2) that the kinetic BSAF in Equation 3 is equal to the lipid normalized concentration in the amphipods, divided by the organic carbon normalized concentration in the sediment at steady-state (t=∞) and at λ = 0 (4). The kinetic BSAF therefore reflects a maximum BSAF

$$\frac{Q(\infty)}{C_S^0} = \frac{k_s}{k_d} \qquad (4).$$

The data of the desorption experiment were analysed according to Cornelissen et al. [13]. In short, amounts of PAHs in the sediment in time were calculated from initial amounts and the desorbed amounts of PAHs that were trapped by the tenax beads in the different time intervals. The desorption from sediment was then described with

$$S_t / S_o = F_{rap}\, e^{-k_{rap} t} + F_{slow} e^{-k_{slow} t} + F_{very\ slow} e^{-k_{very\ slow} t} \qquad (5)$$

in which S_t and S_0 (µg) are the sediment-sorbed amounts at time t (h) and at the start of the experiment, respectively; F_{rap}, F_{slow} and $F_{very\ slow}$ are the fractions of compound present in the rapidly, slowly and very slowly desorbing sediment compartment at time zero, respectively; and k_{rap}, k_{slow} and $k_{very\ slow}$ (h^{-1}) are the rate constant of rapid, slow and very slow desorption, respectively.

The amounts of PAHs present in the aqueous phase were considered negligible compared with the amount in the rapidly and slowly desorbing phases. Values of F_{rap}, F_{slow}, $F_{very\ slow}$, k_{rap}, k_{slow} and $k_{very\ slow}$ were determined by minimalizing the cumulative squared residuals between experimental and calculated values of ln (S_t/S_0) in Equation (5), using Scientist (MicroMath Scientific Software, Salt Lake City, UT, USA). It is assumed that sediment-associated chemicals reside in either the rapidly or the slowly and very slowly desorbing compartment, so that $F_{rap} + F_{slow} + F_{very\ slow} = 1$.

RESULTS

General observations

In the design of the experiment we tried to avoid any toxic effect on the test organisms in the lab-contaminated sediment, as this might influence accumulation. The accumulated

amounts of PAHs in the field + additive and field + extra additive sediment were indeed well below (about 30 to 150 times) the lethal body burden of 1-5 mmol/kg for narcosis. Average survival of the amphipods varied between 91 and 58 % in the field sediment and between 89 and 66 % in the field + additive sediment, and was above 75 % except for the last time point (t=25 days) (see Table 1). No differences among the treatments (t-test, $p<0.05$) were observed in either survival, dry weight, lipid content or total suspended solids (TSS), resulting from the bioturbation of the amphipods, among the treatments (field, field +additive, field + extra additive) (t-test, $p<0.05$). Hence, it can be concluded that toxicity and differences in toxicity between treatments are negligible. The absence of toxicity in our experiments means that the accumulation kinetics were not influenced by toxic effects.

Table 1. Mean percentage survival, mean dry weight in mg and mean lipid content in % w/w of the amphipod *Corophium volutator* in the field and field + additive sediments.

Days of exposure	Percentage survival[1]		Dry weight[2] (in mg/individual)		Lipid content[1] (in % w/w)	
	Field	Field +additive	Field	Field +additive	Field	Field +additive
0					0.82 (0.89; 0.75)	0.82 (0.89; 0.75)
1	91	89	ND	ND		
4	84	83	1.25 (±0.30)	1.42 (±0.10)		
7	86	84	1.24 (±0.32)	1.19 (±0.07)		
12	76	86	1.67 (±0.10)	1.52 (±0.44)		
19	75	76	1.47 (±0.15)	ND	0.79 (0.81; 0.76)	0.92 (0.71; 1.12)
25	58[3]	66	1.59 (±0.42)	1.22 (±0.16)		

[1]number of replicates = 2, (between brackets: first number is the result of the first, the second of the second determination [2]number of replicates = 4 (2 groups of 15 individuals per beaker, two beakers), standard deviation is given in parentheses; [3]based on one replicate; ND = not determined

Average measured concentrations of the PAHs in the three subsamples are shown in Table 2. The lab-contamination of the field + additive subsample resulted in concentrations of the lab-contaminated test compounds that were, on average, 13 times higher than the native background concentrations (see Table 2). Therefore, the native portion of the test compounds is considered negligible compared to the portion that was added in the laboratory. The field + extra additivebatch of sediment had a nominal concentration that was equal to the field + additive sediment for four of the PAHs (phenanthrene fluoranthene, pyrene and benzo[*b*]fluoranthene) and about three times higher for two PAHs (anthracene and benzo[*k*]fluoranthene). The total concentration of testcompounds was only a factor of 1.35 higher in the field + extra spikebatch compared to the field +additive batch (see Table 2). The concentration did not significantly change during the course of the exposure period in beakers with or without amphipods (data not shown, t-test, $p<0.05$).

Table 2. Mean concentrations of PHE = phenanthrene, ANT = anthracene, FLU = fluoranthene, PYR = pyrene, B*a*A = benz[*a*]anthracene, B*b*F = benzo[*b*]fluoranthene, B*k*F = benzo[*k*]fluoranthene, B*a*P = benzo[*a*]pyrene in field sediment (sediment from the estuary Westerschelde, the Netherlands (51° 27'N, 3° 58' E), field + additive and field + extra additive (µg/kg dry sediment).

	PHE	ANT	FLU	PYR	B*a*A[1]	B*b*F	B*k*F	B*a*P[1]	Sum
Field[2]	67 (70;63)	24 (25;22)	132 (136;128)	115 (119;111)	54 (54;54)	93 (97;89)	44 (46;42)	67 (69;65)	596
Field+ additive[3]	773 (114)	306 (51)	2000 (241)	1996 (318)	100 (9)	1005 (150)	575 (85)	129 (11)	6884
Field+ extra additive[2]	1112 (1168; 1055)	957 (976; 937)	2365 (2454; 2277)	2100 (2142; 2277)	98 (101; 95)	939 (956; 922)	1623 (1662; 1585)	138 (141; 136)	9332

[1] Not added to any of the sediments. [2]Mean of concentration at start of the exposure period (between parentheses: first number is the result of the first determination, the second of the second determination) [3]Mean of measured concentration at start and end of the exposure period in beakers with and without amphipods. Standard deviation is given in parentheses.

Accumulation in amphipods

Figure 1 shows the typical uptake profiles of anthracene and benzo[*k*]fluoranthene as representative test compound in *Corophium volutator* in field and field + additive sediment. Two phases are observed. In the first phase, a maximum is being reached within a few days of the exposure time. It is followed by a conspicuous decline in internal concentrations. The rate of the decline is higher for the three ring PAHs phenanthrene and anthracene than for the larger PAHs. The solid line in Figure 1 is the best fit of the data to Equation 2. The fit resulted in parameter estimations of k_s, k_d and λ In Figure 2 the estimates of kd, ks and λ with their standard errors are plotted against log K_{ow}. The values of λ were determined with high precision. High standard errors have been found for the individual estimates of k_d and k_s of the less hydrophobic PAHs because these compounds reach the maximum value of concentration in the amphipods so rapidly that k_s and k_d could not be estimated with high precision. For our main goal of estimation of the kinetic BSAF this is of minor importance. The kinetic BSAF, calculated from the ratio of k_s and k_d, is primarily determined by the maximum concentration in the amphipods. Since the maximum level of accumulation in the amphipods can be accurately described by the model fit (Figure 1), it can be concluded that our approach is valid for estimating the kinetic BSAF.

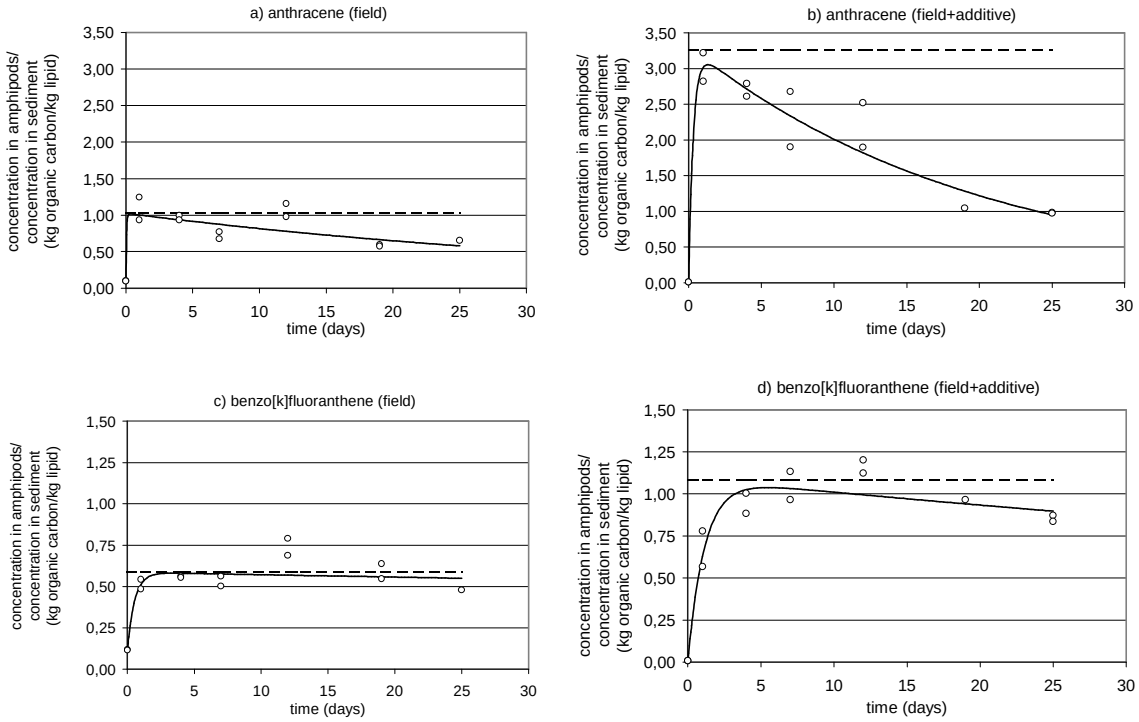
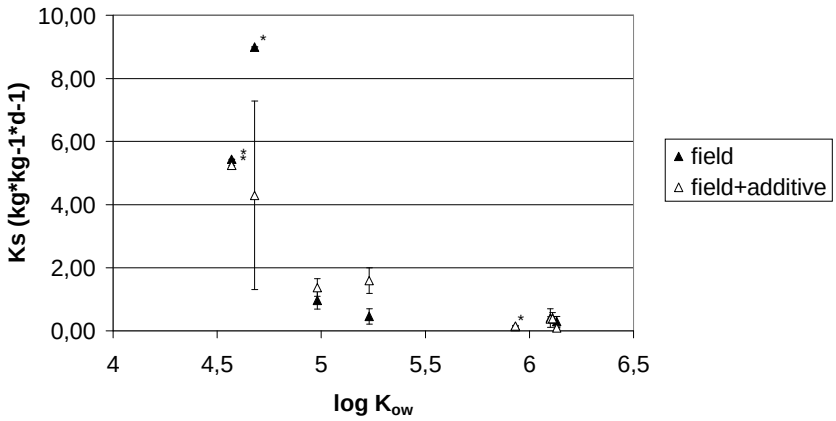
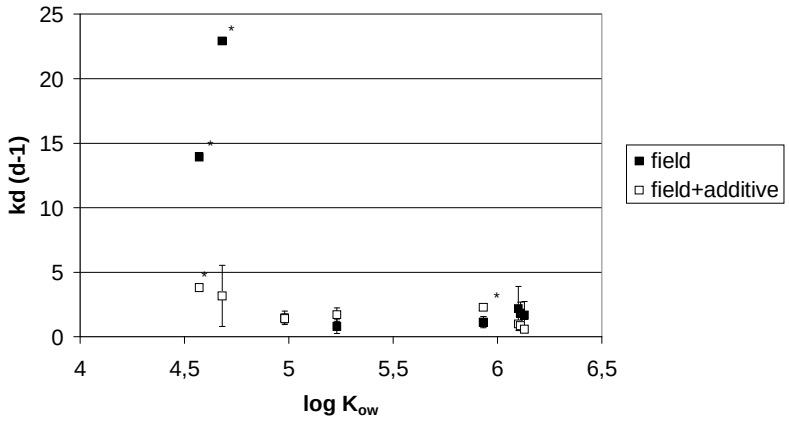


Fig. 1 a, b, d and d. Typical uptake pattern of native PAHs (anthracene and benzo[*k*]fluoranthene) in a field sediment (sediment from the estuary Westerschelde, the Netherlands) and lab-contaminated PAHs (anthracene and benzo[*k*]fluoranthene) in the field + additive sediment in the amphipod *Corophium volutator*. Experimental data (*circles*), fit of data according to Eq. (2) (*line*) and BSAF according to Eq. (3) (*dotted line*).

Biota to sediment accumulation factors of the lab-contaminated PAHs varied between 0.9 and 3.3, while BSAF values of the native PAHs were between 0.5 and 1.7 (Figure 3). Biota to sediment accumulation factors of the lab-contaminated PAHs (phenanthrene, anthracene, fluoranthene, pyrene, benzo[*b*]fluoranthene and benzo[*k*]fluoranthene) were a factor 1.4 to 3.3 higher than the native compounds (Figure 3). Therefore, it can be concluded that the lab-contaminated PAHs were more available to the amphipods than the native PAHs. The BSAF values for the two PAHs that were added at two different concentrations (anthracene and benzo[*k*]fluoranthene) were similar (Figure 3). This observation implies that the accumulation in the amphipods was independent of concentration at this level of total concentration of test compounds.





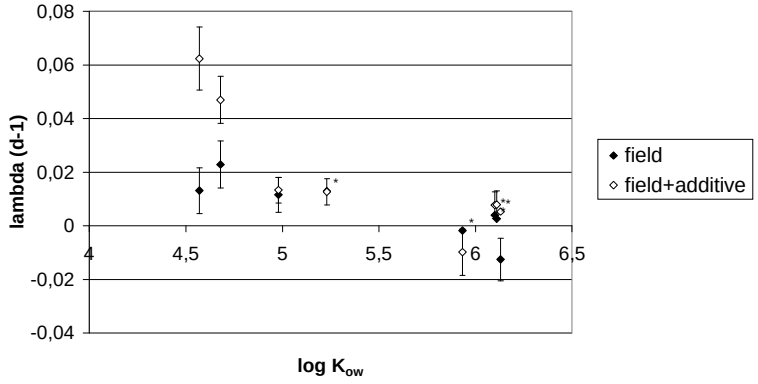


Fig. 2 Estimated values of the parameters k_s, k_d and λ with standard errors for the PAHs in the field sediment and in the field + additive sediment, against log K_{ow} of the PAHs. * = standard error higher than estimated value.

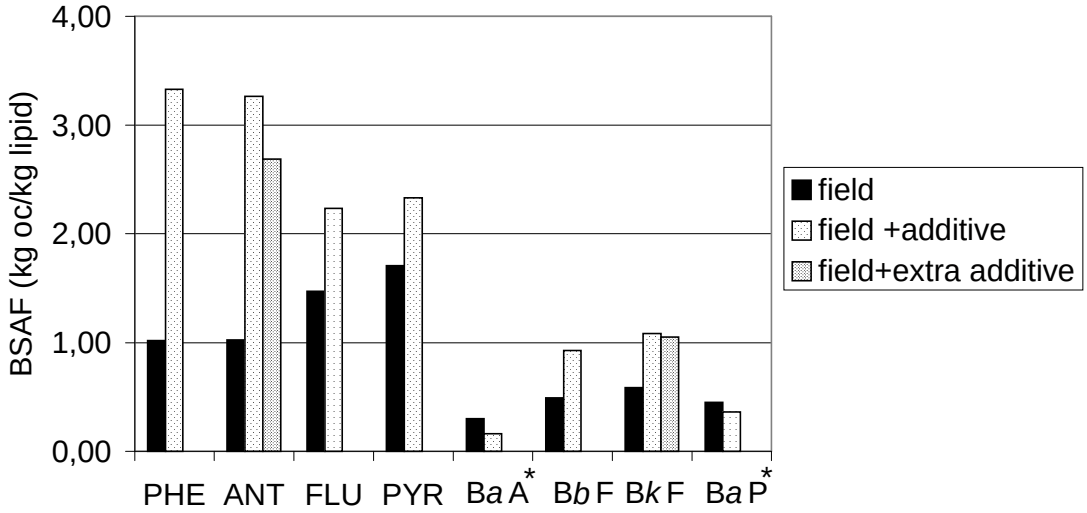


Fig. 3. Biota to sediment bioaccumulation factor (kg o.c./ kg lipid) of PAHs for the amphipod *Corophium volutator* in field, field + additive and field + extra additive sediment (sediment from the estuary Westerschelde, the Netherlands). For abbreviations of PAHs, see footnote Table 2. ANT en BkF were added in field + extra additive at concentrations ca. 3 times higher than in field + additive. *BaA and BaP were not added in any of the three treatments.

Desorption experiments

The rapidly desorbing fraction desorbed with a rate of 0.33 to 3.10 hr^{-1}, whereas the slowly and the very slowly desorbing fraction desorbed with a rate of 1.00E-7 to 0.07 hr^{-1}. The average rapidly desorbing fractions (F_{rap}) of the individual PAHs (phenanthrene, anthracene, fluoranthene, pyrene, benzo[*b*]fluoranthene and benzo[*k*]fluoranthene) in the field and field + additive sediments are shown in Figure 4. The F_{rap} of the lab-contaminated compounds did not change significantly during the exposure period (data not shown, t-test, $p< 0.05$). Therefore, an average of the F_{rap} at the start and the end of the exposure period was calculated. The F_{rap} of the native compounds varied between 0.1 and 0.6. The F_{rap} of the lab-contaminated PAHs was consistently higher, varying between 0.1 and 0.7. The difference was significant for phenanthrene and anthracene, but not for fluoranthene, pyrene benzo[*b*]fluoranthene and benzo[*k*]fluoranthene (t-test, $p< 0.05$). Table 3 shows the rapidly desorbing fractions of lab-contaminated PAHs for sediments with and without amphipods after 25 days of exposure. The rapidly desorbing fractions were not significantly altered in the presence of amphipods (t-test, $p< 0.05$).

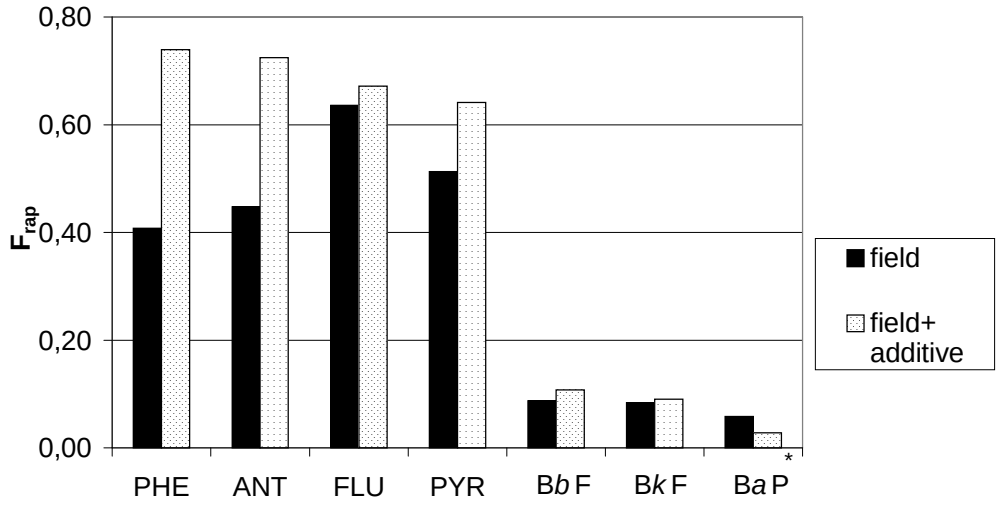


Fig. 4. Average rapidly desorbing fraction (F_{rap}) of PAHs in field and field + additive sediment (sediment from the estuary Westerschelde, the Netherlands). For abbreviations of PAHs, see footnote Table 2. *BaP was not added in any of the three treatments.

Table 3. Rapidly desorbing fractions (F_{rap}) of PAHs in field + additive sediment at the end of the exposure period, with and without exposure of the amphipod *Corophium volutator*. For abbreviations of PAHs, see Table 2.

F_{rap}[1]						
PHE	ANT	FLU	PYR	B*b*F	B*k*F	B*a*P[2]
+ amphipods						
0.77 (0.01)	0.75 (0.08)	0.68 (0.02)	0.69 (0.02)	0.13 (0.02)	0.11 (0.02)	0.04 (0.01)
0.78 (0.01)	0.75 (0.01)	0.68 (0.03)	0.68 (0.02)	0.15 (0.03)	0.12 (0.02)	0.04 (0.01)
- amphipods						
0.79 (0.10)	0.78 (0.01)	0.60 (0.03)	0.65 (0.03)	0.26 (0.04)	0.22 (0.04)	0.02 (0.02)
0.72 (0.10)	0.70 (0.01)	0.67 (0.02)	0.67 (0.02)	0.14 (0.03)	0.12 (0.03)	0.04 (0.00)

[1]Measurements were carried out in duplicate on a pooled sample. First number is the result of the first determination, the second of the second determination. Standard error is given in parentheses; [2] B*a*P was not added to the sediment.

DISCUSSION

Accumulation in amphipods

In the present study a decline in accumulation levels in *Corophium volutator* was observed following an initial phase of first order type uptake kinetics. The decline corresponds with a positive value of fit parameter λ (Figure 2). The decline was most

pronounced for the lower PAHs. A similar shape of the uptake curve was found by other authors, for example for phenanthrene in the freshwater amphipod *Diporeia spp* [40] and for three-ringed PAHs and chlorinated compounds (chlorobenzenes, polychlorinated biphenyls (PCBs), chloroanisoles and pesticides with a molecular weight < 292) in oligochaetes (Kraaij et al., unpublished data). Some explanations can be put forward to explain the observed two-phase accumulation pattern, including biotransformation and a decline in bioavailability ([39] and [23]. Biotransformation did probably not contribute to the observed loss of the PAHs in *Corophium volutator* during the course of our experiment. Concentrations of metabolites of pyrene in subsamples of amphipods that were exposed for two and four weeks to the spiked sediment were below the detection limit of 1 % of the parent compound (personal communication, G. Stroomberg). At the beginning and the end of the experiment, equal amounts of PAHs were measured in the rapidly desorbing fraction. If this fraction relates to bioavailability, no shift in bioavailability was observed during the course of our experiment. We suggest that the pore water in the U-shaped tubes, which are constructed by the amphipods, is diluted with overlying water. This, in combination with biodegradation, might have resulted in reduced exposure of the animals and might have caused the observed drop in internal concentrations of PAHs.

BSAF

The kinetic BSAF values were calculated from the fitted parameters k_s and k_d of Equation 2, derived from the accumulation model proposed by Landrum [39]. The model fit the data well for the lab-contaminated PAHs ($R^2 > 0.9$) and acceptable for the native PAHs ($R^2 > 0.6$, except for phenanthrene and fluoranthene). The calculated values of the kinetic BSAF match graphically determined maximum values of the BSAF (see Fig 1). The BSAF values of the native PAHs of 0,25-1,75 in this study are in the same range as literature values: e.g. Tracey and Hansen [3] calculated an average value of 0,29 for six different organisms. Also the BSAF values of the lab-contaminated PAHs of 0,9-3,3 are within the same order of magnitude of BSAF values found by other authors: e.g. Kukkonen and Landrum [6] found a BSAF value of 0,123-0,171 for freshly added benzo[*a*]pyrene in *Diporeia sp.*

We observed higher BSAFs for all lab-contaminated PAHs compared with the native counterparts. The measured differences were within one order of magnitude. When BSAF is defined as a measure of bioavailability, it can be concluded that the lab-contaminated PAHs are more bioavailable than the native PAHs. Some factors can be put forward to explain the unequal availability of lab-contaminated and native PAHs, including 1) concentration dependent accumulation kinetics, 2) association with soot and 3) sequestration.

Results of Landrum et al. [41] showed a concentration dependency for the uptake clearance coefficient k_s. These authors suggested that enhanced activity of the amphipods at the higher concentrations is one of the most likely explanations for the higher k_s values at these concentrations. Amphipods with higher activity are exposed to larger volumes of pore water. As a consequence, local depletion might be lowered and k_s values increased. The differences in k_s values at the different concentrations in the sediment were within the same order of magnitude as the differences we found in BSAF values for lab-contaminated and native compounds. Yet, the concentration independent uptake of anthracene and benzo[*k*]fluoranthene in the amphipods might indicate that the concentration difference does not account for the observed differences in uptake. It is also expected that the parameters k_s, k_d are constant during the exposure, because toxic effects on physiology or behaviour are not expected.

The lab-contaminated and native PAHs did not enter the sediment in the same way. A fraction of the native PAHs may have been soot associated while deposited. Gustafsson et al. [18] calculated that the partition coefficients of PAHs for soot are relatively high compared to partition coefficients for sediment organic carbon. The strong association between soot and PAHs probably results in a low bioavailability of soot associated PAH. Lamoureux and Brownawell [42] demonstrated a lower assimilation efficiency of BaP by the polychaete *Nereis succinea* in soot amended sediment compared with unamended sediment. Therefore, association to soot of the native PAHs cannot be excluded as an explanation of the relatively low bioavailability of these PAHs in our study.

We intended to investigate the availability of PAHs that differed in contact time with the sediment. The residence times of the native and lab-contaminated PAHs were extremely different (decades vs. days). Time dependent processes like sequestration may have led to a lower availability for the native PAHs compared to the lab-contaminated PAHs. Results of Varanasi et al. [7] strongly suggest that different BSAF values for native and lab-contaminated BaP are related to differences in contact time. In their study, the uptake of native BaP and lab-contaminated BaP was determined in one and the same estuarine sediment. The accumulation factor of the lab-contaminated BaP was 6.1 and 4.3 times higher than the accumulation factor of the native PAH for the marine amphipod *Eohaustorius washingtonianus* and the clam *Macoma nasuta* respectively. Kukkonen and Landrum [6] demonstrated that the uptake clearance coefficient k_s of freshly added PAHs in *Diporeia spp.* was higher than the uptake clearance of PAHs that were added 1 week to 13 months before exposure of the amphipods.

In conclusion: Contact time is one of the factors that may determine bioavailability of hydrophobic compounds to benthic organisms. One of the mechanisms that might be responsible for this contact time dependent accumulation, i.e. sequestration, was indeed

observed in our experiment. This mechanism will be discussed later. Differences in soot association between native and lab-contaminated PAHs may also have induced the observed differential bioavailability.

Desorption kinetics

The measurements of rapidly desorbing fractions of the lab-contaminated compounds being less than unity at the beginning of the experiment indicates that sequestration is already measurable after a very short incubation time. The rapidly desorbing fractions of two out of six PAHs were significantly lower for the native compounds compared with the lab-contaminated compounds. This might be caused by increased sequestration at longer residence times, as was discussed before. The same phenomenon was observed by Cornelissen et al. [13] who found lower rapidly desorbing fractions of chlorobenzenes and PCBs in lab-contaminated sediment that was incubated for 34 days, compared to 2 days incubation. The finding was also confirmed by White et al. [10] who observed lower fractions of phenanthrene that desorbed within 21 days at longer aging periods. The data measured in our study seem to be reliable: The slowly desorbing fractions of the lab-contaminated anthracene, fluoranthene and pyrene (28, 33 and 36 %) are similar to the slowly desorbing fractions of the same lab-contaminated PAHs found by Cornelissen et al. [13] after 34 days of incubation time (24, 40 and 36%).

The amphipods did not change the distribution over rapidly and slowly desorbing fractions, measured in the bulk-sediment. This means that neither ingestion of sediment particles nor bioturbation with subsequent changes of redox conditions and biodegradation in the sediment affected the sequestration on a macro-scale. Yet, it is possible that amphipods did change the sequestration of the PAHs in their micro-environment. However, this change is not measurable on a macro-scale.

Relationship between bioavailability and chemical desorption

The time dependent process of sequestration results in a shift in the distribution over rapidly and slowly desorbing fractions. It has been hypothesised that this shift influences the bioavailability. For microorganisms, uptake and metabolism is solely mediated by the aqueous phase. Changes in desorption behaviour therefore affect biodegradation rates when desorption is the rate limiting process [10, 28]. Sequestration might also influence the accumulation in macrobenthos. The shift to slowly desorbing fractions possibly lowers the pore water concentration [43] and thereby reduces exposure via the aqueous phase. Also, exposure via ingestion might be affected by sequestration as sequestered compounds might become less available in the gut of the organisms.

In order to explore the relationship between desorption kinetics and bioavailability for macrobenthos in historically contaminated and lab-contaminated sediments, we examined the correlation of the rapidly desorbing fraction of PAHs with the BSAF of PAHs in amphipods. The present study shows that ratios of BSAF of lab-contaminated versus native PAHs correspond with ratios of the fraction residing in the rapidly desorbing sediment compartment (Figure 5). The BSAFs of the individual PAHs also showed a good correlation (r^2=0.76) with the rapidly desorbing fractions of the individual lab-contaminated and native PAHs (Figure 6, lower panel). This result, 76 % of the variance in the BSAF values being explained by rapidly desorbing fractions, indicates a strong relationship between desorption and accumulation into the amphipods. The relationship cannot be explained by a coincidental existence of low or high values for both accumulation and rapidly desorbing fractions. This might happen when an unknown process that is controlled by the physico chemical properties of the individual PAHs affects accumulation into the organisms and desorption from sediment in a similar way. The upper panel of Figure 6 confirms the causal relationship between BSAF and rapidly desorbing fraction: The BSAF is related to the rapidly desorbing fraction in a similar manner for all individual PAHs. This result suggests that the established relationship not only holds for individual compounds differing in physico chemical properties, but also for different treatments of one compound. The results in this study are well in line with the scarce literature data on the subject. Lamoureux and Brownawell [9] studied the desorption and accumulation in the deposit feeding clam *Yoldia limatula* of native PAHs, PCBs and linear alkylbenzenes (LAB) from two core sections of a harbour sediment. Biota to sediment accumulation factors of PAHs and LABs were correlated with the fraction that desorbed within 48 hours ($r^2 > 0.78$).

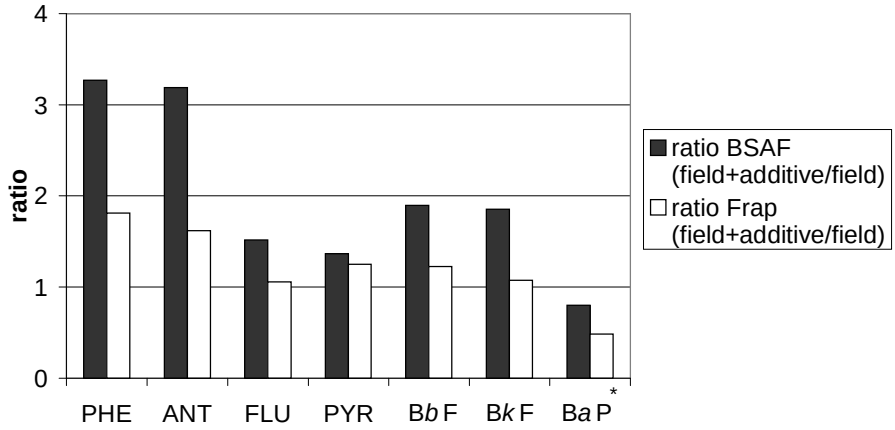


Fig. 5. Ratio of BSAF values in field + additive and field sediment (sediment from the estuary Westerschelde, the Netherlands) of PAHs and the corresponding ratio of rapidly desorbing fraction (F_{rap}) in field + additive and field sediment. For abbreviations of PAHs, see footnote Table 2.* BaP was not added in any of the three treatments.

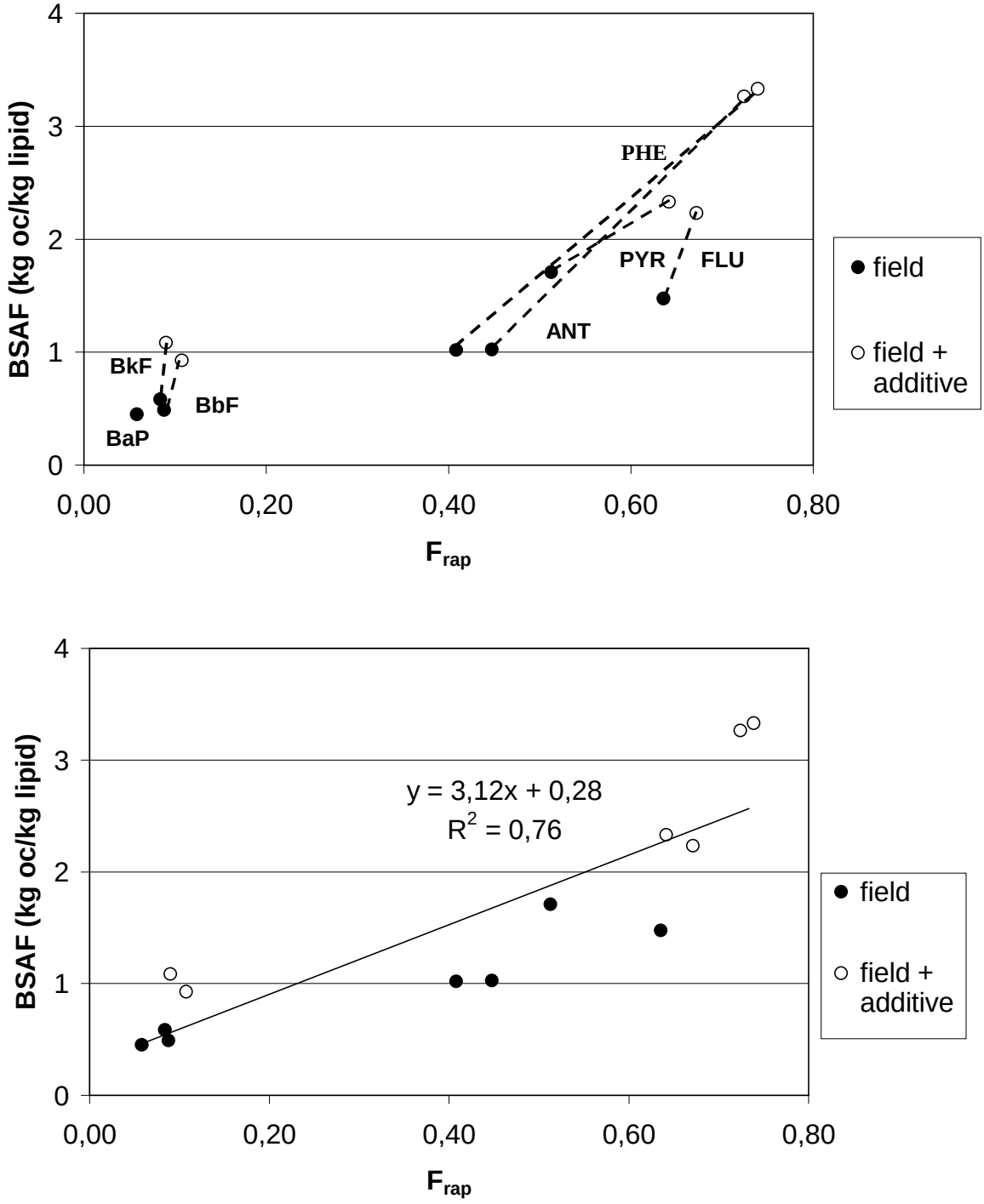


Fig. 6. Experimental BSAF (kg o.c./ kg lipid) of PAHs for the amphipod *Corophium volutator* in field and field + additive sediment (sediment from the estuary Westerschelde, the Netherlands) versus rapidly desorbing fraction F_{rap}. For abbreviations of PAHs, see footnote Table 2. Closed circles are native PAHs in field sediment, open circles are lab-contaminated lab-contaminated PAHs in field + additive sediment. Upper panel: data points for identical PAHs (in field and field + additive sediment) connected by a dotted line. Lower panel: regression line included. R^2 of correlation is 0.76.

The present study demonstrates that desorption measurements might provide a good approximation of differences in bioavailability between compounds, treatments and sediments. We found that differential desorption behaviour can largely explain differences in bioavailability between individual PAHs and between native and lab-contaminated PAHs. Hence, desorption measurements seem to qualify as a promising predictive tool in bioavailability research.

As yet, it is still unclear whether the effect of slow desorption is mediated via the pore water and/or the uptake in the gastro intestinal tract. This question needs to be addressed in order to fully understand the mechanism of the relationship between slow desorption and bioavailability.

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Chapter 3. The effect of contact time on the sequestration and bioavailability of different classes of hydrophobic organic chemicals to benthic oligochaetes (*Tubificidae*).

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ABSTRACT - Differences in bioavailability of hydrophobic organic compounds (HOC) to benthic deposit-feeders have been related to differences in sediment-HOC contact time and sequestration status. As a consequence, it was postulated that contact time and/or sequestration should be incorporated into risk-assessment. In the present study, we investigated the effect of contact time on the bioavailability and sequestration of different classes of HOC. For this purpose, we simultaneously measured the steady-state accumulation into benthic oligochaetes (*Tubificidae*) and the distribution over rapidly and slowly desorbing fractions in laboratory contaminated sediment at different contact times. The decrease in rapidly desorbing fractions (F_{rap}) of PCBs, PAHs and p,p'-DDE after a contact time of 959 days did not exceed a factor of 1.2. Similarly, the reduction in bioavailability was a factor of 2.3 at maximum, indicating that long contact times do not necessarily result in pronounced bioavailability reduction. For chlorobenzenes, the bioavailability was reduced with a factor of 5 to 18. This decrease corresponded with a pronounced reduction in F_{rap}, which was attributed to losses of rapidly desorbing compounds. Over 75 % of the variation in BSAF-values of the PAHs and chlorobenzenes at the three contact times could be explained by differences in F_{rap}, irrespective of the processes at hand. The present study provides evidence of a relationship between sequestration status and bioavailability of HOC to benthic deposit-feeders.

INTRODUCTION

As a result of unrestrained use and emission of hydrophobic organic chemicals (HOC) worldwide, high concentrations of pollutants are encountered in environmental 'sink' compartments such as sediments. Loss processes, such as anaerobic microbial degradation, are usually slow for this type of chemicals in sediments. As a consequence, high concentration levels persist even at reduced input of pollutants. Benthic organisms may be at

risk when accumulation exceeds toxic threshold levels.

The accumulation of HOC cannot be accurately assessed from total concentration measurements, as the bioavailability is largely variable among different sediments, compounds and organisms [3]. Generally, bioavailability has been defined in the literature as the total concentration of chemicals in soil or sediment that is or will potentially be taken up by an organism [19]. Differences in bioavailability can be measured by differences in bioaccumulation. Bioaccumulation is expressed in this paper as the steady state accumulation into the organism divided by the concentration in the sediment, normalised to lipid and organic carbon content, or the 'biota to sediment accumulation factor' (BSAF).

With increasing contact time between sediment and HOC, the bioavailability of a hydrophobic organic compound seems to decrease at a higher pace than the total concentration. Numerous studies were designed in order to elucidate and quantify this ageing or weathering effect on bioavailability. Alexander recently summarized and discussed these studies [44] and concluded that organic compounds become progressively less available for uptake by organisms, exerting effects, biodegradation and bioremediation by microorganisms.

So far, little attention has been given to the effect of contact time on the bioavailability of different compounds and different compound classes. In most studies, only PAHs are used as test compounds. Different compounds do show a wide range of bioavailability, even at similar residence times. For example, the bioavailability of PAHs is generally lower than those of PCBs and chlorobenzenes [3].

Bioavailability might be related to the extent of sequestration, a chemical process that is affected by contact time as well (e.g. [13]). Sequestration has been defined as the formation of relatively slowly desorbing fractions. The slow desorption might be induced by high activation energies at high surface area carbonaceous material (HSACM) such as charcoal, specific ' sites' or ' holes' in the organic matter matrix or diffusional limitations in condensed regions of the organic matter matrix and/or pores in the mineral or organic fraction (e.g. [14-17, 24, 45]). One attempt to characterize sequestration is the determination of rapidly, slowly and very slowly desorbing fractions with distinctly differing desorption rates and sorption behaviour ([17, 46]. Sequestration or slow desorption has been related to biodegradation (e.g. [10, 11, 29], [12]) and recently to the bioavailability to macrobenthos [9, 47].

We investigated the effect of contact time on the bioavailability and sequestration of different chemicals and classes. For this purpose, we performed a controlled study on a broad range of chemicals of different classes: PAHs, chlorobenzenes, PCBs and p,p'-DDE. Relatively nonpolluted sediment was contaminated in the laboratory with a mixture of these compounds and stored at constant conditions. We simultaneously examined the effect of contact time on bioavailability to benthic deposit-feeders and the chemical factor of sequestration by measuring the steady-state accumulation into benthic oligochaetes (*Tubificidae*) at three different sediment-HOC contact times up to three years. Additionally, we quantified the extent of sequestration of the test compounds at these contact times by determining rapidly, slowly and

very slowly desorbing fractions. These fractions were assessed from measurements on the desorption kinetics ([13]). We investigated whether differences in the bioavailability to benthic deposit-feeders, induced by different compound properties or contact time, were related to sequestration.

EXPERIMENTAL APPROACH

Oligochaetes

Oligochaetes were reared at the laboratory on uncontaminated paper pulp. More than 90 % of the culture consisted of the species *Limnodrilus hoffmeisteri* Claparède and *Tubifex tubifex* Müller (both family Tubificidae). Less than 10 % of the culture consisted of *Lumbriculus variegatus* (family Lumbriculidae) individuals (<10 %). We will refer to the oligochaetes as Tubificidae, as more than 90 % of the individuals belonged to this family of oligochaetes. The oligochaetes were acclimatized for at least 24 hours at 10 °C.

Chemicals

Phenanthrene, fluoranthene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, 2-ethylanthracene, 7-methylbenzo[*a*]pyrene, p,p'-DDE (2,2-bis(4-chlorophenyl)-1,1-dichloroethylene), 1,2,3,4-, penta-, and hexachlorobenzene, 2,2',5,5'-tetrachlorobiphenyl (PCB 52), 2,3,5,6-tetrachlorobiphenyl (PCB 65), 2,2',4,5,5'-pentachlorobiphenyl (PCB 101), 2,3,3',5,6-pentachlorobiphenyl (PCB 112), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), 2,2',4,4',5,6'-hexachlorobiphenyl (PCB 154), 2,3,3',4,4'-pentachlorobiphenyl (PCB 105), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138) and 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) were obtained from various commercial sources (PCB numbering according to IUPAC). Octadecyl (C-18) was purchased from JT Baker (Phillipsburg, NJ, USA). Tenax TA (60-80 mesh) was obtained from Chrompack (Bergen op Zoom, The Netherlands). Florisil and silica were obtained from Merck (Darmstadt, Germany) and Alumina was purchased from ICN Biomedicals (Eschwege, Germany).

Sediment treatment and characterization

Sediment was collected from a relatively unpolluted area (Oostvaardersplassen, The Netherlands). Prior to lab-contamination, it was sieved (500 µm mesh size), suspended in copper-free water (ratio 0.71 L/L wet sediment) and homogenized. The batch of sediment was contaminated by dropwise addition of an acetone solution containing the chlorobenzenes, PCBs, PAHs and p,p'-DDE (final ratio 2.3 ml acetone per 100 g dry sediment). During the contamination procedure, the suspension was vigorously stirred by a

concrete mixer. The contaminated sediment was aged by storing the sediment in closed buckets of 10 L at 10 °C.

We tried to avoid any toxic effect on the oligochaete worms by choosing nominal concentrations well below expected acute effect levels. Sediment characterization measurements were performed on blank sediments without oligochaetes in order to monitor changes in the sediment quality during storage. Organic carbon, elemental nitrogen content and elemental oxygen in the organic matter, total phosphate and particle size distribution were determined with standard methods. A polarity index was calculated as the atomic mass ratio of elemental oxygen plus elemental nitrogen divided by elemental carbon (O+N/C) [48].

Desorption experiments

At the beginning of each bioaccumulation experiment, desorption characteristics were studied in a subsample of the stored sediment, according to the method described by Cornelissen et al. [13]. An equivalent of 3 g dry weight sediment was shaken for 10 days with 100 ml copper-free water and 3 g Tenax TA sorbent. This sorbent effectively removes solutes from the aqueous phase. At the beginning of the first and second exposure experiment at 5 and 91 days of contact time respectively, duplicate experiments were carried out. An extra duplicate measurement was carried out during the first exposure experiment after 14 days of contact time in order to study short-term changes in desorption behaviour. At the beginning of the third exposure experiment at 959 days of contact time, a single experiment was performed. The experiments resulted in estimations of rapidly, slowly and very slowly desorbing fractions of compounds in the sediment. It was not possible to determine the rapidly, slowly and very slowly desorbing fractions of the PCBs and p,p'-DDE for some of the exposures because of the low concentrations in the Tenax extracts. Therefore, we did not evaluate the desorption data for these compounds.

Bioaccumulation experiments

At 2, 91 and 959 days after contamination of the sediment, bioaccumulation experiments were started (exposure I, II and III). For each experiment, 500 ml beakers were filled with the contaminated sediment (137 g dry weight per beaker; final dry weight percentage = circa 34 %) and placed in an aquarium at 10 °C. Copper-free tap water was added to the aquarium such that the level of the overlying water was higher than the height of the beakers. The overlying water was aerated mildly to provide the worms with oxygen and to keep the worms in the sediment. Approximately 0.5 g of tubifex was transferred to each beaker. Beakers were taken out of the aquarium after 3 to 6 different times of exposure. Worms were sampled and kept in copper-free water for 25 hours in order to allow worms to empty their guts. The losses of test compounds due to elimination in clean water during this period were calculated from the elimination rate constant k_2. This value was experimentally

determined or estimated, using the model of Sijm and van der Linde [49]. Losses were below 5 %, except for 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, phenanthrene and fluoranthene. Losses were 39.8, 12.4, 29.1 and 7.0 % for 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, phenanthrene and fluoranthene respectively. The concentrations of all compounds were corrected for losses due to elimination in the clean water. In order to facilitate sampling of the worms, the sediment was gently suspended with 170 ml of copper-free water. After emptying their gut, the worms were gently dried with a paper towel, killed in liquid nitrogen and stored at –25 °C until analysis. Lipid contents of the worms at the beginning and end of the exposure were determined according to Folch et al. [35]. Sediment was sampled at the end of each bioaccumulation experiment.

Analyses

Oligochaetes

Oligochaetes were extracted using Matrix Solid Phase Dispersion [37] and prepared for HPLC analyses as described earlier [47]. A subsample of the extract for GC-analyses was transferred to a column filled with two portions of 1.75 g of activated silica to which respectively 0.77 g concentrated sulphuric acid and 0.58 g 1 M potassium hydroxide was added respectively. Both portions were cleaned with acetonitrile prior to use. The column was eluted with 18 ml of acetonitrile and the eluate was concentrated to approximately 0.15 to 1 ml under a gentle flow of nitrogen. Recoveries of the internal standards 2-ethyl-anthracene, 7-methyl-benzo[*a*]pyrene, PCB 112 and PCB 154 were 85.1 %, 76.7 %, 67.2 % and 55.9 % on average. Concentrations of phenanthrene, fluoranthene, benz[*a*]anthracene and chrysene were corrected with the recovery of 2-ethyl-anthracene and concentrations of benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene were corrected with the recovery of 7-methyl-benzo[*a]*pyrene. Concentrations of all chlorinated compounds were corrected with the recovery of PCB 112, except for PCB 138, PCB 153, PCB 156 and PCB180, that were corrected with the recovery of PCB 154.

Sediment and Tenax

Sediments were reflux extracted for 4 hours with hexane and methanol (1/1) according to Smedes and de Boer [50] and prepared for analyses of PAHs as described earlier [47]. A subsample of the extract for GC analysis was transferred to a glass column filled with 1.5 g Al_2SO_3 (7.5 % water content) and 1.5 g SiO_2 (5 % water content) and eluted with 30 ml hexane. The eluate was concentrated to 0.2 to1 ml

Each portion of Tenax was extracted by shaking with 20 ml hexane, and compounds were transferred to acetonitrile prior to analysis with HPLC.

All extracts for PAH analyses were analysed by C18 reversed phase HPLC with

fluorescence detection. Chlorinated compounds were analysed by GC-ECD. Recovery of the internal standard for the PAHs was 79 % for 2-ethylanthracene and 74 % for 7-methylbenzo[*a*]pyrene on average (n=59). Coefficient of variation (c.v.) for the recovery of these two compounds was 10.6 and 8.9 %. Recovery of PCB 112 and PCB 154, the internal standards for the chlorinated compounds, was 60.8 and 64.8 % (n = 34) respectively for the first two experiments and 27.7 and 25.8 % (n=25) for the last experiment. Coefficient of variation (c.v.) of the recovery of PCB 112 and PCB 154 was 23.5 and 23.6 % for the first two experiments and 21.5 and 22.1 % for the last experiment. The relatively low recovery for the PCBs in the last experiment was probably due to incomplete sulphur precipitation. As the coefficient of variation of the recoveries of the PCBs was reasonably low for all experiments, corrections for recoveries were allowed. Concentrations in the sediment were corrected in the same way as for the tubifex samples.

Data analysis

The bioavailability of compounds at different contact times was compared by comparing calculated biota to sediment accumulation factors (BSAF). The BSAF were calculated as

$$BSAF = C_b / C_s \qquad (1)$$

with C_b is the average of the lipid normalized concentration in the oligochaetes and C_s is the average of the organic carbon normalized concentration in the sediment. The concentrations in the oligochaetes were normalized for lipid content by dividing with the average of the lipid content at the start and end of the experiment. For all compounds, an average of the accumulation at approximately 3, 4 and 5 weeks was used for the calculation of the BSAF. Based on results from the first exposure, we calculated that the average accumulation of all compounds at that time was at more than 85 % of equilibrium.

The data of the desorption experiment were fitted to a triphasic desorption model as described by Cornelissen et al. [51]. The fitting procedure resulted in estimations of rapidly, slowly and very slowly desorbing fractions (F_{rap}, F_{slow} and $F_{very\ slow}$) for each compound and contact time.

RESULTS

General observations

The quality of the organic matter did not significantly change between 91 and 959 days of contact time (Table 1). The inconsistent changes in the content of total phosphate between 2

and 959 days of contact time in the sediment are probably due to analytical variance or some heterogeneity in the batch of sediment.

Table 1. Quality measurements on the contaminated sediment at different contact times. (Between parentheses: individual measurements.)

	Contact time (days)		
	2	91	959
C (organic) (% dw)	2.7	3.0	3.2
O (% dw)	1.48(1.45; 1.51)	2.46 (2.51; 2.41)	2.38 (2.30; 2.46)
N (% dw)	0.27	0.30	0.30
Polarity index (O+N/C)	0.65	0.94	0.84
Total phosphate (g/kg dw)	3.0	1.8	4.3
Particle size (% dw)			
< 2 µm	24	23	23
< 16 µm	43	44	43

The concentrations of the PAHs, chlorobenzenes, PCBs and p,p'-DDE, normalized for organic carbon content, remained constant in the 91 days interval after addition of the test compounds to the sediment (two-sided t-test, $p<0.05$) (Table 2). The concentrations of 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, PCB 65, PCB 101, PCB 118, PCB 105 and p,p'-DDE decreased significantly within a contact time of 959 days. The concentrations of all PAHs, except phenanthrene, increased slightly but significantly in this period. The decrease in the concentrations of the chlorinated compounds might be caused by loss processes as evaporation and biodegradation.

The average lipid content of the oligochaetes at the start and the end of the exposure was 3.59 and 2.30 % for the first experiment, 3.15 and 3.18 % for the second experiment and 2.44 % and 1.75 % for the third experiment. The duplicate measurements at the start and the end of the experiments were all within 20 % of the average value. We used an average of 2.95, 3.17 and 2.09 % for the first, second and third exposure respectively. The maximum accumulated sum of moles of all compounds per kg wet weight of oligochaetes met our criterium, as this maximum concentration was approximately 10 to 40 times below the lethal body burden of 2-8 mmol/kg for narcosis [52] for aquatic organisms. It also stayed below (1.5 to 3 times) the body residue for effects on growth and reproduction of 0.3-0.6 mmol/kg that was determined for the oligochaete *Lumbriculus variegatus* ([53]).

The precision in the measurements of the concentrations in the biota was sufficiently high: The average coefficient of variation (c.v.) in the measurements of the concentration in the biota was below 14 % for the PAHs and below 40 % for the chlorinated compounds.

Table 2 Mean concentrations of test compounds in sediment (mg/kg organic carbon) at contact time 0, 91 and 959 days at the end of the exposure experiments.

	Contact time (days)					
	2		91		959	
PAHs						
PHE	58.4	(50.7; 66.1)[1]	45.5	(46.6; 44.4)[1]	43.9	(1.29)
FLU	67.3	(64.8; 69.7)[1]	57.0	(58.6; 55.3)[1]	71.2	(2.53)
B*a*A	63.8	(55.4; 72.1)[1]	50.5	(51.5; 49.5)[1]	68.7	(2.44)
CHR	62.4	(54.5; 70.4)[1]	49.5	(50.6; 48.3)[1]	69.3	(3.89)
B*b*F	71.8	(61.7; 82.0)[1]	58.3	(58.7; 58.0)[1]	84.4	(9.92)
B*k*F	67.9	(58.4; 77.5)[1]	55.5	(55.7; 55.3)[1]	77.6	(3.82)
B*a*P	58.9	(50.9; 66.9)[1]	47.3	(47.1; 47.5)[1]	64.5	(2.68)
chlorobenzenes						
1,2,3,4	0.93	(0.70; 1.16)[1]	0.77	(0.72; 0.82)[1]	0.23	(0.05)
penta	1.15	(0.95; 1.34)[1]	1.03	(0.81; 1.26)[1]	0.41	(0.02)
hexa	1.05	(0.89; 1.21)[1]	0.92	(0.76; 1.09)[1]	0.25	(0.06)
PCBs						
PCB 52	1.33	(1.17; 1.48)[1]	1.39	(1.62; 1.16)[1]	1.20	(0.44)
PCB 65	0.90	(0.77; 1.03)[1]	0.87	(0.88; 0.86)[1]	0.78	(0.03)
PCB 101	0.63	(0.54; 0.73)[1]	0.55	(0.56; 0.53)[1]	0.42	(0.05)
PCB 118	1.59	(1.36; 1.83)[1]	1.29	(1.44; 1.15)[1]	0.75	(0.09)
PCB 153	1.43	(1.26; 1.60)[1]	1.21	(1.41; 1.01)[1]	0.77	(0.06)
PCB 105	1.72	(1.48; 1.96)[1]	1.35	(1.46; 1.23)[1]	0.85	(0.05)
PCB 138	1.29	(1.13; 1.45)[1]	1.05	(1.24; 0.86)[1]	0.63	(0.03)
PCB 156	1.30	(1.11; 1.48)[1]	0.97	(1.13; 0.81)[1]	0.68	(0.04)
PCB 180	1.08	(0.97; 1.20)[1]	0.83	(1.01; 0.64)[1]	0.45	(0.03)
pesticides						
p,p'-DDE	1.08	(0.94; 1.22)[1]	0.85	(0.86; 0.85)[1]	0.66	(0.02)

1Measurement in duplicate (others in triplicate), between parentheses: individual measurements (others: SD); PHE = phenanthrene, FLU = fluoranthene, B*a*A = benz[*a*]anthracene, CHR = chrysene, B*b*F = benzo[*b*]fluoranthene, B*k*F = benzo[*k*]fluoranthene, B*a*P = benzo[*a*]pyrene

Bioavailability

Typical accumulation curves of PAHs, chlorobenzenes and PCBs at the different contact times are plotted in Figure 1. For some compounds, a decline in accumulation was observed after approximately 3 weeks. We did not correct for this decline as the decline was generally smaller than 15 % of the absolute BSAF- values. Figure 1 demonstrates that accumulation is quite constant at the first two contact times of 2 and 91 days. Between 91 and 959 days of contact time, the BSAF declines for some compounds.

The BSAF values of all compounds at the different contact times are shown in Table 3. The effect of contact time on the BSAF was markedly different for the compounds of the different classes. The average contact time reduction factor, expressed as the ratio of the BSAF at a contact time of 2 and 91 days (exposure I and II) and 959 days (exposure III) was 12.7 for the chlorobenzenes, 1.5 for the PAHs, 2.2 for p,p'-DDE and 1.2 for the PCBs (Table 3).

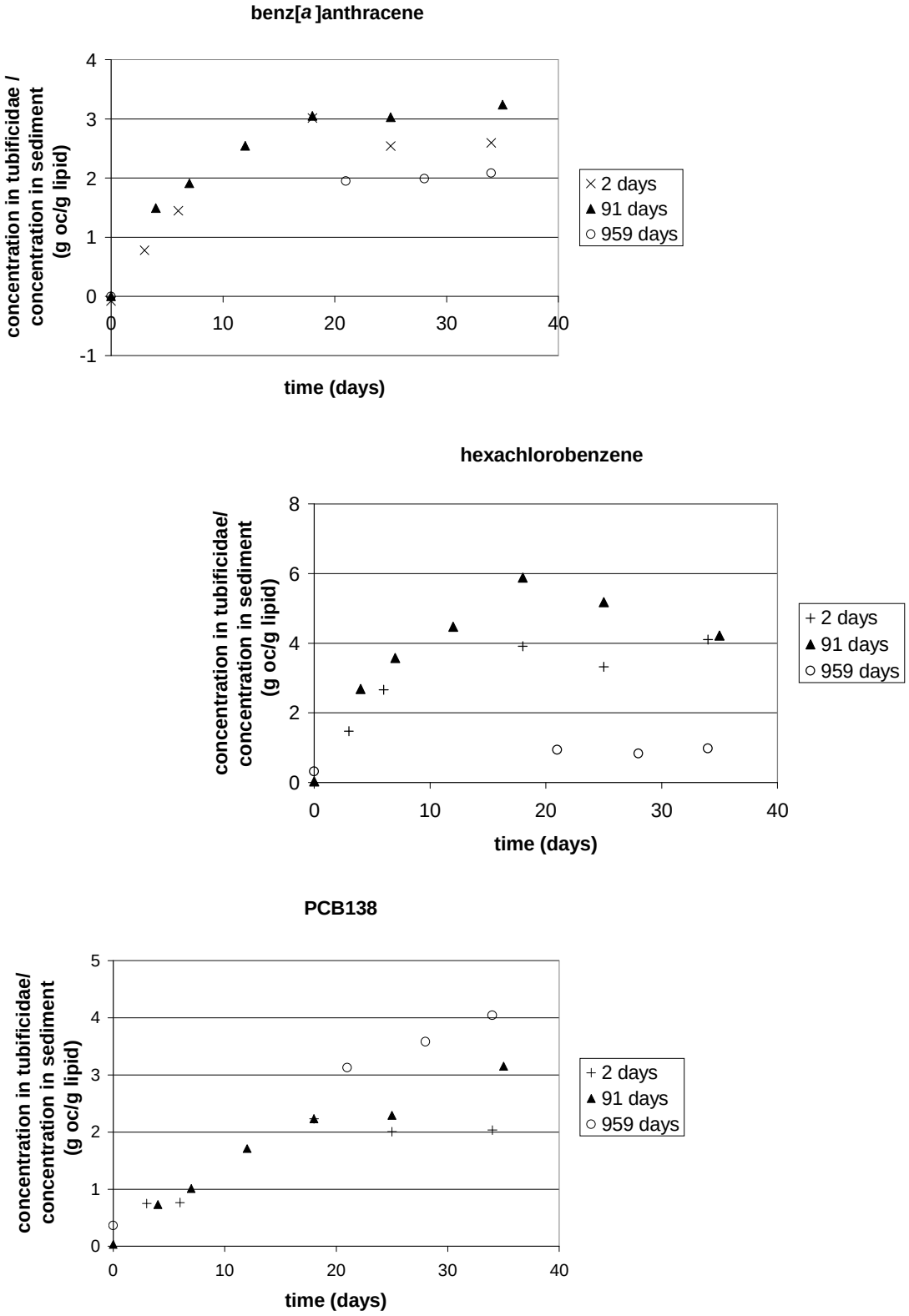

Fig. 1 Typical accumulation curves of a) the PAHs benz[*a*]anthracene, b) the chlorobenzene hexachlorobenzene and c) the PCB 2,2',3,4,4',5'-hexachlorobiphenyl in oligochaetes (*Tubificidae*) in lab-contaminated sediment at different contact times of the contaminant in the sediment. Accumulation expressed as lipid normalized concentration in biota divided by organic carbon normalized concentration in the sediment (µg organic carbon/µg lipid).

Table 3 Average Biota to sediment accumulation factors (BSAF) for oligochaetes (*Tubificidae*) in laboratory contaminated sediment, at different contact times. Contact time reduction factor for BSAF calculated as the ratio of the average BSAF at contact time = 2 and 91 days divided by BSAF at contact time = 959 days. First two columns: log K_{ow} and molecular weight (M).

	log K_{ow}	M	BSAF at 2 d	BSAF at 91 d	BSAF at 959 d	contact time reduction factor
PAHs						
PHE	4.57[1]	178	5.98	6.63	2.70	2.33
FLU	5.23[1]	202	4.25	4.68	2.42	1.85
B*a*A	5.91[1]	228	2.72	3.11	2.01	1.45
CHR	5.81[1]	228	2.97	3.07	1.99	1.52
B*b*F	6.10[1]	252	1.06	1.35	1.05	1.15
BkF	6.11[1]	252	1.31	1.43	1.14	1.20
B*a*P	6.13[1]	252	1.41	1.28	1.03	1.31
chlorobenzenes						
1,2,3,4	4.64[2]	216	3.00	4.47	0.21	18.2
penta	5.18[2]	250	4.22	6.25	0.35	15.0
hexa	5.73[2]	285	3.78	5.09	0.92	4.85
PCBs						
PCB 52	6.10[3]	292	3.90	*7.65*	1.78	2.19
PCB 65	5.94[6]	292	4.41	3.91	2.48	1.68
PCB 105	6.61[5]	326	1.95	3.60	2.64	1.05
PCB 118	6.61[5]	326	3.29	7.84	4.25	1.31
PCB 138	6.70[4]	361	1.48	2.26	3.59	0.52
PCB 153	6.90[3]	361	2.34	4.24	4.66	0.71
PCB 156	7.06[5]	361	2.09	2.56	2.61	0.89
pesticides						
p,p'-DDE	6.96[2]	318	7.59	12.7	4.68	2.16

[1] in [54], [2] in [55], [3] selected, in [56], [4] in [57], [5] estimated, in [55], [6]average of selected values for tetrachlorobiphenyl congeners (n=12) in [56]. For abbreviations of PAHs: see Table 2.

Sequestration

Figure 2 displays the typical distribution of benz[*a*]anthracene between the rapidly, slowly and very slowly desorbing fractions at different contact times. Sequestration seems to take place fast as significant fractions of slowly and very slowly desorbing fraction are measured within a few weeks after addition of the test compounds to the sediment. The sequestration process then moves on at a much slower pace: With increasing contact time, a small shift towards very slowly desorbing fractions is observed. The estimations of the rapidly and very slowly desorbing fractions of all test compounds are shown in Table 4 and 5. The distribution over rapidly, slowly and very slowly desorbing fractions remained virtually unaltered within a contact time of 91 days. The rapidly desorbing fraction had

decreased with a factor of 1.2 to 2 for the 3-4 ringed PAHs and hexachlorobenzene after a contact time of 959 days. The decrease in rapidly and increase in very slowly desorbing fractions, expressed as reduction and enhancement factors, was higher for hexachlorobenzene than for the PAHs.

The relatively small rapidly desorbing fractions at 5 days, compared with 14 days of contact time is ascribed to incomplete equilibration of the system after addition of the test compounds.

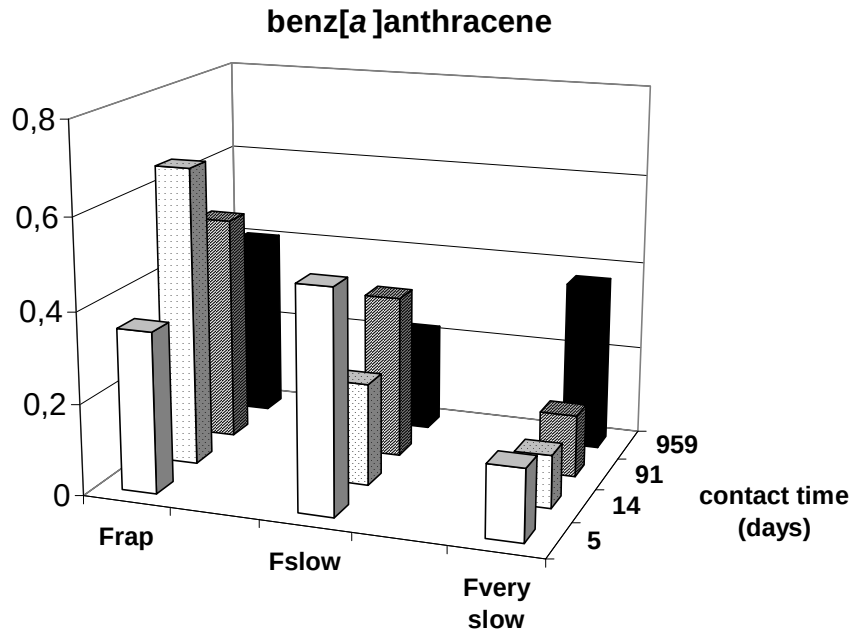

Fig. 2 Typical average rapidly (F_{rap}), slowly (F_{slow}) and very slowly desorbing fractions ($F_{very\ slow}$) of benz[*a*]anthracene in lab-contaminated sediment at different contact times of the contaminant in the sediment.

Table 4 Rapidly desorbing fractions (F_{rap}) in laboratory contaminated sediment, at different contact times. Contact time reduction factor for F_{rap} calculated as the ratio of the average F_{rap} at contact time = 5, 14 and 91 days and the F_{rap} at contact time = 959 days.

	F_{rap} at				contact time reduction factor
	5 d	14 d	91 d	959 d	
PAHs					
PHE	0.68 (0.68; 0.69)	0.63 (0.54; 0.72)	0.74 (0.74; 0.74)	0.55	1.2
FLU	0.61 (0.59; 0.62)	0.76 (0.75; 0.77)	0.70 (0.69; 0.70)	0.56	1.2
B*a*A	0.36 (0.29; 0.42)	0.66 (0.66; 0.66)	0.50 (0.49; 0.51)	0.41	1.2
CHR	0.35 (0.29; 0.40)	0.69 (0.68; 0.69)	0.50 (0.50; 0.50)	0.43	1.2
B*b*F	0.20 (0.07; 0.33)	0.32 (0.37; 0.28)	0.22 (0.21; 0.22)	0.28	0.9
B*k*F	0.14 (0.06; 0.21)	0.30 (0.37; 0.24)	0.23 (0.15; 0.30)	0.32	0.7
B*a*P	0.07 (0.05; 0.08)	0.29 (0.33; 0.25)	0.19 (0.18; 0.20)	0.28	0.6
chlorobenzenes					
penta	0.67 (0.71; 0.63)	0.72 (0.80; 0.63)	0.59 (0.58; 0.61)		
hexa	0.56 (0.59; 0.54)	0.80 (0.84; 0.77)	0.67 (0.66; 0.68)	0.35	1.9

For abbreviations of PAHs: see footnote Table 2.

Table 5 Very slowly desorbing fractions ($F_{very\ slow}$) in laboratory contaminated sediment, at different contact times. Contact time enhancement factor for $F_{very\ slow}$ calculated as the ratio of $F_{very\ slow}$ at contact time = 959 days divided by average $F_{very\ slow}$ at contact time = 5, 14 and 91 days. For log K_{ow} and molecular weight (M): see Table 3.

	$F_{very\ slow}$ at				contact time enhancement factor
	5 d	14 d	91 d	959 d	
PAHs					
PHE	0.16 (0.16; 0.18)	0.12 (0.18; 0.07)	0.15 (0.14; 0.15)	0.32	2.2
FLU	0.17 (0.20; 0.14)	0.13 (0.13; 0.12)	0.14 (0.14; 0.14)	0.26	1.8
B*a*A	0.16 (0.15; 0.17)	0.12 (0.12; 0.11)	0.14 (0.14; 0.14)	0.37	2.7
CHR	0.10 (0.10; 0.10)	0.09 (0.09; 0.08)	0.14 (0.14; 0.15)	0.36	3.3
B*b*F	0.25 (0.24; 0.26)	0.22 (0.22; 0.21)	0.22 (0.22; 0.22)	0.48	2.1
B*k*F	0.22 (0.22; 0.22)	0.19 (0.19; 0.19)	0.19 (0.18; 0.19)	0.46	2.3
B*a*P	0.31 (0.27; 0.35)	0.20 (0.19; 0.20)	0.26 (0.24; 0.28)	0.50	2.0
chlorobenzenes					
penta	0.0 (0.0; 0.0)	0.20 (0.10; 0.30)	0.23 (0.20; 0.26)		
hexa	0.09 (0.01; 0.18)	0.10 (0.05; 0.16)	0.12 (0.12; 0.12)	0.50	4.7

For abbreviations of PAHs: see footnote Table 2.

DISCUSSION

Bioavailability

The results of the present study demonstrate that contact time affects the bioavailability of different compounds to a different extent. A contact time of 959 days strongly reduced the bioavailability of chlorobenzenes. The bioavailability of most PCBs, PAHs and p,p'-DDE was also lowered after 959 days, but the reduction in bioavailability was generally small (factor 2 or less). To date, we did not find other publications in which the relative effect of contact time on the bioavailability of different classes of compounds has been reported. Belfroid et al. [8] found a relatively high difference in bioavailability between field and freshly added chlorobenzenes (factor 2 to 30), compared with the difference between field and laboratory contaminated PAHs in other studies (factor 4 to 6; 1.4 to 3.3) ([7, 47]). However, the studies on chlorobenzenes and PAHs are not completely comparable because of differences in sediment, organism and experimental set-up.

The final results on the effect of contact time on the bioavailability to oligochaetes in the present study fit flush in collections of reported data in the literature. Landrum et al. [5] found a reduction of uptake clearance rate k_s for amphipods with a factor of 0.7 to 5.9 and 0.9 to 5.6 for PAHs after a contact time of 60 and 150 days respectively. Kukkonen and Landrum [6] determined a reduction factor for the BSAF of benzo[*a*]pyrene for amphipods of 1.3, 1.3, and 1.6 after 7, 180 and 390 days respectively. Recalculated kinetic BSAF values (k_s/k_e) for oligochaetes reportedly declined with a factor of 1.4 and 2.0 for pyrene and benzo[*a*]pyrene respectively after 28 days of contact time in a study of Leppänen and Kukkonen [58]. We found a reduction in the bioavailability of PAHs to oligochaetes of 1.2-2.3 after a contact time of 959 days. Although the final effects of contact time on the bioavailability are quite similar among these studies, some disagreement can be found in the time scale of the effect of residence time: The effect of residence time was most pronounced in the initial days and seemed to level off later in most studies, whereas in our study, a measurable decline was yet found after a prolonged period of several years.

The observed decline in bioavailability in the present study was measured in an interval in which no significant change in the quality of the sediment organic was observed. As a consequence, the effect of contact time on the bioavailability was not related to structural or compositional changes during storage of the sediment.

Sequestration

The effect of contact time on sequestration was most pronounced for hexachlorobenzene. In Figure 3, concentrations of rapidly, slowly and very slowly desorbing hexachlorobenzene are shown for the different contact times. These concentrations were calculated from

$$C_{s,i} = C_s * F_i \quad \text{(i = rap, slow or very slow)} \qquad (2)$$

with $C_{s,i}$ = concentration of rapidly, slowly or very slowly desorbing compound, C_s = total concentration of compound in sediment (mg/kg organic carbon) and F_i = rapidly, slowly or very slowly desorbing fraction.

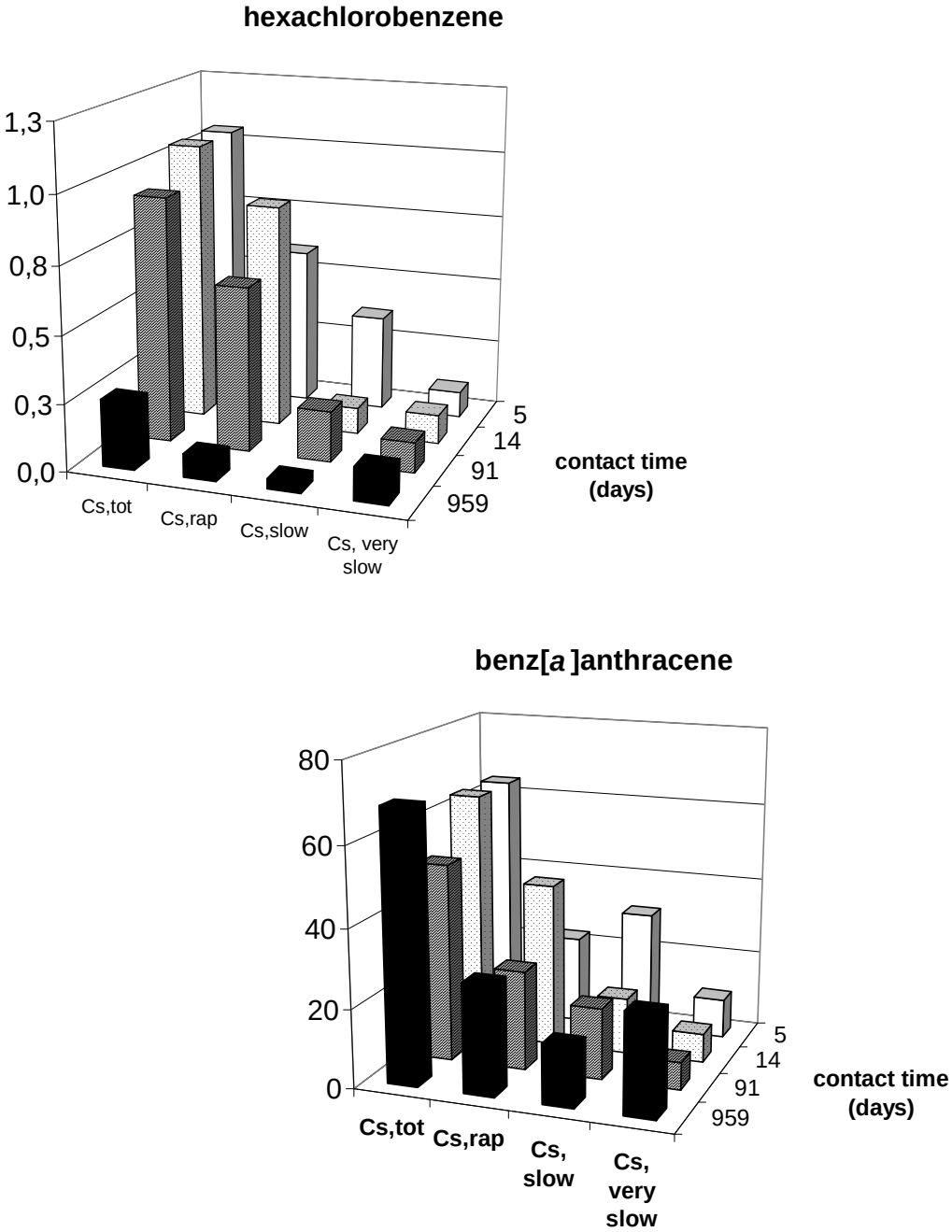

Fig. 3 Average total concentrations and concentrations of rapidly, slowly and very slowly hexachlorobenzene and benz[*a*]anthracene (mg/kg organic carbon) in lab-contaminated sediment at different contact times (days) of the contaminant in the sediment.

The figure shows that 1) the total concentration of hexachlorobenzene in the sediment decreased with a factor of approximately 4, 2) the decline in the total concentration is caused by losses from the rapidly and slowly desorbing compounds and 3) the concentration of hexachlorobenzene in the very slowly desorbing compartment increased only slightly in time. It has been shown before that loss processes as biodegradation are selectively occuring for rapidly desorbing fractions (e.g. [11]). The effect of contact time on the distribution over rapidly, slowly and very slowly desorbing compartments is essentially similar for hexachlorobenzene and the PAHs, as is illustrated for hexachlorobenzene and benz[*a*]anthracene in Figure 3: A contact time of 959 days resulted in a small increase of chemicals residing in the very slowly desorbing compartment for both compounds. In conclusion, the conspicuous decline in rapidly desorbing fractions of chlorobenzenes in time was a result of loss processes of rapidly desorbing compounds rather than a redistribution over the rapidly, slowly and very slowly desorbing fraction.

Significant fractions of slowly and very slowly desorbing fraction were measured in our study within a few weeks after addition of the test compounds to the sediment (Table 4). The rapidly desorbing fraction decreased with a factor of 1.2 for the 3-4 ringed PAHs and 1.9 for the hexachlorobenzene respectively after a contact time of 959 days. Cornelissen et al. ([13]) observed slowly desorbing fractions of chlorobenzenes and PCBs of 0.1 to 0.3 after 2 days of contact time in OVP-sediment. These fractions increased with a factor of 1.4-3.1 within 34 days of contact time. A much stronger effect of contact time was found by Ten Hulscher et al [59] in Lake Ketelmeer sediment, where the rapidly desorbing fraction of hexachlorobenzene dropped from approximately 0.7 after two days of contact time to 0.5 and less than 0.05 after 180 and 620 days respectively. The concentration of hexachlorobenzene in the sediment was almost constant in this experiment. It can be concluded that the effect of contact time on sequestration is quite variable for different sediments.

Bioavailability & sequestration

The results of the present study confirm the hypothesis that sequestration is related to the bioavailability of HOC for benthic deposit-feeders. The markedly higher effect of contact time on the bioavailability of chlorobenzenes compared with the effect for PAHs corresponded with a relatively high decrease in the rapidly desorbing fraction. In Figure 4, the BSAF of the PAHs and chlorobenzenes at the three exposures was plotted against the rapidly desorbing fractions. The BSAF values varied about one order of magnitude. The figure demonstrates a highly significant relationship between bioavailability and sequestration. More than 75 % of the variability in BSAF for the different compounds and exposures could be explained by differences in rapidly desorbing fractions. In former studies, rapidly desorbing fractions [47] or fractions desorbing within 48 hours [9] has been

related to the bioavailability of HOC to other benthic deposit-feeders. Lamoureux and Brownawell [9] studied the desorption and accumulation of native PAHs, PCBs and linear alkylbenzenes (LAB) from sediment in the deposit-feeding clam *Yoldia limatula*. The BSAF of PAHs and LABs were correlated with the fraction that desorbed within 48 hours (r^2 > 0.78). Kraaij et al [47] demonstrated a good correlation (r^2 of 0.76) between the bioavailability of native and laboratorium-added PAHs for the amphipod *Corophium volutator* and rapidly desorbing fractions.

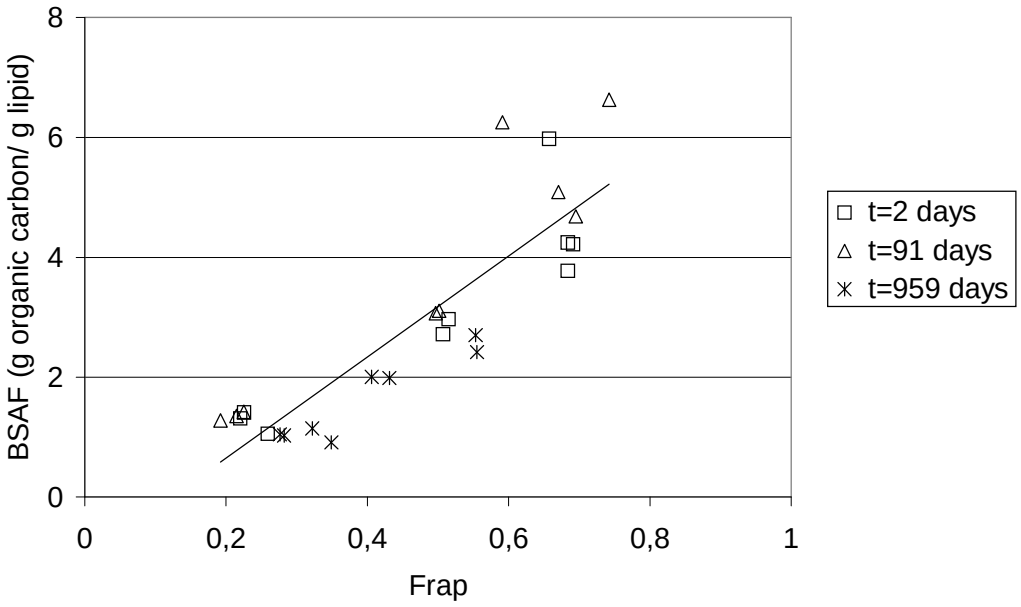

Fig. 4 Average Biota to sediment accumulation factor (BSAF) (g organic carbon/g lipid) of chlorobenzenes (CB) and PAHs for oligochaetes (*Tubificidae*) in lab-contaminated sediment versus average rapidly desorbing fraction F_{rap} at the first, second and third exposure, starting at a contact time of 2, 91 and 959 days respectively. Regression line for all compounds included. R^2 of regression = 0.77.

In conclusion, the effect of contact time on the bioavailability of hydrophobic organic compounds is different for different classes of compounds. The reduction in bioavailability after a contact time of 959 days was a factor 1 to 2 for PCBs, PAHs and p,p'-DDE, while this contact time reduced the bioavailability of chlorobenzenes with a factor of 5 to 18. The results correspond with the sequestration measurements: A contact time of 959 days resulted in a relatively high decrease in the rapidly desorbing fractions for the chlorobenzenes, compared with the PAHs. The pronounced decrease was attributed to the relatively strong impact of loss processes, such as biodegradation and volatilization, on the rapidly desorbing compartment of the chlorobenzenes.

The present study confirms that sequestration measurements are indicative of

differences in the bioavailability of HOC to benthic deposit-feeders, induced by differences in compound properties, and contact time. The relationship between desorption and bioavailability is as yet based on circumstantial evidence from a limited number of experiments. Causality of the relationship has to be confirmed in order to justify a broader applicability on different organisms, sediments and chemicals. In further publications, we will report on the processes involved in the relationship between desorption and bioavailability.

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Chapter 4 Direct evidence of sequestration in sediments affecting the bioavailability of hydrophobic organic chemicals to benthic deposit-feeders

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Abstract - Current risk-assessment of hydrophobic organic contaminants (HOC) in sediments is hampered by a high variability in accumulation of HOC by deposit-feeders from sediment organic carbon. Recent literature suggests that the variability can be attributed to differences in sequestration in the sediment. In the present study, we investigated whether this relationship is causal. We determined biota to sediment accumulation factors (BSAF) and sequestration of PAHs and PCBs in a manipulated sediment as well as in an original, nonmanipulated sediment. Sequestration was measured as the distribution over rapidly and slowly desorbing fractions. The manipulation, 48 hours suspending with Tenax, strongly reduced the rapidly desorbing fraction while other factors such as contact time and sediment and compound properties remained constant. In this manner, sequestration was isolated as a variable. The BSAF values showed a decrease proportional to the decrease in rapidly desorbing fractions. The results provide direct evidence of a causal relationship between sequestration and bioavailability to deposit-feeders.

INTRODUCTION

Sediments have been polluted with hydrophobic organic contaminants (HOC) worldwide. High levels of pollutants have been measured in harbours, industrialized or urbanized coastal zones and in sedimentation areas of (formerly) heavily polluted rivers (e.g. [43, 60]. The accumulation of HOC in benthic organisms may lead to detrimental effects on benthic communities and to biomagnification in food chains (e.g. [21]5]). As high levels of HOC in sediment often persist in time, remediation efforts are sometimes necessary in order to sustain or restore ecosystems. The efficiency of the use of resources in such environmental remediation projects greatly benefits from sound site-specific assessments of the bioaccumulation of the contaminants.

The biota to sediment accumulation factors (BSAF), i.e. the ratio of the concentration in lipids of organisms and the concentration in sediment organic carbon, is frequently used as a measure of bioaccumulation. Differences in BSAF reflect differential bioavailability.

The bioavailability of hydrophobic organic compounds (HOC) to benthic deposit-feeders is determined by a complex interaction of biological, ecological and chemical factors such as habitat, feeding behaviour and sorption/desorption processes in the sediment (e.g. [32],[19]). As a result, bioavailability might vary between sediments. Literature reviews on BSAF values revealed a variability of about 2-3 orders of magnitude ([3, 4]). Several research projects have been initiated in order to develop tools to estimate the bioavailability of HOC in soils or sediments. Bioavailability has for example been related to fractions that were extracted with mild solvents or solid phase extraction disks ([34, 61-64]), mild selective supercritical fluid extraction (SFE) [65], in vitro gut-fluid extraction [33, 66, 67], fractions that associated with water-soluble cyclodextrines [68],[69] or persulfate oxidated fractions [70].

Recently, evidence has been provided that the bioavailability of HOC to microorganisms and benthic deposit-feeders is strongly related to sequestration. Sequestration is defined in this paper as the formation of relatively slowly desorbing fractions. Slow desorption might be induced by high activation energies at specific 'sites' or diffusional limitations in pores or condensed regions of the organic matter matrix (e.g. [14, 15, 24]). One attempt to characterize sequestration is the determination of rapidly, slowly and very slowly desorbing fractions with distinctly different desorption rates and sorption behaviour ([17, 46]. Some authors demonstrated that the degradation of hydrophobic compounds was strongly related to the amount of chemical being desorbed within a specified lapse of time (e.g. [10, 29]), [11]). Lamoureux and Brownawell [9] showed a good correlation between the bioavailability of PAHs and linear alkylbenzenes in sediment-cores to deposit-feeding clams and the fraction desorbing in 24 hours. In a previous study, we demonstrated that 76 % of the variability of BSAF of native and freshly added PAHs could be explained by differences in rapidly desorbing fractions [47].

As the reported relationship might have been coincidental, causality in the relationship between sequestration and bioavailability still needs to be verified. Direct evidence of such a relationship would imply a more general applicability. In the present study we tested the hypothesis of a causal relationship between sequestration and the bioavailability of HOC to benthic deposit-feeders (*tubificidae*). To that end we performed a controlled experiment with sediment that had been contaminated in the laboratory with a wide range of PAHs and PCBs approximately 2.5 years prior to the experiment.. A portion of the sediment was artificially manipulated in order to reduce the rapidly desorbing fraction while keeping other factors constant. Tubificidae were exposed to the manipulated sediment as well as to the original, nonmanipulated sediment. In both sediments, we determined the BSAF as well as the distribution over rapidly, slowly and very slowly desorbing fractions and investigated the relationship between BSAF and the rapidly desorbing fraction.

EXPERIMENTAL APPROACH

Oligochaetes

Tubifex were reared at the laboratory on noncontaminated paper pulp. The culture consisted of the species *Limnodrilus hoffmeisteri* Claparède and *Tubifex tubifex* Müller (both family Tubificidae). At the start of the experiment, the culture was contaminated with some *Lumbriculus variegatus* (family Lumbriculidae) individuals (<10 %). As the percentage of impurity was low, we will refer to 'tubifex' in this paper. The oligochaetes were acclimatized for at least 24 hours at 10 °C.

Chemicals

Phenanthrene, fluoranthene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, 2-ethylanthracene, 7-methylbenzo[*a*]pyrene, 2,2',5,5'-tetrachlorobiphenyl (PCB 52), 2,2',4,5,5'-pentachlorobiphenyl (PCB 101), 2,3,3',5,6-pentachlorobiphenyl (PCB 112), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), 2,2',4,4',5,6'-hexachlorobiphenyl (PCB 154), 2,2',4,4',6,6'-hexachlorobiphenyl (PCB 155), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138) and 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180) were obtained from various commercial sources (PCB numbering according to IUPAC). Tenax TA (60-80 mesh; 177-250 µm) was obtained from Chrompack (Bergen op Zoom, The Netherlands). Florisil was obtained from Merck (Darmstadt, Germany) and Alumina was purchased from ICN Biomedicals (Eschwege, Germany).

Sediment sampling, characterization and lab-contamination

Sediment was collected from a relatively unpolluted area (Oostvaarderplassen, the Netherlands). The organic carbon content was determined using an element analyzer (Carlo Erba NA 1500, Milan, Italy) after removal of carbonates with phosphoric acid. The measurements were performed on sediments at the beginning of the experiment. Prior to lab-contamination, the batch of sediment was suspended with copper-free water (ratio 0.71 L/L wet sediment) in order to facilitate homogenisation. The suspension was lab-contaminated by dropwise addition of an acetone solution containing the test compounds (final ratio 2.3 ml acetone per 100 g dry sediment). During the lab-contamination procedure, the suspension was vigorously mixed by a concrete mixer. The contaminated sediment was stored for 959 days at 10 °C.

Stripping procedure

Triplicate sediment samples (30 g dry weight) were suspended in separation funnels with 300 ml copper-free water and 30 g Tenax was added. The Tenax efficiently removes

compounds that have desorbed to the water phase. The separation funnels with the sediment/Tenax suspension were shaken for 48 hours on a shaking machine. The time span of stripping was optimized in a preceding experiment with different stripping times (6, 16.5 and 48 hours). It was found that in a time-span of 48 hours, most of the rapidly desorbing compounds were stripped from the sediment, while the total concentration was only reduced by a factor of approximately 2 to 5. After completion of the shaking interval, the floating Tenax was separated from the sediment. The remaining Tenax in the funnel was washed three times in order to remove some remaining sediment particles. The collected manipulated sediment was gently centrifuged to retrieve a similar density as the untreated sediment and to discard remaining Tenax beads. The treated sediment was visually almost completely free from Tenax beads. The procedure was repeated three times in a two-week interval before exposure of the oligochaetes in order to obtain the necessary amount of treated sediment. All batches of treated sediment were collected in one bucket and thoroughly mixed before filling out beakers. The time interval between treatment and start of the exposure was one week.

Bioaccumulation experiments

250 ml beakers were filled with either treated or untreated sediment (68.5 g dry weight; final density approximately 34 %). The filled beakers were placed in two separate aquaria at 10 °C and copper-free tap-water was added. The level of the overlying water was higher than the height of the beakers so that the overlying water was homogeneously mixed for all beakers. Overlying water was aerated mildly to keep the worms in the sediment. Approximately 0.25 g of tubifex were transferred to each beaker. Three beakers containing untreated sediment and tubifex and two beakers with treated sediment and tubifex were taken out of the aquaria at 21, 28 and 34 days of exposure. The sediment was gently suspended with 170 ml of copper-free water in order to facilitate sampling of the worms. Worms were sampled and kept in copper-free water for 25 hours in order to allow worms to empty their guts. The losses of test compounds due to elimination in clean water during this period were calculated from the elimination rate constant k_2. This value was experimentally determined or estimated, using the model of Sijm and van der Linde [49]. Losses were below 5 %, except for 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, phenanthrene and fluoranthene. Losses were 39.8, 12.4, 29.1 and 7.0 % for 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, phenanthrene and fluoranthene respectively. The concentrations of all compounds were corrected for losses due to elimination in the clean water. The worms were gently dried with a paper towel, killed in liquid nitrogen and stored at –25 ° C. The lipid content of the worms at the beginning and end of the exposure was determined in duplicate samples of worms according to Folch et al. [35]. The average lipid content of the oligochaetes at the start and the end of the exposure was 2.44 (average of 2.30 and 2.57 %) and 1.75 % (average of 1.75 and 1.75 %). We used an average lipid content 2.09 % in further

calculations. Sediment was sampled for concentration measurements at the end of the exposure period.

Desorption experiment

Sediment desorption characteristics were studied according to Cornelissen et al. ([13]). An equivalent of 3 g dry weight sediment was shaken for 10 days with 100 mL copper free water and 3 g Tenax TA (60-80 mesh; 177-250 µm). Tenax effectively removes solutes from the aqueous phase. During the desorption experiment the Tenax beads were replaced at 10 predetermined time points. The desorption experiment was performed with untreated sediment at the start of the exposure ('U (0)'), treated sediment at the start of the exposure ('T (0)') and treated sediment at the end of the exposure ('T (35)'). The measurement on the latter batch ('T (35)') was included in order to check forshifts in the distribution over rapidly and slowly desorbing fractions in the treated sediment during the exposure interval.

Analyses

Oligochaetes

Oligochaetes were extracted using Matrix Solid Phase Dispersion [37] and prepared for PAH analyses as described earlier [47]. A subsample of the extract for PCB-analyses was transferred to a column filled with two portions of 1.75 g of activated silica to which respectively 0.77 g concentrated sulphuric acid and 0.58 g 1 M potassium hydroxide was added respectively. Both portions were cleaned with acetonitrile prior to use. The column was eluted with 18 ml of acetonitrile and the eluate was concentrated to approximately 0.15 to 1 ml under a gentle flow of nitrogen. Recoveries of the internal standards 2-ethyl-anthracene, 7-methyl-benzo[*a*]pyrene, PCB 112 and PCB 154 were 92.6 %, 80.5 %, 67.2 % and 55.9 % on average, with a coefficient of variation of 21.1, 27.5, 27.3 and 25.9 % (n=25). Concentrations of phenanthrene, fluoranthene, benz[*a*]anthracene and chrysene were corrected with the recovery of 2-ethyl-anthracene and concentrations of benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene were corrected with the recovery of 7-methyl-benzo[*a*]pyrene. Concentrations of all chlorinated compounds were corrected with the recovery of PCB 112, except for PCB 138, PCB 153, PCB 156 and PCB180, that were corrected with the recovery of PCB 154.

Sediment and Tenax

Sediments were reflux extracted for 4 hours with hexane and methanol (1/1) [50] and prepared for analyses of PAHs as described earlier [47]. A subsample of the extract for GC analysis was transferred to a glass column filled with 1.5 g Al_2SO_3 (7.5 % water content) and 1.5 g SiO_2 (5 % water content) and eluted with 30 ml hexane. The eluate was concentrated to

0.2-1 ml.Each portion of Tenax was extracted by shaking with 20 ml hexane, and compounds were transferred to acetontrile prior for PAH analysis with HPLC.

All extracts for PAH analyses were analysed by C_{18} reversed phase HPLC with fluorescence detection. Chlorinated compounds were analysed by GC-ECD. Recovery of the internal standard for the PAHs was 77.0 % for 2-ethylanthracene and 69.2 % for 7-methylbenzo[*a*]pyrene on average. Coefficient of variation (c.v.) for the recovery of these two compounds was 9.7 and 6.8 % on average (n=6). Recovery of PCB 112 and PCB 154 was 27.7 and 25.8 %. Coefficient of variation (c.v.) of the recovery of PCB 112 and PCB 154 was 21.5 and 22.1 % respectively (n = 6). The relatively low recovery for the PCBs was probably due to incomplete sulphur precipitation. As the coefficient of variation of the recoveries of the PCBs was reasonably low, corrections for recoveries were allowed. Concentrations in the sediment were corrected in the same way as for the tubifex samples.

Data analysis

The bioavailability of compounds at different contact times was compared by comparing calculated biota to sediment accumulation factors (BSAF). The BSAFs were calculated by dividing the average of the lipid normalized concentration in the oligochaetes by the organic carbon normalized concentration in the sediment. The concentrations in the oligochaetes were normalized for lipid content by dividing with the average of the lipid content at the start and end of the experiment. For all compounds, an average of the accumulation at approximately 3, 4 and 5 weeks was used for the calculation of the BSAF. Based on results from the first exposure, we calculated that the average accumulation of all compounds at that time was at more than 85 % of equilibrium. The data of the desorption experiment were fitted to a triphasic desorption model as described by Cornelissen et al. [51]. The fitting procedure resulted in estimations of rapidly, slowly and very slowly desorbing fractions (F_{rap}, F_{slow} and $F_{very\ slow}$) for each compound and contact time.

RESULTS

Sediment properties, treatment and sequestration

The organic carbon contents of the untreated and the treated sediment were 3.2 % and 4.2 %, respectively. We attributed this difference to remaining very small amounts of Tenax beads in the treated sediment or to some heterogeneity in the sediment batch.

The treatment of the lab-contaminated sediment with Tenax resulted in a decline in the total concentration of HOC in the sediment (Table 1). The concentration of PAHs, PCBs and hexachlorobenzene in the treated sediment was on average 3.6, 3.2 and 2.5 times lower than in the untreated sediment.

The effect of the treatment on the distribution over rapidly, slowly and very slowly

desorbing compounds is demonstrated in Fig.1. In this graph, the concentrations of the rapidly, slowly and very slowly desorbing compounds in the untreated and the treated sediment are plotted for some of the PAHs and PCBs. These concentrations were calculated by multiplying the total concentration in the sediment organic carbon (Table 1) with the rapidly, slowly or very slowly desorbing fractions (Table 2) respectively. Figure 1 illustrates the removal of rapidly desorbing PAHs and PCBs in the treatment. The concentrations of rapidly desorbing compounds in the treated sediment declined with an average factor of 17.9 and 8.5 for PAHs and PCBs during the treatment, which was much higher than the decline in total concentrations. Also, a significant part of the slowly desorbing PAHs and most of the slowly desorbing PCBs were stripped. In contrast, the concentrations of the very slowly desorbing fractions of PAHs and PCB were hardly affected by the treatment.. Within the 35 days exposure, a small reverse trend was observed: Concentrations of rapidly and slowly desorbing compounds increased and concentrations of very slowly desorbing compounds decreased slightly. We accounted for this redistribution process by using the average values of the estimates of the concentrations or fractions at the start and end of the exposure in further calculations.

Table 1. Average concentrations of test compounds in treated (48 hours shaking of aqueous suspension with Tenax) and untreated lab-contaminated sediment (mg/kg organic carbon). Standard deviation between brackets (n=3). PHE = phenanthrene, FLU = fluoranthene, B*a*A = benz[*a*]anthracene, CHR = chrysene, B*b*F = benzo[*b*]fluoranthene, B*k*F = benzo[*k*]fluoranthene, B*a*P = benzo[*a*]pyrene.

	untreated		treated	
PAHs				
PHE	43.93	(1.29)	14.52	(0.23)
FLU	71.16	(2.53)	17.28	(0.45)
B*a*A	68.74	(2.44)	17.37	(1.47)
CHR	69.26	(3.89)	15.07	(1.08)
B*b*F	84.44	(9.92)	25.36	(0.77)
B*k*F	77.64	(3.82)	22.48	(0.46)
B*a*P	64.52	(2.68)	23.44	(1.41)
Chlorobenzenes				
hexachlorobenzene	0.25	(0.06)	0.10	(0.02)
PCBs				
PCB 52	1.20	(0.44)	0.31	(0.02)
PCB 101	0.42	(0.05)	0.14	(0.01)
PCB 118	0.75	(0.09)	0.22	(0.03)
PCB 153	0.77	(0.06)	0.26	(0.02)
PCB 138	0.63	(0.03)	0.22	(0.04)
PCB 180	0.45	(0.03)	0.15	(0.02)

Table 2 Rapidly, slowly and very slowly desorbing fractions (F_{rap}, F_{slow} and $F_{very\ slow}$) in untreated sediment at the start of the exposure period (U(0)), treated sediment (48 hours shaking of aqueous suspension with Tenax) at the start of the exposure period (T(0)) and treated sediment at the end of the exposure period (T(E)). For abbreviations of PAHs, see Table 1.

	F_{rap}			F_{slow}			$F_{very\ slow}$		
	U(0)	T(0)	T(E)	U(0)	T(0)	T(E)	U(0)	T(0)	T(E)
PAHs									
PHE	55.3	6.23	8.48	12.7	6.56	12.6	32.0	87.2	79.0
FLU	55.5	9.55	6.90	18.8	0.00	8.79	25.7	90.5	84.3
B*a*A	40.6	5.66	N.D[1]	22.2	7.87	N.D.	37.2	86.5	N.D.
CHR	43.1	5.67	7.09	20.5	8.87	11.4	36.4	85.5	81.5
B*b*F	27.6	5.89	8.69	24.1	11.5	15.4	48.3	82.6	75.9
B*k*F	32.2	5.77	9.09	21.5	11.6	15.8	46.3	82.6	75.1
B*a*P	28.2	5.10	7.92	21.8	11.8	15.9	50.0	83.1	76.2
CB									
hexa	34.9	N.D.	N.D.	15.4	N.D.	N.D.	49.7	N.D.	N.D.
PCBs									
52	41.9	N.D.	16.3	27.7	N.D.	41.0	30.4	N.D.	42.7
101	41.9	5.42	11.1	20.7	0.00	28.6	37.4	94.6	60.3
118	31.5	12.6	8.60	21.6	0.00	18.0	46.9	87.4	73.4
138	40.2	13.4	8.53	15.3	0.00	19.8	44.5	86.7	71.8
153	38.2	8.80	17.9	16.3	0.00	14.8	45.5	91.2	67.4
180	29.1	19.9	7.48	21.7	0.00	16.3	49.2	80.1	76.3

1N.D. = not determined

Bioaccumulation

In Figure 2, biota to sediment accumulation factors (BSAF) of PAHs and PCBs for the oligochaetes in the untreated and treated sediment are plotted. The BSAF values in the treated sediment were a factor of 3 to 9 for the PCBs and 3 to 35 for the PAHs lower than in the untreated sediment.

Sequestration and bioaccumulation

In Figure 3 and 4, the BSAF of PAHs and PCBs in the untreated and treated sediment are plotted versus the rapidly desorbing fractions F_{rap}. The data points of pairs of measurements for one compound in two sediments are connected by a line. The figures demonstrate that the BSAF is strongly related to the rapidly desorbing fraction for each PAH and PCB. The reduction in the rapidly desorbing fraction of these compounds in the treated sediment is accompanied by an almost proportional reduction in BSAF.

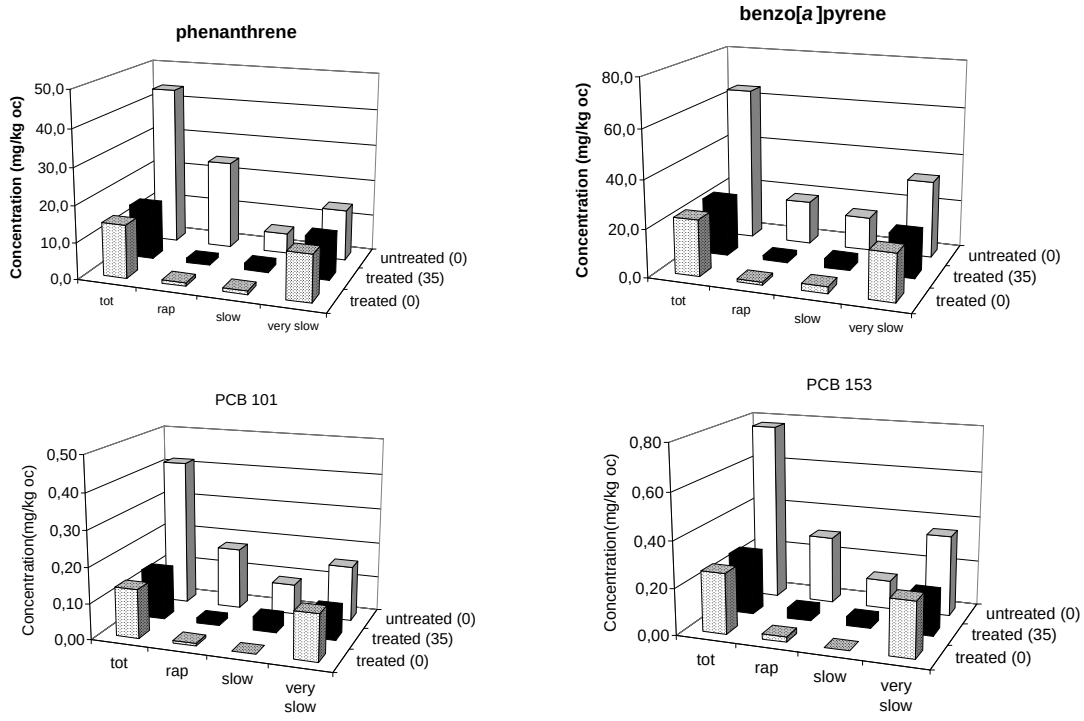

Fig. 1 Total concentrations and concentrations (in mg/kg organic carbon) of rapidly, slowly and very slowly desorbing compounds (tot, rap, slow and very slow) of two PAHs (phenanthrene and benzo[*a*]pyrene) and two PCBs (PCB 101 and PCB 153) in treated (48 hours shaking of aqueous suspension with Tenax) and untreated lab-contaminated sediment at 0 and 35 days after the start of the bioaccumulation experiment.

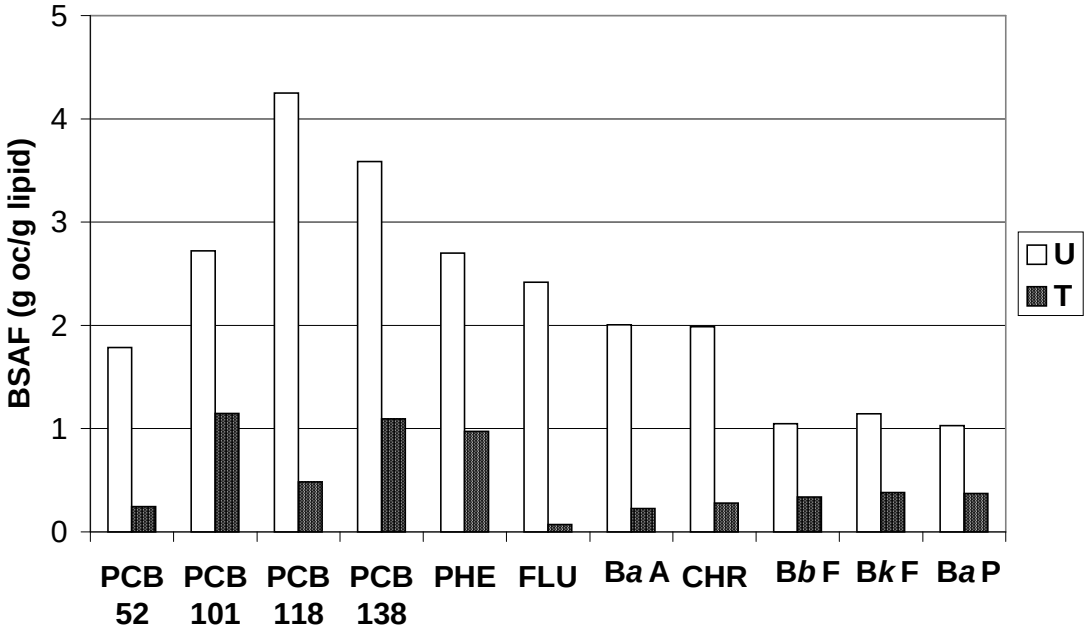

Fig. 2 Biota to sediment accumulation factor (BSAF) (g organic carbon/g lipid) of PAHs and PCBs in untreated (U) and treated (T) (48 hours shaking of aqueous suspension with Tenax) lab-contaminated sediment. For abbreviations of PAHs, see Table 1.

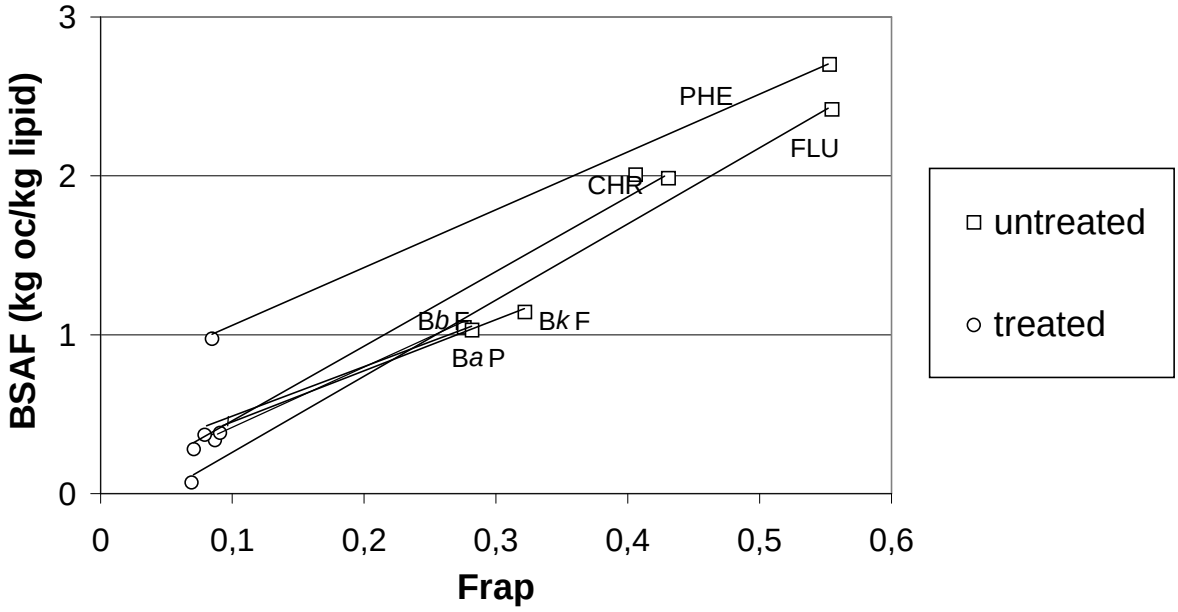

Fig. 3 Biota to sediment accumulation factor (BSAF) (in g organic carbon/g lipid) of PAHs in treated (48 hours shaking of aqueous suspension with Tenax) and untreated lab-contaminated sediment versus rapidly desorbing fractions. The data points for identical PAHs (in untreated and treated sediment) are connected by a line. For abbreviations of PAHs, see Table 1.

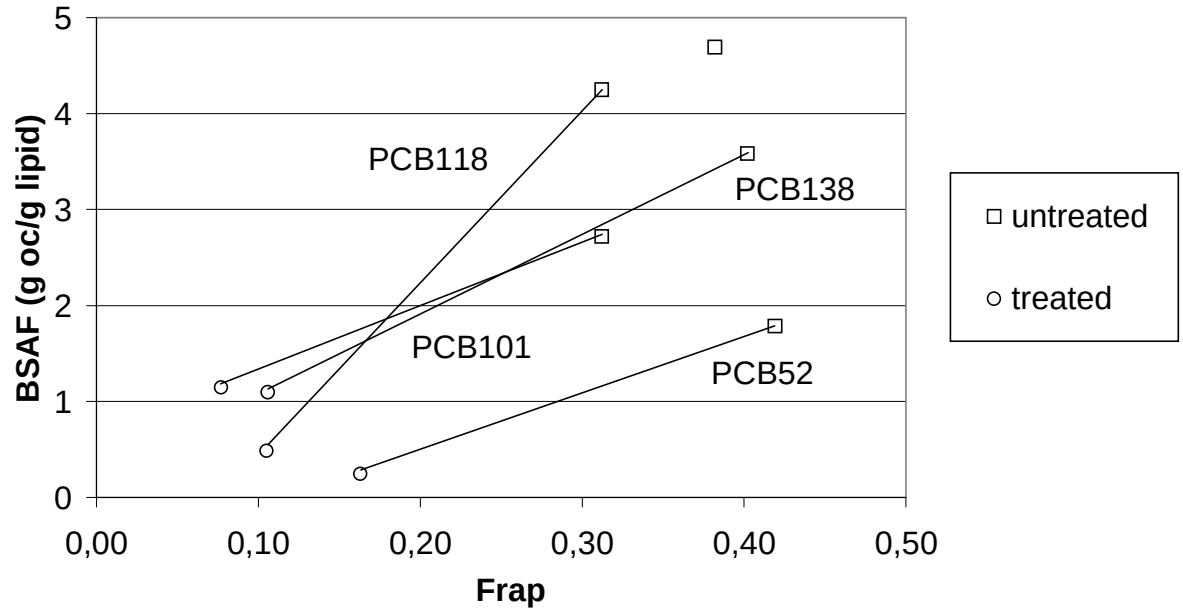

Fig. 4 Biota to sediment accumulation factor (BSAF) (in g organic carbon/g lipid) of PCBs in treated (48 hours shaking of aqueous suspension with Tenax) and untreated lab-contaminated sediment versus rapidly desorbing fractions. The data points for identical PCBs (in untreated and treated sediment) are connected by a line.

In Figure 5, bioaccumulation and sequestration measurements for the PAHs and PCBs are plotted in one graph. The measurements of the PCBs are more scattered than those of the PAHs. The scattering is probably due to the lower concentration levels of the PCBs in the sediments, which resulted in some analytical uncertainty. (see table 1). Hence, estimations of the rapidly desorbing fractions are less accurate for the PCBs than for the PAHs.

The bioaccumulation and sequestration measurements of the PAHs and PCBs were fitted to an empirical linear equation of the form

$$BSAF = a \cdot F_{rap} + b \qquad (1)$$

resulting in the following regression equations:

$$BSAF = 4.52 \cdot F_{rap} - 0.01 \qquad (R^2 = 0.93;\ n = 12) \text{ for PAHs} \qquad (2)$$

and

$$BSAF = 8.91 \cdot F_{rap} - 0.03 \qquad (R^2 = 0.57;\ n = 8) \text{ for PCBs} \qquad (3).$$

The regression equations are plotted in Figure 5. The value of R^2 indicates that approximately 93 and 57 % of the variance in the BSAF values of PAHs and PCBs respectively in the manipulated and nonmanipulated sediment is explained by differences in the rapidly desorbing fraction.

The estimated desorption rates of the rapidly as well as the slowly desorbing fraction (0.17 to 1.81 and 0.01-0.10 hr^{-1} respectively) indicate that desorption of both fractions is sufficiently high to enable uptake in the organisms within the experimental time-frame. The present results do not yield a clear indication of the relative bioavailability of the rapidly and slowly desorbing fractions as high values of F_{rap} coincide with high values of F_{slow}. We investigated the relationship between F_{rap}, instead of $F_{rap} + F_{slow}$, and BSAF because the freely dissolved concentrations in the pore water are proportional to the rapidly desorbing fraction ([71]). As uptake from the pore water seems to be a major route of accumulation of HOC in oligochaetes in sediments with refractory organic matter as the sediment used in our experiments, we hypothesized that F_{rap} is a relevant measure of bioavailability. In addition, the density of the organisms (approximately 1 g lipid per 500 g organic carbon) was relatively low. As a result, the rapidly desorbing fraction was not depleted and could fully sustain HOC uptake into the test organisms..

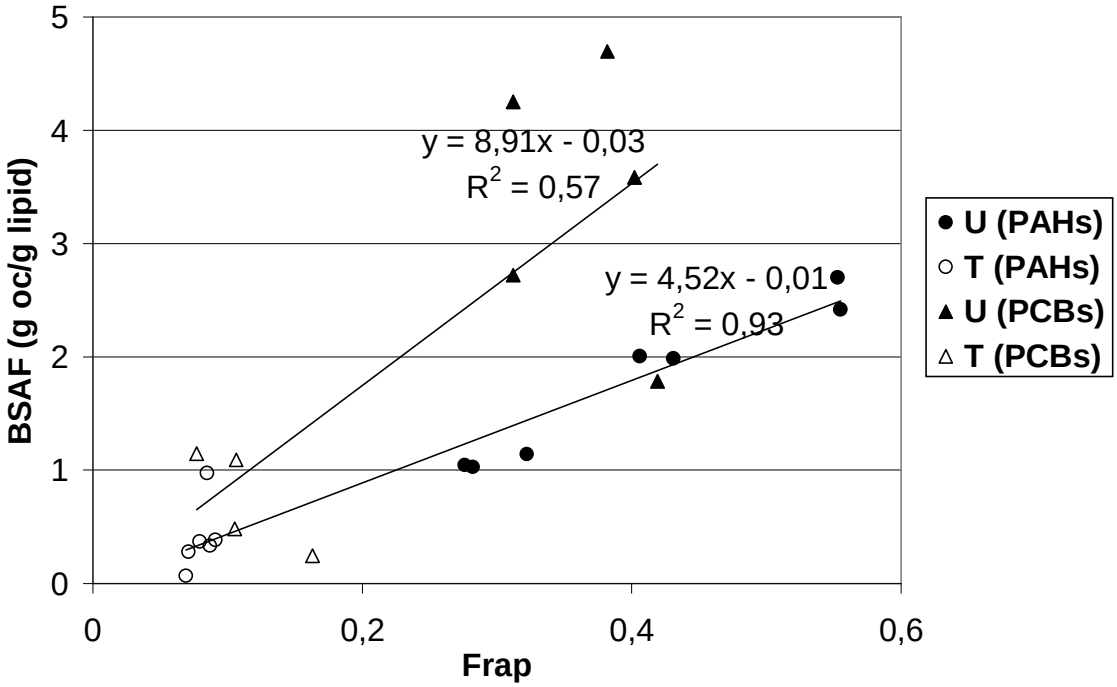

Fig. 5 Biota to sediment accumulation factor (BSAF) (in g organic carbon/g lipid) of both PAHs and PCBs in treated (48 hours shaking of aqueous suspension with Tenax) (T) and untreated (U) lab-contaminated sediment versus rapidly desorbing fractions. Trendlines and regression equations included for the measurements of the PCBs and the PAHs.

DISCUSSION

Sediment

In the present study, accumulation was measured in a manipulated sediment and compared to the accumulation in the original sediment. The quality of the sediment was kept virtually constant. The accumulation experiments with the treated and untreated sediment were performed simultaneously, with oligochaetes from one batch, in order to further ensure similarity of the exposure. Consequently, the two sediments only differed in concentration levels and sequestration status. The differences in total concentration and sequestration were artificially induced by treating one of the sediments with Tenax. The impact of the Tenax manipulation on the concentration of rapidly desorbing compounds was much higher than on the total concentration.

During the 35-days exposure of the oligochaetes, most of the concentration of rapidly and slowly desorbing compounds in the treated sediment increased and the concentration of very slowly desorbing compounds decreased (Figure 1). We expected no such a change in the untreated sediment because of two reasons: 1. No significant change in the rapidly desorbing fraction of PAHs was observed within 137 weeks preceding the exposure. 2. Biota did not change the rapidly desorbing fraction of PAHs in a lab-contaminated sediment (Chapter 2).

Schlebaum et al. [72] also observed redistribution of pentachlorobenzene from a slowly desorbing compartment to the rapidly desorbing compartment after gas-purging of the sediment. These results indicate that redistribution from slowly to rapidly desorbing compartments may occur. Interestingly, the redistribution process is not instantaneous, indicating a retarded transport between the compartments.

Bioaccumulation

The BSAF-values of all PAHs and PCBs in the manipulated sediment were much lower than in the untreated sediment. As differences in BSAF are equivalent to differences in bioavailability, we conclude that the manipulation of the sediment reduced the bioavailability to the deposit-feeding tubificidae. We will now address the factors that may have caused this reduction in bioavailability.

Sequestration and bioavailability

The manipulation of the sediment resulted in a decrease in total concentration levels and a much higher decrease in rapidly and slowly desorbing fractions. Formerly reported studies (e.g. [21]) suggest that BSAF is independent of total concentrations of HOC in sediment organic carbon. The quality of the sediment, compound properties, contact time and composition of the tubifex culture were kept virtually constant. We conclude that the strong reduction in BSAF in the manipulated sediment is due to the strong decrease in rapidly and slowly desorbing fractions as no other factors could have interfered and attributed to the established relationship. The present study confirms therefore that the strong relationship between sequestration and bioavailability to deposit-feeders that was found in this and other studies([9], chapter 2 and 3) is causal.

Results from a previous study also indicated mechanistic causality between sequestration and bioavailability (Chapter 2). It was demonstrated that the bioavailability of seven identical PAHs, were either native or added in the laboratory, was related to the rapidly desorbing fractions. The present study is more conclusive as all compounds were added in the laboratory and the way of contamination does not interfere with the results. Also, the applied range in sequestration was higher than in the former study.

The findings of the present study are not fully in accordance with results on solubilization of PAHs in the gastro-intestinal tract by Weston and Mayer [66]. These authors measured a similar or even higher solubilization of native phenanthrene and benzo[*a*]pyrene compared to freshly added PAHs. These findings do not correspond with our results, as the sequestration of native compounds is usually similar or higher than the sequestration of compounds added in the laboratory. Combined measurements on sequestration and digestive fluid extractability would provide more insight in the solubilization of sequestered compounds in the gastro-intestinal tract of deposit-feeders.

The relationships between BSAF and rapidly desorbing fractions were reasonably similar for PCBs and PAHs. This finding suggeststhat the variability in BSAF values of PCBs and PAHs ([4], [3]) might be partly explained by differences in sequestration status, rather than differences in compound properties.

The intercepts of the regression equations of BSAF versus the rapidly desorbing fractions F_{rap} for the PAHs and PCBs are small. Assuming that the intercept is negligible, the ratio of BSAF and F_{rap} is estimated by the regression coefficient 'a' (see equation (1)). The ratio of BSAF and F_{rap} might be more or less constant for different deposit-feeders, treatments and sediments. In a former study on the accumulation of native and freshly added PAH in amphipods (*Corophium volutator*), a ratio of 3.1 was found, which is close to the ratio of 4.5 that was found for PAHs in the present study [47]. In the present study, we derived the relationship $BSAF = a \cdot F_{rap} + b$, that can be used in risk-assessment. Recently, a relatively quick method of determining rapidly desorbing fractions was developed [73]. Regression equations for one HOC compound class and organism may be extrapolated to other classes and organisms. The regression equations for PAHs and PCBs and oligochaetes in the present study and for PAHs and amphipods in a former study were reasonably similar, suggesting that extrapolation might provide a satisfactory estimation of BSAF values. The risk assessment for deposit-feeders might be soundly conservative for the whole community of macrobenthos: Deposit-feeders are likely to be at highest risk as they are exposed to sediment-associated chemicals by both passive diffusion from the pore and or overlying water as well as by ingestion of sediment particles.

The results of the present study demonstrate that the relationship between bioavailability for deposit-feeders and sequestration, measured as the distribution over rapidly, slowly and very slowly desorbing fractions, is causal. This implies broad applicability of the relationship in risk assessment.

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Chapter 5 Equilibrium partitioning of non-sequestered fractions of hydrophobic organic chemicals between sediment, pore water and benthic deposit-feeders (*Tubificidae*).

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ABSTRACT - In the present paper, we tested equilibrium partitioning of non-sequestered, rapidly desorbing compounds between sediment, pore water and tubificidae. To that end, we determined freely dissolved concentrations of a range of HOC in pore water using matrix-SPME and bioconcentration factors for tubificidae in water. These data were employed to interpret measurements on bioaccumulation in tubificidae and sequestration in the same sediment as reported in the preceding chapter. Bioaccumulation, based on equilibrium partitioning, was estimated by multiplying the pore water concentrations and the bioconcentration factors. Experimental and estimated steady state accumulation was similar within an average factor of 0.8 and 1.8 for the chlorobenzenes and the PAHs respectively. The freely dissolved concentrations in the pore water were proportional to the concentrations of rapidly desorbing compounds, confirming linear sorption behaviour for the rapidly desorbing compartment. We conclude that steady-state accumulation of HOC in benthic deposit-feeders can be fully reconciled with equilibrium partitioning of rapidly desorbing compounds between sediment, pore water and deposit-feeders. The bioaccumulation of sediment associated HOC in deposit-feeders can be estimated from measurements of freely dissolved concentrations in the pore water.

INTRODUCTION

High concentrations of hydrophobic organic chemicals (HOC) are encountered worldwide in sediments (e.g. [1]). Loss processes are usually slow for this type of chemicals in sediments. As a consequence, high concentration levels persist even at reduced input of pollutants. Benthic organisms may be at risk when accumulation surpasses toxic threshold levels. The accumulation of HOC cannot be accurately assessed from total concentration measurements, as biota to sediment accumulation factors (BSAF) are largely variable among different sediments and compounds (e.g. [3]).

The differences in BSAF can for a large part be attributed to differences in extent of sequestration. Lamoureux and Brownawell [9] found that the BSAF of PAHs and LABs were

correlated with the fraction that desorbed within 48 hours ($r^2 > 0.78$). Kraaij and co-authors [47] demonstrated a good correlation (r^2 of 0.76) between the BSAF of native and laboratorium-added PAHs and rapidly desorbing fractions. Moreover, direct evidence of a causal relationship between sequestration and BSAF was reported in a preceding chapter ([74]). The differences in BSAF, induced by sequestration, might be mediated by freely dissolved concentrations in the pore water, as uptake from pore water is an important uptake pathway.

The key question in the assessment of bioaccumulation is if HOC distribute between pore water and biota according to the equilibrium partitioning theory (EqP). EqP assumes that distribution over biota and pore water can be described with a constant aqueous bioconcentration factor (BCF). If so, bioaccumulation in sediments with differing extents of sequestration could simply be assessed from freely dissolved concentrations in the pore water by multiplication with this BCF. In spite of numerous efforts to validate the EqP (e.g. [75, 76]), considerable uncertainty concerning the applicability of the EqP still remains. This is largely due to the indirect methods of measuring freely dissolved concentrations in the pore water.

Another question that needs to be addressed in order to understand the biota-pore water/sediment system is the distribution over sequestered, non-sequestered compartments and the pore water. It is assumed that distribution over the non-sequestered, rapidly desorbing fraction and pore water can be characterized with a constant partition coefficient $K_{oc,rap}$ (e.g. [17, 45]). Measurements on the freely dissolved concentration in the pore water are essential for validating this hypothesis, but direct measurements are not available up to date.

One of the implicit assumptions of the EqP is a constant K_{oc} value for the total sediment, independent of sequestration status. The hypothesis of linear partitioning for a non-sequestered, rapidly desorbing fraction would violate the EqP as it predicts that the K_{oc} is a variable determined by the rapidly desorbing fraction (F_{rap}), according to

$$K_{oc} = K_{oc,rap}/F_{rap} \qquad (1)$$

In a former experiment, sediment was contaminated in the laboratory with PAHs, PCBs, chlorobenzenes and p,p'-DDE. One sub-sample was treated with Tenax in order to reduce the rapidly desorbing fraction, while sediment-HOC contact time, and sediment quality remained constant. Oligochaetes (*Tubificidae*) were exposed to the sediments and analysed. In addition, the distribution of compounds between the rapidly, slowly and very slowly desorbing fractions were measured, performing desorption experiments with subsamples of the sediments. BSAF of PAHs as well as PCBs were proportional to the rapidly desorbing fraction in the two sediments.

In the present study we employed pore water and aqueous bioconcentration measurements to reinterpret the relationship between sequestration and BSAF. We tested equilibrium partitioning between biota and pore water on one hand, and 'rapidly desorbing' fractions and pore water on the other hand, by measuring bioconcentration factors and in-situ freely dissolved concentrations in the pore water with a recently developed technique, matrix-

SPME [77]. The measurements were performed for a range of compounds of different classes in an unmanipulated lab-contaminated sediment as well as in a manipulated sediment. These measurements were combined with the bioaccumulation and sequestration measurements in the same sediments from a preceding study [74]. The combination of measurements enabled us to study the distribution of HOC in the sediment, pore water and biota system and the effect of sequestration on this distribution in a comprehensive way.

EXPERIMENTAL APPROACH

General outline of the experiments

Freely dissolved concentrations in the pore water were determined using matrix-SPME. The measurements were performed in manipulated and unmanipulated lab-contaminated sediment. The sediment contaminated in the laboratory with hydrophobic organic chemicals 959 days prior to the start of the experiments (see preceding chapter). A portion of the sediment was manipulated by suspending the sediment with water and Tenax for 48 hours in order to reduce the rapidly desorbing fraction. The pore water measurements were finished simultaneous with bioaccumulation experiments in the same sediments. Results on the bioaccumulation, sediment concentrations and the distribution over rapidly and slowly desorbing fractions in the treated and untreated sediments were discussed in detail in the previous chapter [74].

In addition to these experiments, oligochaetes from the same culture were exposed to water that was contaminated in the laboratory with chlorobenzenes and PCBs, and analysed. This aquatic experiment yielded bioconcentration factors (BCF) for some test-compounds for the test-species. The present paper gives an integrative interpretation of the pore water and bioconcentration measurements as well as the previously reported results on the manipulated and untreated sediment.

Oligochaetes

Tubifex were reared at the laboratory on noncontaminated paper pulp. The culture consisted of the species *Limnodrilus hoffmeisteri* Claparède and *Tubifex tubifex* Müller (both family Tubificidae). At the start of the experiment, the culture was contaminated with some *Lumbriculus variegatus* (family Lumbriculidae) individuals (<10 %). As the percentage of impurity was low, we will refer to ‘tubifex’ in this paper. The oligochaetes were acclimatized for at least 24 hours at 10 °C.

Chemicals

Test compounds phenanthrene, fluoranthene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, 1,2,3,4-tetrachlorobenzene,

pentachlorobenzene, hexachlorobenzene, p,p'-DDE (2,2-bis(4-chlorophenyl)-1,1-dichloroethylene), 2,2',5,5'-tetrachlorobiphenyl (PCB 52), 2,3,5,6-tetrachlorobiphenyl (PCB 65), 2,2',4,5,5'-pentachlorobiphenyl (PCB 101), 2,3,3',4,4'-pentachlorobiphenyl (PCB 105), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138) 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) and 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180) and internal standards 2-ethylanthracene, 7-methylbenzo[*a*]pyrene, 2,3,3',5,6-pentachlorobiphenyl (PCB 112) and 2,2',4,4',5,6'-hexachlorobiphenyl (PCB 154), were obtained from various commercial sources (PCB numbering according to IUPAC). Log K_{ow} and m/z masses used for GC-MS detection are listed in Table 2 and 1 respectively. Octadecyl (C-18) was purchased from JT Baker (Phillipsburg, NJ, USA). Florisil was obtained from Merck (Darmstadt, Germany) and Alumina was purchased from ICN Biomedicals (Eschwege, Germany).

Pore water measurements

Freely dissolved concentrations in the pore water were measured with matrix-SPME, developed by Mayer et al. [77]. Matrix-SPME is a direct probe of in-situ concentrations of freely dissolved compounds and can be used to precisely quantify analytes up to the pg/l range. In short, pieces of an optical fiber with a glass core diameter of 200 µm and a poly-(dimethylsiloxane) (PDMS) coating of 15 µm, supplied by Fiberguide Industries (Striling, NJ), were cut to a length of 100 mm and washed twice with methanol and ultrapure water prior to exposing them to sediment. To that end, three vials of 40 ml were filled with 35 g of untreated sediment three days after the start of the tubifex accumulation period. Two fibers were exposed to each vial with sediment by inserting the fibers through the silicone/PTFE septum of the caps. The vials were then placed on a shaking device (200 rpm, 3-mm orbit) at 25 ºC and sampled within two days after completion of the oligochaete exposure experiment. The exposure time was 33 days, thus exceeding the equilibration period for such hydrophobic compounds reported by Mayer et al. [77]. The timing of the fiber sampling was set reasonably simultaneous with the last biota sampling so that steady-state concentrations in fiber and organism could be compared in identical sediment batches, aged for identical times. The mass of compounds in the fiber was measured by GC-MS. Solvent standards were used to calibrate the amounts of test compounds injected into the GC. No measurable amounts of test compounds were detected upon inserting duplicate blank fibers into the injector. The concentration in the fiber was calculated as n_{PDMS}/V_{PDMS} or the ratio of the desorbed mass of compounds in the PDMS coating and the volume of the part of the PDMS coating that was thermally desorbed (= lower part of 56 ± 2 mm). Subsequently, freely dissolved pore water concentrations of the test compounds were calculated as the ratio of the concentration in the PDMS coating and partition coefficients for the PDMS and water ($K_{PDMS,\ water}$) that were determined in our laboratory [78]. For some compounds, an experimental $K_{PDMS,\ water}$ was not available. For these compounds, a $K_{PDMS,\ water}$ was calculated using the regression equation in [78].

In the treated sediment, the uptake kinetics and the reequilibration processes following the Tenax treatment step would be interfering and result in a poorly defined signal. Therefore, we chose to expose fibers to the untreated and treated sediment for two days at the end of the exposure experiment and determine the mass of compounds in the PDMS coating for the treated and the untreated sediment. The pore water concentrations in the treated sediment were then calculated as

$$C_{pore,treated} = C_{pore,untreated} * \frac{n_{PDMS,treated}}{n_{PDMS,untreated}} \quad (2)$$

with C_{pore} is freely dissolved concentration in pore water (pg/l). Three vials of 15 ml were filled with 11 g of untreated sediment and treated sediment at three days after the start of the biota accumulation period and kept at 10 ºC. Then, two fiber were exposed to each vial containing sediment. The vials were subsequently agitated at 25 ºC on the shaking device and sampled after 45 to 48 hours. The approach gives an estimation of the freely dissolved concentration at the end of the bioaccumulation experiment. The concentrations in the treated sediment at the start and end of the experiment might be slightly different, as the sequestration status at the start and end of experiment (see preceding chapter) is different. We will compare the pore water concentrations at the end of the experiment with the steady-state accumulation and the rapidly desorbing fraction at the end of the experiment.

From the two exposed fibers present in each vial, one was used for injection while the other served as a back up. The fiber was carefully withdrawn via the septum. Within 10-20 s after sampling, the fiber was inserted into the injector of a Varian 3400 CX gas chromatograph equipped with a 1078 programmable injector, and a Saturn 2000 Ion Trap mass-spectrometric detector. The insertliner of the injector had an internal diameter (I.D.) of 0.8 mm and was operated in a splitless mode, with a splitless time of 15 min. The initial temperature of the injector of 60 ºC was held for 0.2 minutes, then increased rapidly at 150 ºC/min to 250 ºC, and remained at that temperature for 15 minutes before cooling down. We used a 30-m DB5-MS capillary column, with an I.D. of 0.25 mm and a film thickness of 0.25 µm. The oven temperature was initially held at 70 ºC for 15 minutes, then increased to 290 ºC at a heating rate of 10 ºC/min. The final temperature was held for 3 minutes. Detection was based on mass spectrometry, using a Varian Saturn 2000 ion trap. The mass spectrometer was operated in the EI (Electron Impact) (electron energy 70 eV) and SIS (Selected Ion Storage) cluster analysis mode with a scan time of 0.6 s and an ACG target value of 20.000. The ions employed for detection of the test compounds are listed in Table 2.

Bio-concentration experiment

Copper free water was contaminated with pentachlorobenzene, hexachlorobenzene and

PCB 65 using the generator column method ([79]). The collected water was diluted with copper free water. The concentrations were chosen so that accumulation in the tubifex would be detectable without exerting toxic effects. A 25-L aquarium was filled with 16.5 L of the contaminated water and closed with a glass plate. Twelve batches of approximately 0.5 g and one batch of approximately 3 g tubifex were placed in glass petri dishes covered with metal gauze, which were put into the aquarium. The water in the aquarium was gently homogenized by two stirring bars on the bottom of the aquarium between the petri dishes. At 2, 19, 21.5, 25, 42.5, 44, 67.5 and 139.5 hours, single or duplicate samples of 200 ml water were taken and one or two petri dishes with 0.5 g of tubifex were sampled. At the start of the experiment, 200 ml water was sampled and two subsamples of 0.5 g tubifex were set aside for blank analysis. Lipid content was determined on two subsamples of 1.5 g tubifex that were put aside at the start of the experiment and on two samples of 1.5 g tubifex, exposed together in one petri dish for 139.5 hours. The average lipid content of the oligochaetes at the start and the end of the exposure was 4.7 (average of 4.8 and 4.6 %) and 4.3 % (average of 4.1 and 4.4 %). We used an average lipid content 4.5 % in further calculations.

Analyses

Oligochaetes

Oligochaetes were extracted using Matrix Solid Phase Dispersion [37]. A subsample of the extract for GC-analyses was transferred to a column filled with two portions of 1.75 g of activated silica to which respectively 0.77 g concentrated sulphuric acid and 0.58 g 1 M potassium hydroxide was added respectively. Both portions were cleaned with acetonitrile prior to use. The column was eluted with 18 ml of acetonitrile and the eluate was concentrated to approximately 0.15 to 1 ml under a gentle flow of nitrogen.

Water

Water samples of the bio-concentration experiment were extracted with 10 ml hexane. The separated water was again extracted with 5 ml hexane. The hexane extracts were combined and concentrated under a gentle flow of nitrogen to approximately 1 ml.

Data analysis

The data of the bio-concentration experiment were fitted with the first-order model for uptake into organisms, using the solver function of Microsoft ®Excel 97 SR1. The fit resulted in parameter estimations of the uptake and elimination rate constants k_1 (L/(kg· hr) and k_2 (1/hr). A kinetic BCF was calculated as the ratio of k_1 and k_2. For hexachlorobenzene, k_2 could not be estimated correctly. Therefore, a k_2 for hexachlorobenzene and tubificidae that was determined in a separate elimination experiment (see chapter 3) was used for the

calculation of the kinetic BCF of hexachlorobenzene.

The bio-concentration experiment was performed with a selected group of HOC only. For the estimation of BCF-values of all compounds, the experimental BCF-values and BCF values from the literature were extrapolated. For this purpose, we selected lipid normalized data for oligochaetes (Tubifidae [80] *Eisenia andrei* [81]) from water-exposure experiments in the literature. The experimental and literature log BCF values were linearly related to the log K_{ow}-values of the compounds (Figure 2), as was also found for other taxonomic groups as fish (e.g. [82]). The regression equation was used to calculate BCF values for all test compounds.

The sediment bioaccumulation data for the tubificidae in the preceding chapter were reinterpreted as follows: We calculated the steady-state accumulation of HOC in the oligochaetes according to equilibrium partitioning of rapidly desorbing compounds with:

$$C_b = BCF \cdot C_p \qquad (3)$$

with C_b = concentration in biota lipid (µg/kg lipid), BCF is lipid normalized bioconcentration factor (L/kg lipid) and C_p is the concentration in the pore water (µg/L).

Theory

The equilibrium partitioning theory (EqP) describes the distribution of HOC between sediment and biota with three compartments: biota, sediment organic carbon and pore water [21] (Figure 1). The model assumes that the pore water and the organic carbon are in equilibrium, and that the distribution over organic carbon and pore water can be described by a constant partition coefficient K_{oc}:

$$K_{oc} = C_s/C_p \qquad (4)$$

with C_s is the concentration in the sediment organic carbon (µg/g oc)

As a consequence, it is assumed that at steady state accumulation from the sediment pore water and from the sediment organic carbon via ingestion is equal to accumulation from pore water exposure only. If it is assumed that the partition coefficient between biota and pore water is constant, the assumption can be written as:

$$BCF = C_b/C_p \qquad (5).$$

Combination of equation (3), (4) and (5) results into:

$$BSAF = BCF/K_{oc} \quad (6)$$

or $$C_b = BCF \cdot C_s/K_{oc} \quad (7)$$

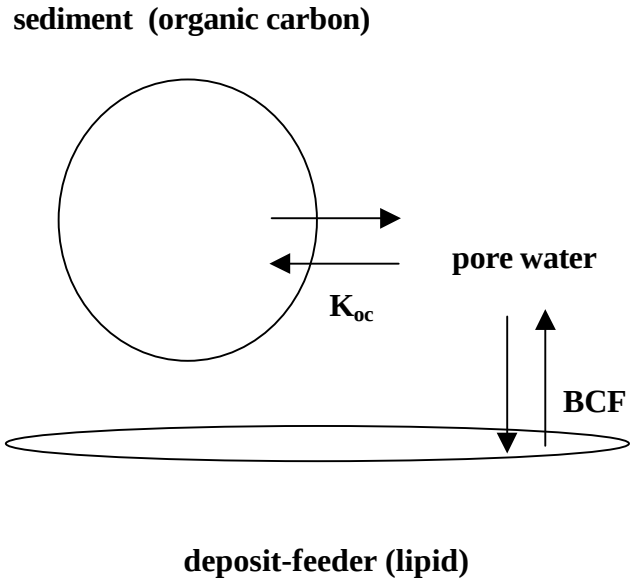


Fig. 1 Model of distribution of hydrophobic organic chemicals in sediments according to the equilibrium partitioning model (EqP). K_{oc} = partition coefficient between sediment and pore water (l/kg oc); BCF = bioconcentration factor (L/kg lipid).

RESULTS & DISCUSSION

Porewater measurements

Matrix-SPME was employed to measure in-situ pore water concentrations. The freely dissolved concentrations of the HOC in the pore water are shown in Table 1. The measured concentrations were in the pico- to nanogram per liter range for the chlorinated compounds (approximately 2-5000 pg/l) and in the nano- to microgram per liter range for the PAHs (approximately 1- 4000 ng/l). The precision of the measurements was satisfactory : The average coefficient of variation was 8.2 and 15.0 % in the untreated and treated sediment respectively, benzo[*b*]fluoranthene and benzo[*k*]fluoranthene exempted. The manipulation of the sediment resulted in pore water concentrations that were a factor of 7.5 to 19 and 14 to 59 lower than in the unmanipulated sediment for the chlorinated compounds and the PAHs respectively.

Table 1 Freely dissolved concentration in pore water (C_p) (pg/l) in untreated (U) and treated (T) (48 hour suspension with Tenax) lab-contaminated sediment. Standard deviation is given in parentheses. m/z used in GC-MS detection.

	m/z	C_p (pg/l)			
		U		T	
Chlorobenzenes					
1,2,3,4	216	1208	(172)	N.D.[1]	
penta	250	1500	(266)	N.D.	
hexa	285	420	(44)	30[2]	
PCB's					
52	292	2851	(143)	169	(26)
65	292	5234	(483)	275	(41)
101	326	560	(4)	30	(5)
105	326	524	(31)	N.D.	
118	326	825	(82)	83	(12)
138	361	270	(5)	35	(2)
153	361	421	(29)	55	(12)
156	361	127	(8)	N.D.	
180	396	123	(11)	16	(5)
PAHs					
PHE	178	3627727	(349705)	61359	(4782)
FLU	202	649415	(39832)	17092	(2274)
B*a*A	228	51083	(1095)	3096[2]	
CHR	228	73195	(1764)	N.D.	
B*b*F	252	9688	(7442)	N.D.	
B*k*F	252	7777	(6327)	N.D.	
Other compounds					
p,p'-DDE	318	664	(39)	50	(7)

[1] not determined; [2] one measurement; PHE = phenanthrene, FLU = fluoranthene, B*a*A = benz[*a*]anthracene, CHR = chrysene, B*b*F = benzo[*b*]fluoranthene, B*k*F = benzo[*k*]fluoranthene.

Bioconcentration factors

In Table 2, the measured bioconcentration factors (BCF) for pentachlorobenzene, hexachlorobenzene and PCB 52 are listed, together with estimated BCF-values for the other compounds. The bioconcentration factors we determined for tubificidae matched the literature values (Figure 2). Linear regression analysis of all data resulted in the regression equation for oligochaetes

$$\log BCF = 1.05 \cdot \log K_{ow} - 0.20 \qquad (8).$$

This regression equation was used to estimate the BCF values of the test compounds in Table 2. All experimental as well as literature values of BCF are within approximately half a log unit (approximately factor 3) of the regression equation. Therefore, we expect that the

estimated values are reasonably accurate. Biotransformation of pyrene in tubificidae is negligible. Concentrations of metabolites of pyrene in subsamples of tubificidae that were exposed for two and five weeks to lab-contaminated sediment were below 1 % of concentrations of the parent compound (personal communication, G. Stroomberg). Therefore no significant deviations of BCF-values for PAHs are expected. No experimental bioconcentration data were available for compounds with a log K_{ow} higher than 5.9 and lower than 3.7. Therefore, BCF values were calculated for compounds in the log K_{ow} range of 3.7 to 5.9 exclusively.

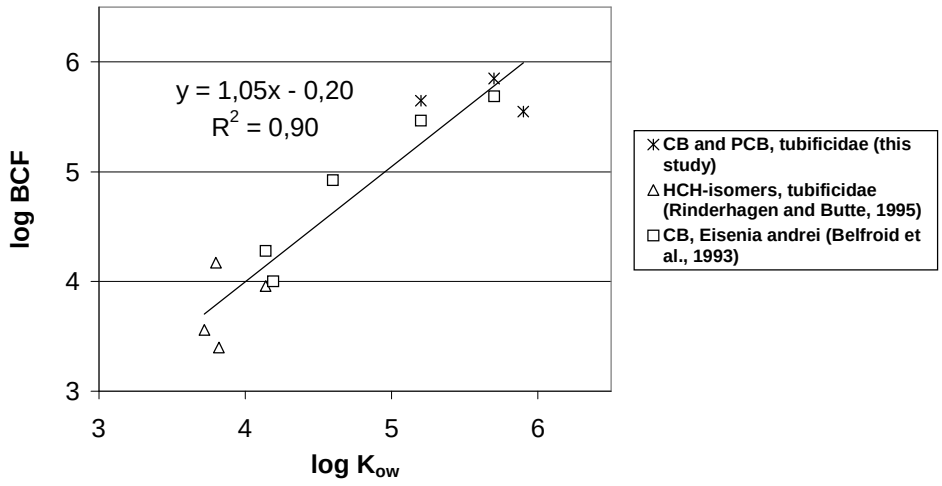


Fig. 2 Measured and selected experimental literature bioconcentration factors (BCF) (ml/g lipid) of hydrophobic organic chemicals in oligochaetes plotted versus log K_{ow}. Line represents linear regression, fitted for all data. CB = chlorobenzenes, PCB = polychlorinated biphenyls, HCH = hexachlorocyclohexane. References in [80] and [81].

Partitioning between pore water and biota

We will now evaluate the bioaccumulation data in combination with the pore water concentrations and bioconcentration data in order to test equilibrium partitioning. At equilibrium partitioning, steady-state accumulation can be calculated as the pore water concentration multiplied by the bio-concentration factor. In Figure 3, calculated accumulation of PAHs and chlorobenzenes for the treated and the untreated sediment is plotted versus the measured accumulation in these sediments. The figure demonstrates close similarity between the calculated and the measured accumulation values. Differences between experimental steady state and modeled equilibrium partitioning concentration are within approximately half a log-unit. In addition, the calculated values do not systematically over- or underestimate the actual bioaccumulation for any of the sediments or chemicals.

The good agreement is robust as a broad range of chemicals in the pore water was tested. Moreover, the relationship holds for the treated as well as the untreated sediment with two different concentration levels in the pore water. Therefore, the results indicate that equilibrium partitioning between the pore water and the oligochaetes provides a satisfactory description of steady-state bioaccumulation of HOC from sediments.

Table 2 Log K_{ow} and bioconcentration factors (BCF) of test compounds for tubificidae.

	log K_{ow}	log BCF (ml/g lipid)
Chlorobenzenes		
1,2,3,4	4.6[1]	4.67[5]
penta	5.2[1]	5.65[4]
		5.24[5]
hexa	5.7[1]	5.85[4]
		5.82[5]
PCBs		
65	5.9[2]	5.55[4]
		6.46[5]
PAHs		
PHE	4.57[3]	4.60[5]
FLU	5.23[3]	5.29[5]
B*a*A	5.91[3]	6.01[5]
CHR	5.81[3]	5.90[5]

[1] in [55]; [2] average of selected values for tetrachlorobiphenyl congeners (n=12) in [56] ; [3] in [54]; [4]experimental value; [5]calculated with regression equation: log BCF = 1.05 · log K_{ow} – 0.20, based on literature values for oligochaetes (see text for explanation). For abbreviations of PAHs: See footnote table 1.

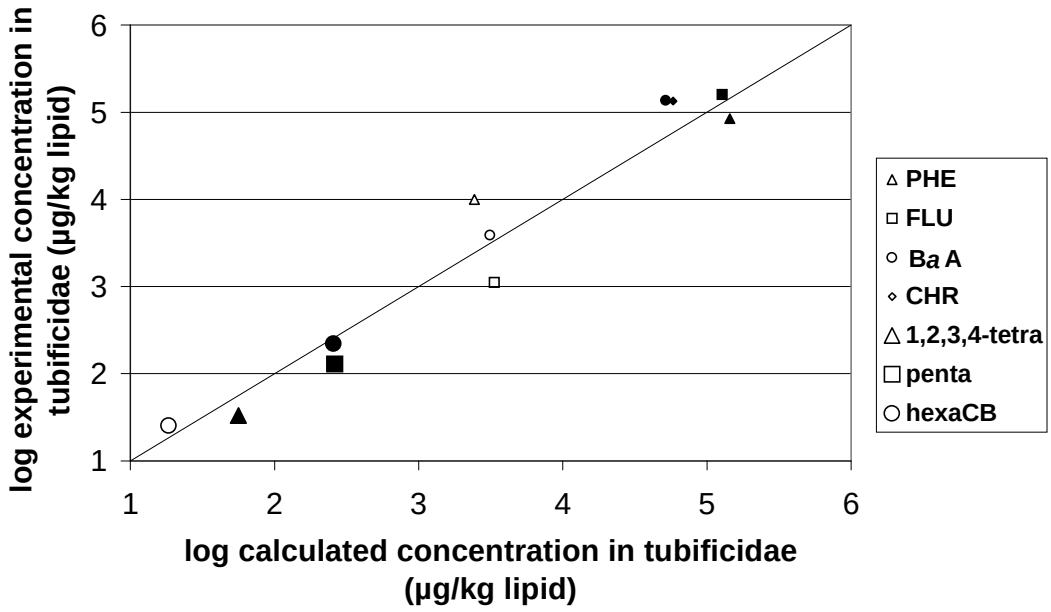


Fig. 3 Measured versus calculated steady-state accumulation of chlorobenzenes and PAHs in oligochaetes (*tubificidae*). The estimated steady-state accumulation is calculated by multiplying the freely dissolved concentration in the pore water with the bioconcentration factor (BCF). One to one line included. Closed symbols: measurements in untreated lab-contaminated sediment, open symbols: measurements in treated lab-contaminated sediment (48 hours shaking of aqueous suspension with Tenax). For abbreviations of PAHs: See footnote table 1.

Partitioning between sediment and pore water

Simultaneously, we tested linear partitioning between the rapidly desorbing compartment and the pore water by measuring freely dissolved concentrations of PAHs and PCBs in the pore water as well as concentrations of rapidly desorbing PAHs and PCBs. These concentrations were calculated by multiplying the total concentration in the sediment organic carbon with the rapidly desorbing fraction, reported in the preceding chapter. In Figure 4 B, the partition coefficient for the rapidly desorbing fraction $K_{oc,rap}$, defined as the concentration of rapidly desorbing compounds divided by the freely dissolved concentration, is plotted for the treated and the untreated sediment. Both the sequestration status as well as the freely dissolved concentrations in the pore water of the treated and untreated sediment are highly different, while the $K_{oc,\ rap}$ values for the treated and untreated sediment are reasonably similar, indicating linear partitioning. The ratio of the $K_{oc,rap}$ for the treated and the $K_{oc,rap}$ for the untreated sediment is close to unity for all compounds, varying between 0.6 and 3.0. In addition, no systematic differences in $K_{oc,rap}$ between the treated and untreated sediment are found. In contrast, the partition coefficient for the total sediment K_{oc} is consistently higher for the treated sediment, compared with the untreated sediment (Figure 4A). The K_{oc} values for the treated and untreated sediment show a much higher variability

than the $K_{oc,rap}$, with a ratio of K_{oc} for the treated and untreated sediment varying between 2.5 and 19.5. In conclusion, the variation in the partition coefficient for sediments differing in sequestration status is highly reduced when the partition coefficient is based on concentrations of rapidly desorbing compounds instead of total concentrations of compounds.

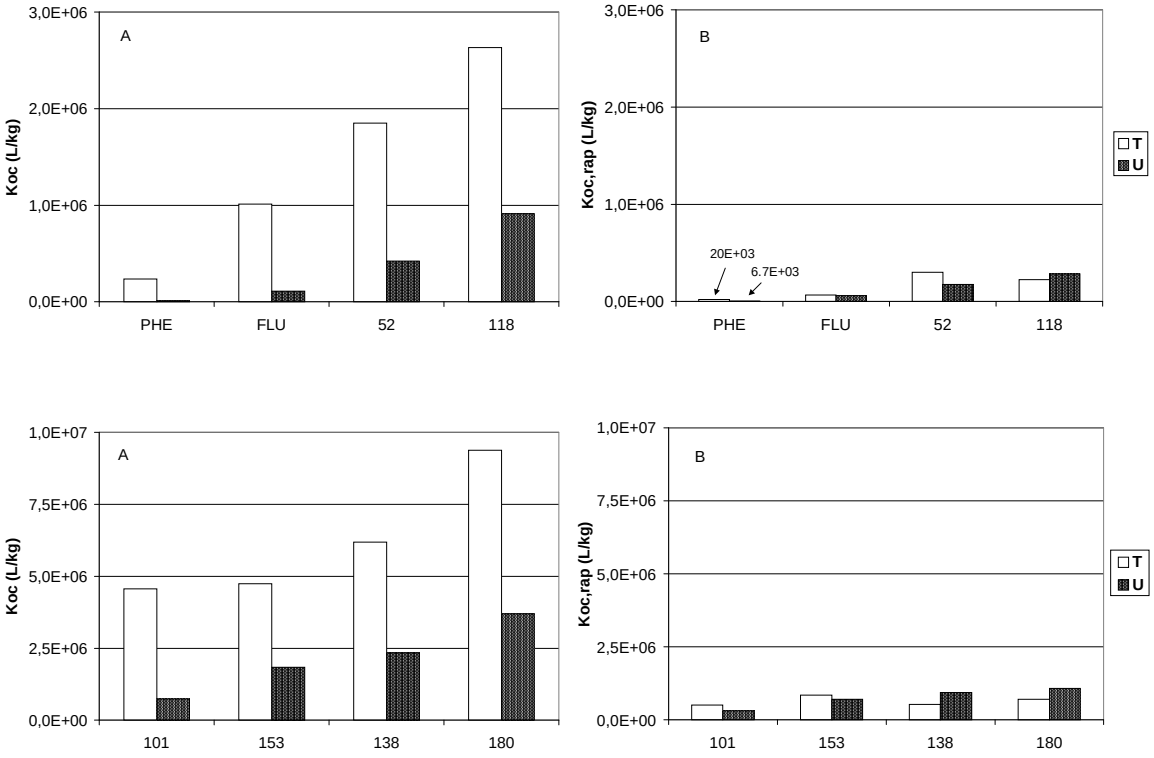


Fig. 4 K_{oc} (A) or $K_{oc,rap}$ (B) for PCBs and PAHs in untreated (U) and treated (T) (48 hours shaking of aqueous suspension with Tenax) lab-contaminated sediment. $K_{oc,rap}$ calculated as $K_{oc} \cdot F_{rap}$, with F_{rap} = rapidly desorbing fraction. For abbreviations of PAHs: See footnote table 1.

In Figure 5 the $K_{oc,rap}$ for chlorinated compounds (chlorobenzenes, PCBs and p,p'-DDE) and PAHs in the two sediments is plotted versus the log K_{ow} of the compounds. The log $K_{oc,rap}$ is related to the log K_{ow} of the compound ($R^2 = 0.87$). A regression line of log K_{oc} versus log K_{ow} values according to Karickhoff [83] is added for comparison. The experimental $K_{oc,\ rapid}$-values in the present study are lower than the K_{oc}-values from Karickhoff. We suggest that significant sequestration is already encountered within the time-span of the determination of the literature K_{oc}-values of Karickhoff (hours/days) (e.g. [13], [84]). The presence of slowly and very slowly compartments might explain that the K_{oc} values in the literature are higher than the $K_{oc,\ rapid}$ in the present study.

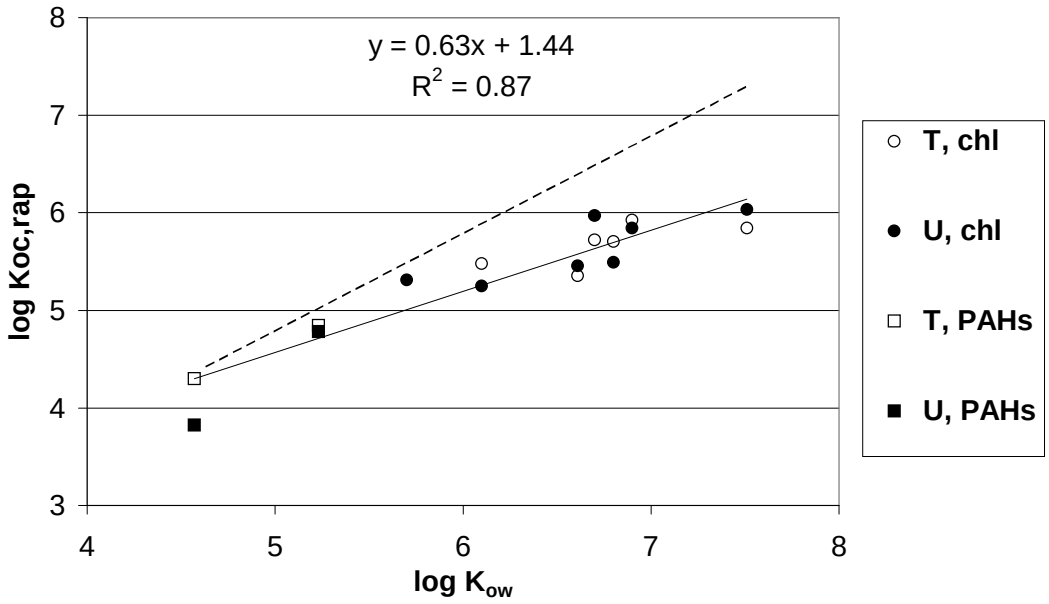


Fig. 5 Partition coefficients between the rapidly desorbing compartment and pore water ($K_{oc,\ rap}$, g oc/L) versus the log K_{ow}. Closed symbols: measurements on untreated sediment (U), open symbols: measurements on treated sediment (T) (48 hours shaking of aqueous suspension with Tenax). Chl = chlorinated compounds (hexachlorobenzene and polychlorinated biphenyls, PAHs = polyaromatic hydrocarbons. Unbroken line represents regression curve. Broken line represents estimated literature regression for K_{oc} versus log K_{ow} according to Karickhoff ([83]). Log K_{ow} from [56], [55] (estimated and calculated values), [57] and [54].

Evaluation of equilibrium partitioning between deposit-feeders, pore water and sediment

The concept of equilibrium partitioning can be summarized as follows (see Figure 6): Rapidly desorbing, non-sequestered, hydrophobic organic compounds partition between the rapidly desorbing sediment compartment, pore water and deposit-feeding organisms. At steady state, the distribution is at thermodynamic equilibrium, with equal fugacities in the biota, pore water and the sediment. The distribution can be characterized by partition coefficients of HOC between organic carbon of the rapidly desorbing fraction and pore water ($K_{oc,\ rapid}$) and between the organism lipid and pore water (BCF). We suggest that compounds exchange between the slowly desorbing compartment and the pore water and the rapidly desorbing compartment, but that this does not affect the partition coefficient $K_{oc,rap}$.

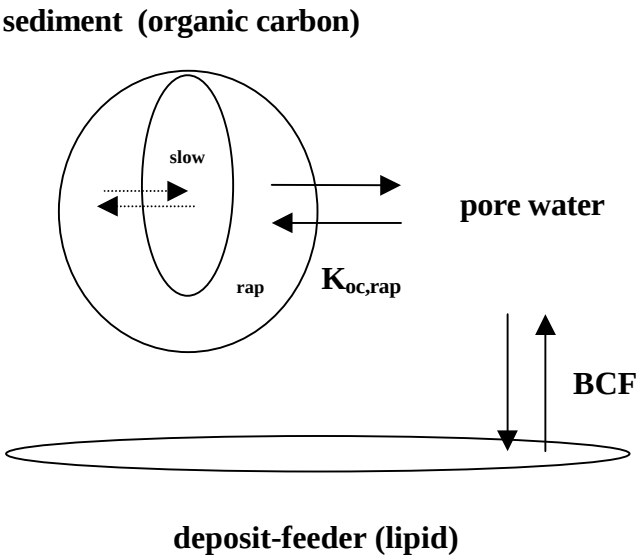


Fig. 6 Model of distribution of hydrophobic organic chemicals in sediments. rap = rapidly desorbing compartment; slow = slowly desorbing compartment; $K_{oc,rap}$ = partition coefficient between rapidly desorbing compartment and pore water (l/kg oc); BCF = bioconcentration factor (L/kg lipid).

The adapted concept of equilibrium partitioning of non-sequestered compounds is based on the traditional equilibrium partitioning (EqP) model as it describes accumulation with a thermodynamic equilibrium between organisms, pore water and sediment. The improvement in the new concept is the incorporation of sequestration into the model. The new model assumes linear partitioning between pore water and the rapidly desorbing compartment, with constant $K_{oc,rap}$ while traditional EqP applications are based on a constant K_{oc} of the whole sediment organic carbon. The new concept implies that either accurate pore water measurements or measurements of the rapidly desorbing fraction in combination with the total concentration in the sediment are needed to characterize the biota-pore water - sediment system. In traditional use of EqP, pore water measurements were made redundant by applying constant K_{oc}-values from the literature. Figure 7 demonstrates the limitations of traditional EqP-estimations, while applying traditional EqP on the data of the present study. In Figure 7, we plotted the steady state accumulation, calculated according to the traditional EqP concept, versus the experimentally determined accumulation. The EqP-values were calculated with equation (7). Following EqP assumptions, we applied a constant K_{oc} value for both sediments. The K_{oc} values were calculated from the frequently used QSAR by Karickhoff et al. in [83] as is common practice. Figure 7 shows that the traditional EqP model systematically overestimated experimental bioaccumulation for the two tested sediments. It has to be noted here that EqP might also underestimate accumulation, for

example in sediments with extremely high rapidly desorbing fractions, e.g. $F_{rap} = 1$. In this case, equation (1) can be written as

$$K_{oc} = K_{oc,rap} \quad (9).$$

As average literature K_{oc}-values were found to be higher than $K_{oc,rap}$ values (see Figure 4), these values would underestimate pore water concentrations and accumulation in deposit-feeders. Moreover, we demonstrated that K_{oc} is not a constant. Actual K_{oc} values might be calculated by dividing experimental or literature $K_{oc,\ rapid}$ values with experimentally determined rapidly desorbing fractions, as was postulated by Cornelissen and co-workers [71].

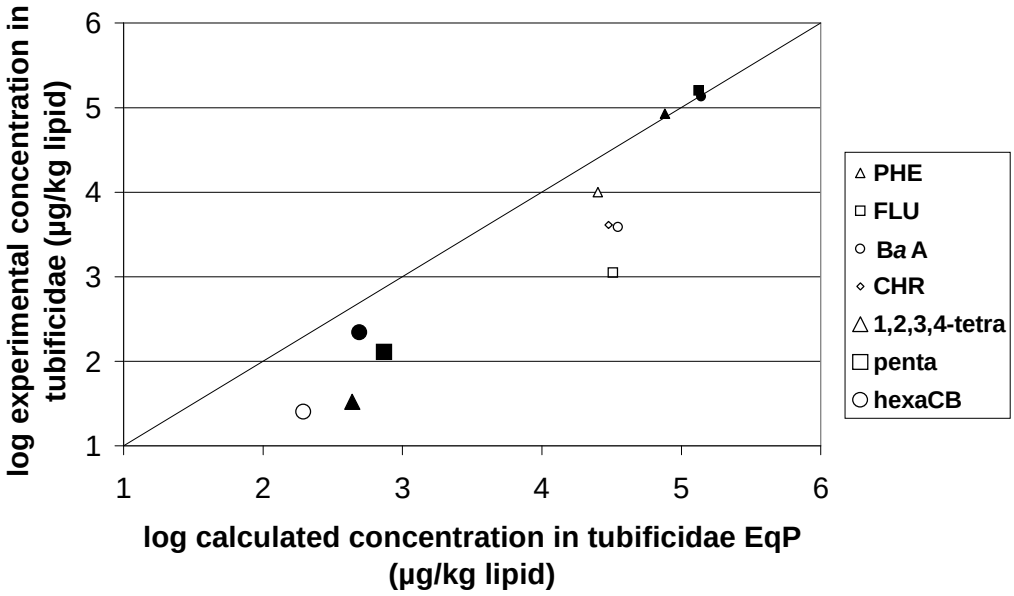


Fig. 7 Measured versus calculated steady-state accumulation of chlorobenzenes and PAHs in oligochaetes (*tubificidae*) according to EqP. The estimated steady-state accumulation accumulation is calculated by dividing the bioconcentration factor (BCF) by a estimated K_{oc} according to Karickhoff ([83]). One to one line included. Closed symbols: measurements in untreated lab-contaminated sediment, open symbols: measurements in treated lab-contaminated sediment (48 hours shaking of aqueous suspension with Tenax). For abbreviations of PAHs see footnote Table 1.

We will now address some possible limitations of the new model. Firstly, the validity of equilibrium partitioning for highly lipophilic compounds ($\log K_{ow} > 5.9$) and moderately lipophilic ($\log K_{ow} < 3.7$) needs to be confirmed.

Secondly, the model implies that ingestion of sediment particles does not result in a significantly higher uptake than water exposure exclusively. In theory, this can be explained by the low reduction in organic matter content in the gastro-intestinal tract for most

sediments ([85]). As a consequence, sorption capacity of the sediment remains unaltered and fugacity of a compound is not enhanced. Eventually a chemical equilibrium is reached between sediment, pore water and organism. The concept might not be applicable for sediments with highly digestible organic matter. These sediments are found for example in the highly enriched Baltic Sea, in post algal bloom episodes [86]. In these cases, organic matter in the GI-tract might be significantly reduced and resulting BSAF-values might be higher than based on equilibrium partitioning. In addition, some organisms appear to have enhanced uptake mechanisms. Weston and Mayer proposed that the digestive fluid of a polychaete and an echiuran acts as an efficient desorption medium and is selectively retained in the gastro-intestinal tract of deposit-feeders while the sediment particles are excreted [33, 66].

Implications

The results of the present paper provide evidence that steady-state accumulation of HOC in benthic deposit-feeders can be described with equilibrium partitioning between pore water and deposit-feeders. The freely dissolved pore water concentrations are affected by sequestration and cannot be predicted from total sediment concentration measurements. This implies that literature Koc values should not be used to calculate pore water concentrations. Instead, freely dissolved pore water concentrations can be obtained from direct measurements, using sensitive methods such as matrix-SPME. We propose that a multiplication of pore water concentrations and bioconcentration factors yields a valid estimation of steady-state bioaccumulation and that sediment analyses are not required.

Chapter 6 Discussion

In this thesis, the connection between the chemical process of sequestration, measured as the distribution over rapidly and slowly desorbing fractions, and the bioavailability of hydrophobic organic chemicals to benthic deposit-feeders has been elucidated. This discussion will address the major findings.

Results from the literature suggested a relationship between rapidly desorbing fractions of hydrophobic organic contaminants (HOC) and the bioavailability of these compounds to bacteria and deposit-feeders (e.g. [9],[10] [11, 12]). These findings prompted us to question the validity of the equilibrium partitioning theory (EqP) [21]. This model describes bioaccumulation in sediments by simple constant partitioning processes between sediment, pore water and biota, irrespective of the distribution over rapidly and slowly desorbing fractions.

Initially, we hypothesized that with increasing sediment contact time (ageing), fractions of rapidly desorbing compounds are reduced. This reduction in rapidly desorbing fractions might cause a reduction in bioavailability. However, we arrived at the following conclusion:

Contaminant-sediment contact time ('ageing') is not a strong determinant of bioavailability to deposit-feeders.

A prolonged contact time of almost three years did not strongly reduce the bioavailability of laboratory added PAHs, PCBs and p,p'-DDE in freshwater oligochaetes or change the distribution over rapidly, slowly and very slowly desorbing fractions in the sediment (Chapter 3). Differences in bioavailability were measured by comparing 'Biota to sediment accumulation factor' (BSAF), equivalent to the concentration in the organism lipid divided by the concentration in the sediment organic carbon. Also, the bioavailability of field contaminated PAHs in marine amphipods was only slightly lower (factor 2) than freshly added PAHs (Chapter 2), indicating again that prolonged ageing does not have a major impact on bioavailability. An exception was found for chlorobenzenes: A contact time of almost three years strongly reduced the bioavailability of these compounds. Interestingly, the reduction in bioavailability could be largely attributed to relatively high losses of rapidly desorbing compounds due to biodegradation and evaporation, compared to losses of slowly desorbing compounds.

In contrary of recent advocating of incorporation of contact-time in risk-assessment [44], we therefore concluded that contact-time is not a useful input-variable. Instead, we

decided to focus on the relationship between bioavailability and the distribution over rapidly, slowly and very slowly desorbing fractions itself, instead of further investigating the factor of contact time that might control this distribution. This change of perspective led to the following conclusion.

Bioavailability of HOC in deposit-feeders is closely related to the rapidly desorbing fraction in sediments.

Results in the literature provided only circumstantial evidence on the relationship between desorption kinetics and bioavailability. The experiments in Chapter 2, 3 and 4 show strong and proportional relationships between bioavailability and the rapidly desorbing fraction of sediment associated hydrophobic organic compounds. We found that different classes of compounds, PCBs, PAHs and chlorobenzenes, conformed to such relationships. In addition, the relationship was established for two different organisms, freshwater and marine sediment, two different sediment treatments and laboratory added and native compounds. We demonstrated causality of the relationship by performing a very controlled experiment, described in Chapter 4.

Recent development of new analytical methodology enabled us to perform accurate measurements on the freely dissolved pore water concentrations simultaneous with the measurements on bioaccumulation and the distribution over rapidly and slowly desorbing fractions (Chapter 5). The results of Chapter 4 were reinterpreted with the results of these pore water measurements (Chapter 5) and compared with the traditional EqP framework. The evaluation led to the following conclusion:

Equilibrium partitioning describes HOC bioaccumulation in deposit-feeders

The unique combination of measurements on bioaccumulation, pore water concentrations and distribution over rapidly and slowly desorbing fractions resulted in new insights into the partitioning behaviour of a wide range of HOC between sediments and deposit-feeders (Chapter 5). The new concept can be summarized as follows (see Fig 1): At steady state, partitioning of hydrophobic organic compounds between the rapidly desorbing sediment compartment, pore water and deposit-feeding organism is at thermodynamic equilibrium. The distribution can be characterized by partition coefficients of HOC between organic carbon of the rapidly desorbing fraction and pore water ($K_{oc,\ rapid}$) and between the organism lipid and pore water (bioconcentration factor or BCF). As we assumed instantaneous equilibrium, kinetics are not incorporated into the model. It is further assumed that the distribution over the rapidly and slowly compartment, including the very slowly

desorbing compartment, is constant.

In our concept, the rapidly desorbing fraction determines bioavailability because it equilibrates with the pore water. An increase in slowly desorbing fractions leads to a lower bioavailability as it is equivalent with a decrease in the rapidly desorbing fraction equilibrating with the pore water. We underline that both the pore water and sequestration measurements were needed to understand the processes leading to bioaccumulation.

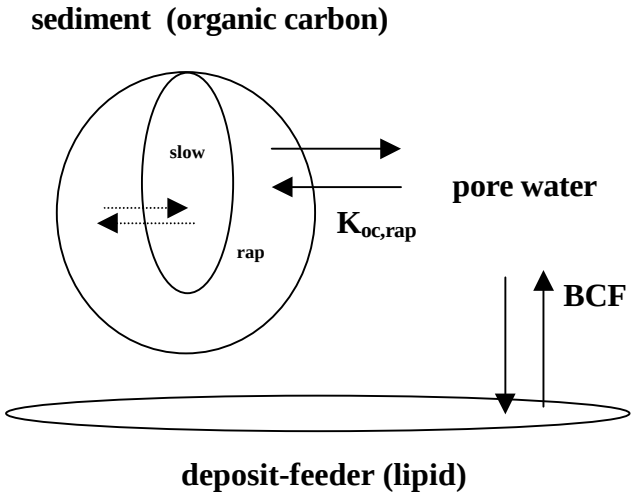


Fig. 1 Model of distribution of hydrophobic organic chemicals in sediments. rap = rapidly desorbing compartment; slow = slowly desorbing compartment; $K_{oc,rap}$ = partition coefficient between rapidly desorbing compartment and pore water (L/kg organic carbon); BCF = bioconcentration factor (L/kg lipid).

The major conclusions of the research project can be integrated:

Equilibrium partitioning is consistent with the relationship between rapidly desorbing fractions and bioavailability

The close relationship between rapidly desorbing fractions and bioavailability that was suggested in the literature and confirmed in our experiments (Chapter 2 through 4) can now be interpreted mechanistically: Rapidly desorbing fractions are linearly and causally related to pore water concentrations and consequentially to aqueous exposure and bioaccumulation.

The new model is coherent with the regression equations from Chapter 4, generalized as

$$BSAF = a \cdot F_{rap} + b \quad (1)$$

with F_{rap} = the rapidly desorbing fraction.

In Chapter 5 it was found that:

$$C_b = BCF \cdot C_{pore} \quad (2)$$

with C_b = concentration in organisms (µg/g lipid), C_{pore} = freely dissolved concentration in the pore water (µg/L) and BCF = bioconcentration factor (L/g lipid).

Combination of equation (2) with the definitions

$$K_{oc,rap} = C_{sed,rap}/C_{pore} \quad (3)$$

and

$$BSAF = C_b/C_{sed} \quad (4)$$

and

$$C_{sed,rap} = C_{sed} \cdot F_{rap} \quad (5)$$

with $K_{oc,rap}$ = partition coefficient for the organic carbon of the rapidly desorbing compartment, $C_{sed,rap}$ = concentration of rapidly desorbing compounds in the sediment (µg/g organic carbon) and C_{sed} = total concentration in the sediment (µg/g organic carbon)

yields

$$BSAF = (BCF/K_{oc,\,rap}) \cdot F_{rap} \quad (6).$$

The intercepts of equation (1) were very small (Chapter 4). Assuming that b is negligible, combination of equation (1) and (6) results into:

$$a = BCF/K_{oc,\,rap} \quad (7).$$

The bioaccumulation of HOC can be estimated using two approaches

The steady-state bioaccumulation in deposit-feeders can be assessed from rapidly

desorbing fractions, in combination with total concentration measurements. Measurements of the rapidly desorbing fraction (F_{rap}) yield an estimated concentration of HOC in deposit-feeders as follows: The concentrations in the organisms can be calculated from the concentration in the sediment with the definition of BSAF (equation (4)). The BSAF is calculated from F_{rap} with regression equation (1). Some estimated values of the parameters a and b for oligochaetes have been reported in Chapter 4. Assuming that b is negligible, combination of equation (1) and (4) yields :

$$C_b = a \cdot F_{rap} \cdot C_{sed} \qquad (8).$$

The steady-state concentration in the organisms can be estimated from pore water concentrations with equation (2)

$$C_b = BCF \cdot C_{pore} \qquad (2).$$

The BCF value in the equation can either be determined experimentally, or derived from literature values.

The new concept of equilibrium partitioning differs from traditional EqP

The modified concept differs from the traditional concept of equilibrium partitioning (EqP) by adoption of the notion that only a fraction of the sorbed compounds distributes between sediment and pore water according to equilibrium partitioning. In the traditional concept, desorption from the organic matter was described with a one-compartment model, assuming constant desorption behaviour for all sorbed compounds, and equilibrium partitioning of HOC between the total of sediment organic matter and pore water. The new concept is based on equilibrium partitioning between the organic matter of the rapidly desorbing fraction and pore water. In contrary to the traditional concept of EqP, K_{oc} is not constant, but depends on the distribution over rapidly and slowly desorbing fractions, according to:

$$K_{oc} = K_{oc,rap}/F_{rap} \qquad (9).$$

In current applications of traditional EqP, K_{oc}-values are often estimated from regression equations in the literature, for example in Karickhoff et al.[83]. These K_{oc}-values are probably obtained from experiments with sediments with a more or less average sequestration status. With equation (9), it can be seen that the highest differences between estimated and real-life values will be found in sediments with extremely high or low rapidly desorbing fractions.

In some cases, the model may not be fully applicable. We will now discuss some situations for which extra measurements are appropriate.

Possible limitations

The concept might not be applicable for sediments with highly digestible organic matter. These sediment are found for example in the highly enriched Baltic Sea, in post algal bloom episodes [86]. In these cases, organic matter in the GI-tract might be significantly reduced and resulting BSAF-values might be higher than based on equilibrium partitioning. The digestibility of the organic carbon of the sediments we used in our experiments is not extremely high. For this and most other sediments, it is expected that the organic matter reduction in the gastro-intestinal tract is very low (e.g. [85]) and the sorption capacity of the sediment remains unaltered. As a consequence, we expect that the fugacity of a compound is not enhanced in the GI-tract [87, 88], and eventually a chemical equilibrium is reached between sediment, pore water and organism. As yet, equilibrium partitioning cannot be validated for highly hydrophobic organic compounds (log K_{ow} > 5.9). In Chapter 5 it was mentioned that no BCF –values are available for deposit-feeders for these chemicals. Also, local dilution of pore water with overlying water in the microenvironment of deposit-feeders might cause deviations from the concept of equilibrium partitioning of rapidly desorbing compounds (e.g. [32]). The possible effect of dilution on local pore water concentrations is still highly uncertain. The possible limitations of the proposed concept should be kept in mind when the proposed modified concept of equilibrium partitioning is applied.

Sediment risk assessment can be improved

In this thesis, a new concept of the connection between chemical processes and bioavailability of HOC to deposit-feeders was presented. The improved understanding of these interactions led to a refinement of the traditional equilibrium partitioning theory (EqP). We demonstrated that bioaccumulation of sediment associated HOC in deposit-feeders can be assessed using pore water concentrations. Any measurements of the solid sediment phase, either total concentration, sorption or sequestration measurements, do not add to the assessment of bioaccumulation and can be omitted. Risk analysis can instead be focussed on correct pore water concentrations. The proposed method estimates bioaccumulation, independent of sediment properties. As a consequence, the method does not suffer from sediment to sediment extrapolation uncertainties that are associated with assessment methods, based on sediment measurements. We showed that sequestration is tightly connected with bioavailability as it influences pore water concentrations. Pore water

concentrations are set by linear partitioning of the rapidly desorbing compounds. Therefore pore water concentrations are proportional to the rapidly desorbing fraction, i.e. the sequestration status of the sediment. This finding implies that pore water concentrations cannot be assessed from total sediment concentrations, without additional sequestration measurements. In conclusion, we propose that steady-state accumulation in benthic organisms can be estimated from pore water concentrations. The combination of the advantageous properties and the foundation on understanding of basic processes leading to bioavailability suggest broad applicability of the proposed method.

References

1. Meador, J, Stein, J, Reichert, W, and Varanasi, U. 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Rev Environ Contam Toxicol* 143: 80-164.
2. Vlas, J de. 1979. Secondary production by tail regeneration in a tidal flat population of lugworms (*Arenicola marina*) cropped by flatfish. *Netherlands Journal of Sea Research* 13: 362-393.
3. Tracey, G and Hansen, D. 1996. Use of biota-sediment accumulation factors to assess similarity of nonionic organic chemical exposure to benthically-coupled organisms of differing trophic mode. *Arch Environ Contam Toxicol* 30: 467-475.
4. Besten, P den. 1996. Biologische beschikbaarheid van contaminanten in verouderd sediment. Resultaten bioaccumulatie-bioassays met Oligochaeten in sediment uit Dordtsche Biesbosch en Geulhaven., Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling: Lelystad. Werkdocument 95.176X, WSC-Ecotoxicologie 95.03.
5. Landrum, P, Eadie, B, and Faust, W. 1992. Variation in the bioavailability of polycyclic aromatic hydrocarbons to the amphipod *diporeia (SPP.)* with sediment aging. *Environ Toxicol Chem* 11: 1197-1208.
6. Kukkonen, J and Landrum, P. 1998. Effect of particle-xenobiotic contact time on bioavailability of sediment-associated benzo(*a*)pyrene to benthic amphipod, *Diporeia spp. Aquatic toxicology* 42: 229-242.
7. Varanasi, U, Reichert, W, Stein, J, Brown, D, and Sanborn, H. 1985. Bioavailability and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment from an urban estuary. *Environ Sci Technol* 19: 836-841.
8. Belfroid, A, Seinen, W, Berg, M van de, Hermens, J, and Gestel, K van. 1995. Uptake, bioavailability and elimination of hydrophobic compounds in earthworms (*Eisenia andrei*) in field contaminated soil. *Environ Toxicol Chem* 14: 605-612.
9. Lamoureux, E and Brownawell, B. 1999. Chemical and biological availability of sediment-sorbed hydrophobic organic contaminants. *Environ Toxicol Chem* 18: 1733-1741.
10. White, J, Hunter, M, Nam, K, Pignatello, J, and Alexander, M. 1999. Correlation between biological and physical availabilities of phenanthrene in soils and soil humin in aging experiments. *Environ Toxicol Chem* 18: 1720-1727.
11. Cornelissen, G, Rigterink, H, Ferdinandy, M, and Noort, P van. 1998. Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. *Environ Sci Technol* 32: 966-970.
12. Beurskens, J, Dekker, C, and Velde, L van der. *Extended abstract*. in *COST 641 Workshop*. 1990. Copenhagen.
13. Cornelissen, G, Noort, P van, and Govers, H. 1997. Desorption kinetics of

chlorobenzenes, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: Sediment extraction with tenax and effects of contact time and solute hydrophobicity. *Environ Toxicol Chem* 16: 1351-1357.

14. Pignatello, J and Xing, B. 1996. Mechanisms of slow sorption of organic chemicals to natural particles. *Environ Sci Technol* 30: 1-11.

15. Luthy, R, Aiken, G, Brusseau, M, Cunningham, S, Gschwend, P, Pignatello, J, et al. 1997. Sequestration of hydrophobic organic contaminants by geosorbents. *Environ Sci Technol* 31: 3341-3347.

16. Chiou, C and Kile, D. 1998. Deviations from sorption linearity on soils of polar and nonpolar organic compounds at low relative concentrations. *Environ Sci Technol* 32: 338-343.

17. Cornelissen, G. 1999. Mechanism and consequences of slow desorption of organic compounds from sediments. PhD thesis. University of Amsterdam, Amsterdam, The Netherlands.

18. Gustafsson, O, Haghseta, F, Chan, C, Macfarlane, J, and Gschwend, P. 1997. Quantification of the dilute sedimentary soot phase: Implications for PAH speciation and bioavailability. *Environ Sci Technol* 31: 203-209.

19. Belfroid, A, Sijm, D, and Gestel, C van. 1996. Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. *Environ Rev* 4: 276-299.

20. Shea, D. 1988. Developing national sediment quality criteria. *Environ Sci Technol* 22: 1256-1261.

21. Di Toro, D, Zarba, C, Hansen, D, Berry, W, Swartz, R, Cowan, C, et al. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ Toxicol Chem* 10: 1541-1583.

22. Loonen, H, Muir, D, Parsons, J, and Govers, H. 1997. Bioaccumulation of polychlorinated dibenzo-p-dioxins in sediment by oligochaetes: Influence of exposure pathway and contact time. *Environ Toxicol Chem* 16: 1518-1525.

23. Harkey, G, Hoof, P, and Landrum, P. 1995. Bioavailability of polycyclic aromatic hydrocarbons from a historically contaminated sediment core. *Environ Toxicol Chem* 14: 1551-1560.

24. Pignatello, J. 2000. The measurement and interpretation of sorption and desorption rates for organic compounds in soil media. *Advances in agronomy* 69: 1-73.

25. Alexander, M. 1995. How toxic are toxic chemicals in soil? *Environ Sci Technol* 29: 2713-2717.

26. Cornelissen, G, Noort, P van, and Govers, H. 1998. Mechanism of slow desorption of organic compounds from sediments: a study using model sorbents. *Environ Sci Technol* 32: 3124-3131.

27. Bosma, T, Middeldorp, P, Schraa, G, and Zehnder, A. 1997. Mass transfer limitation of biotransformation: Quantifying bioavailability. *Environ Sci Technol* 31: 248-252.

28. Carmichael, L, Christman, R, and Pfaender, F. 1997. Desorption and mineralization kinetics of phenanthrene and chrysene in contaminated soils. *Environ Sci Technol* 31: 126-132.

29. White, J and Alexander, M. 1996. Reduced biodegradability of desorption-resistant fractions of polycyclic aromatic hydrocarbons in soil and aquifer solids. *Environ Toxicol Chem* 15: 1973-1978.

30. Davies, N, Edwards, P, Lawrence, M, Taylor, M, and Simkiss, K. 1999. Influence of particle surfaces on the bioavailability to different species of 2,4-dichlorophenol and pentachlorophenol. *Environ Sci Technol* 33: 2465-2468.

31. Meador, J, Casillase, E, Sloan, C, and Varanasi, U. 1995. Comparative bioaccumulation of polycyclic aromatic hydrocarbons from sediment by two infaunal invertebrates. *Mar Ecol Prog Ser* 123: 107-124.

32. Lee II, H. 1991. Chapter 5. A clam's eye view of the bioavailability of sediment-associated pollutants. In RA B, eds, *Organic Substances and Sediments in Water: Biological*. Lewis Publishers, Ann Arbor, Mi, USA, pp. 73-93.

33. Weston, D and Mayer, L. 1998. Comparison of in vitro digestive fluid extraction and traditional in vivo approaches as measures of polycyclic aromatic hydrocarbon bioavailability from sediments. *Environ Toxicol Chem* 17: 830-840.

34. Kelsey, J, Kottler, B, and Alexander, M. 1997. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. *Environ Sci Technol* 31: 214-217.

35. Folch, J, Lees, M, and Stanley, G. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226: 497-509.

36. Ciarelli, S, Straalen, N van, Klap, V, and Wezel, A van. 1999. Effects of sediment bioturbation by the estuarine amphipod Corophium volutator on fluoranthene resuspension and transfer into the mussel, *Mytilus edulis L*. *Environ Toxicol Chem* 18: 318-328.

37. Long, A, Crouch, M, and Barker, S. 1991. Multiresidue matrix solid phase dispersion (MSPD) extraction and gas chromatographic screening of nine chlorinated pesticides in catfish (Ictalurus punctatus) muscle tissue. *J Assoc Off Anal Chem* 74: 667-670.

38. Bakker, M, Vorenhout, M, Sijm, D, and Kollöffel, C. 1999. Dry deposition of atmospheric polycyclic aromatic hydrocarbons in three Plantago species. *Environ Toxicol Chem* 18: 2289-2294.

39. Landrum, P. 1989. Bioavailability and toxicokinetics of polycyclic hydrocarbons sorbed to sediments for the amphipod Pontoporeia hoyi. *Environ Sci Technol* 23: 588-595.

40. Landrum, P, Dupuis, W, and Kukkonen, J. 1994. Toxicokinetics and toxicity of sediment-associated pyrene and phenanthrene in *Diporeia spp.:* Examination of equilibrium-partitioning theory and residue-based effects for assessing hazard. *Environ Toxicol Chem* 13: 1769-1780.

41. Landrum, P, Eadie, B, and Faust, W. 1991. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia sp. Environ Toxicol Chem* 10: 35-46.

42. Lamoureux E, Brownawell B. 1998 *Sediment soot content as a control on bioavailability of hydrophobic organic compounds*. Abstracts, 19th Annual meeting, Society of Environmental Toxicology and Chemistry, Charlotte, NC, USA, November 15-19, p 31.
43. Hulscher, T ten, Noort, P van, and Velde, L van der 1997. Equilibrium partitioning theory overestimates chlorobenzene concentrations in sediment-porewater from Lake Ketelmeer, the Netherlands. *Chemosphere* 35: 2331-2344.
44. Alexander, M. 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ Sci Technol* 34: 4259-4265.
45. Chiou, C, Kile, D, Rutherford, D, Sheng, G, and Boyd, S. 2000. Sorption of selected organic compounds from water to a peat soil and its humic-acid and humin fractions: Potential sources of the sorption nonlinearity. *Environ Sci Technol* 34: 1254-1258.
46. Hulscher, T ten, Vrind, B, Heuvel, H van de, Velde, L van der, Noort, P van, Beurskens, J, and Govers, HAJ. 1999. Triphasic desorption of highly resistant chlorobenzenes, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons in field contaminated sediment. *Environ Sci Technol* 33: 126-132.
47. Kraaij, R, Ciarelli, S, Tolls, J, Kater, B, and Belfroid, A. 2001. Bioavailability of lab-contaminated and native PAHs to the amphipod Corophium volutator relates to chemical desorption. *Environ Toxicol Chem* In press.
48. Xing, B, McGill, W, and Dudas, M. 1994. Sorption of a-naphtol and organic sorbents varying in polarity and aromaticity. *Chemosphere* 28: 145-153.
49. Sijm, D and Linde, A van der. 1995. Size-dependent bioconcentration kinetics of hydrophobic organic chemicals in fish based on diffusive mass transfer and allometric relationships. *Environ Sci Technol* 29: 2769-2777.
50. Smedes, F and Boer, J de. 1995. Guidelines for the determination of chlorobiphenyls in sediment. *Quim Anal* 13: 100-108.
51. Cornelissen, G, Noort, P van, Parsons, J, and Govers, H. 1997. The temperature dependence of slow adsorption and desorption kinetics of organic compounds in sediments. *Environ Sci Technol* 31: 454-460.
52. Wezel, A van and Opperhuizen, A. 1995. Narcosis due to environmental pollutants in aquatic organisms: residue-based toxicity, mechanisms and membrane burdens. *Critical Reviews in Toxicology* 25: 255-279.
53. Landrum, P. Comparison among three invertebrate species of the body residue for lethal and sublethal effects.Abstracts, 19th Annual meeting, Society of Environmental Toxicology and Chemistry, Charlotte, NC, USA, November 15-19, p 10.
54. Maagd, PGJ de, Hulscher, DTEM ten, Heuvel, H van de, Opperhuizen, A, and Sijm, DTHM. 1998. Physicochemical properties of polycyclic aromatic hydrocarbons: aqueous solubilities, n-octanol/water partition coefficients, and henry's law constants. *Environ Toxicol Chem* 17: 251-257.

55. Bruijn, J de, Busser, F, Seinen, W, and Hermens, J. 1989. Determination of octanol/water partition coefficients for hydrophobic organic chemicals with the "slow-stirring" method. *Environ Toxicol Chem* 8: 499-512.

56. Shiu, W and Mackay, D. 1986. A critical review of aqueous solubilities, Henry's law constants and octanol-water partition coefficients of the polychlorinated biphenyls. *J Phys Chem Ref Data* 15: 911-929.

57. Brodsky, J and Ballschmiter, K. 1988. Reversed phase liquid chromatography of PCBs as a basis for the calculation of water solubility and log Kow for polychlorobiphenyls. *Fresenius Z Anal Chem* 331: 295-301.

58. Leppänen, M and Kukkonen, J. 2000. Effect of sediment-chemical contact time on availability of sediment-associated pyrene and benzo[a]pyrene to oligochaete worms and semipermeable membrane devices. *Aquat Toxicol* 49: 227-241.

59. Hulscher, T ten, Vrind, B, Heuvel, H van de, Noort, P van, and Govers, H. 2000. The influence of long contact times on desorption kinetics of spiked HCB and PCB-28 from Lake Ketelmeer sediment. Extended Abstracts, 220th National meeting, American Chemical Society, Washington, DC, August 20-24, Vol. 40, p 231-233. .

60. Paine, M, PM Chapman, PJ Allard, MH Murdoch en D Minifie. 1996. Limited bioavailability of sediment PAH near an aluminium smelter: Contamination does not equal effects. *Environ. Toxicol. Chem.* 15 (11), 2003-2018.

61. Hatzinger, P and Alexander, M. 1995. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ Sci Technol* 29: 537-545 .

62. Chung, N and Alexander, M. 1999. Effect of concentration on sequestration and bioavailability of two polycyclic aromatic hydrocarbons. *Environ Sci Technol* 33: 3605-3608.

63. Tang, J, Robertson, B, and Alexander, M. 1999. Chemical-extraction methods to estimate bioavailability of DDT, DDE and DDD in soil. *Enviro Sci Technol* 33: 4346-4351.

64. Tang, J and Alexander, M. 1999. Mild extractability and bioavailability of polycyclic aromatic hydrocarbons in soil. *Envrion Toxicol Chem* 18: 2711-2714.

65. Hawthorne, S and Grabanski, C. 2000. Correlating selective supercritical fluid extraction with bioremediation behavior of PAHs in a field treatment plot. *Environ Sci Technol* 34: 4103-4110.

66. Weston, D and Mayer, L. 1998. In vitro digestive fluid extraction as a measure of the bioavailability of sediment-associated polycyclic aromatic hydrocarbons: Sources of variation and implications for partitioning models. *Environ Toxicol, Chem* 17: 820-829.

67. Weston, D, Penry, D, and Gulmann, L. 2000. The role of ingestion as a route of contaminant bioaccumulation in a deposit-feeding polychaete. *Arch Environ Contam Toxicol* 38: 446-454.

68. Reid, B, Stokes, J, Jones, K, and Semple, K. *Assessing bioavailability of soil associated contaminats by a chemical means*. 2001. Poster, Annual meeting, Society of Environmental Toxicology and Chemistry, Brighton, UK.

69. Reid, B, Stokes, J, Jones, K, and Semple, K. 2000. Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. *Environ Sci Technol* 34: 3174-3179.
70. Cuypers, C, Grotenhuis, T, Joziasse, J, and Rulkens, W. 2000. Rapid persulfate oxidation predicts PAH bioavailability in soils and sediments. *Environ Sci Technol* 34: 2057-2063.
71. Cornelissen, G, Rigterink, H, Vrind, B, Hulscher, T ten, Fernandy, M, and Noort, P van. 1997. Two-stage desorption kinetics and in situ partitioning of hexachlorobenzene and dichlorobenzenes in a contaminated sediment. *Chemosphere* 35: 2405-2416.
72. Schlebaum, W, Schraa, G, and Riemsdijk, W van. 1999. Influence of nonlinear sorption kinetics on the slow-desorbing organic contaminant fraction in soil. *Environ Sci Technol* 33: 1413-1417.
73. Cornelissen, G, Hulscher, TEM ten, Rigterink, H, Vrind, B, and Noort, P van. 2001. A simple Tenax method to determine the chemical availability of sediment-sorbed organic compounds. *Environ Toxicol Chem* 20: 706-711.
74. Kraaij, R, Belfroid, A, Cornelissen, G, Seinen, W, and Tolls, J. Direct evidence of sequestration in sediments affecting the bioavailability of hydrophobic organic chemicals to benthic deposit-feeders. In preparation.
75. Leppänen, M and Kukkonen, J. 1998. Relative importance of ingested sediment and pore water as bioaccumulation routes for pyrene to oligochaete (*Lumbriculus variegatus, Muller*). *Environ Sci Technol* 32: 1503-1508.
76. Kaag, N, Foekema, E, Scholten, M, and Straalen, N van. 1997. Comparison of contaminant accumulation in three species of marine invertebrates with different feeding habits. *Environ Toxicol Chem* 16: 837-842.
77. Mayer, P, Vaes, W, Wijnker, F, Legierse, K, Kraaij, R, Tolls, J and Hermens, JLM. 2000. Sensing dissolved sediment porewater concentrations of persistent and bioaccumulative pollutants using disposable solid phase microextraction fibers. *Environ Sci Technol* 34: 5177-5183.
78. Mayer, P, Vaes, W, and Hermens, J. 2000. Absorption of hydrophobic compounds into the poly(dimethylsiloxane) coating of solid-phase microextraction fibers: High partition coefficients and fluorescence microscopy images. *Anal Chem* 72: 459-464.
79. Opperhuizen, A. 1986. Bioconcentration in fish and other distribution processes of hydrophobic chemicals in aqueous environments. PhD thesis. University of Amsterdam, Amsterdam, The Netherlands.
80. Rinderhagen, M and Butte, W. 1995. Kinetics of accumulation and elimination of isomeric hexachlorocyclohexanes by tubificids. *SAR and QSAR in Environ Research* 4: 131-138.
81. Belfroid, A, Wezel, A van, Sikkenk, M, Gestel, K van, Seinen, W, and Hermens, J. 1993. The toxicokinetic behavior of chlorobenzenes in earthworms (Eisenia andrei): Experiments in water. *Ecotoxicol Environ Safety* 25: 154-165.

82. McCarty, L. 1986. The relationship between aquatic toxicity QSARS and bioconcentration for some organic chemicals. *Environ Toxicol Chem* 5, 1071-1080.
83. Karickhoff, S, Brown, D, and Scott, T. 1979. Sorption of hydrophobic pollutants on natural sediments. *Water Res* 13: 241-248.
84. Kraaij, H, Tolls, J, Sijm, D, Cornelissen, G, Heikens, A, and Belfroid, A. The effect of contact time on the sequestration and bioavailability of different classes of hydrophobic organic chemicals to benthic oligochaetes (*tubificidae*). Submitted.
85. Kukkonen, J and Landrum, P. 1995. Measuring assimilation efficiencies for sediment/bound PAH and PCB congeners by benthic organisms. *Aquat Toxicol* 32: 75-92.
86. Gunnarsson, J, Bjork, M, Gilek, M, Granberg, M, and Rosenberg, R. 2000. Effects of eutrophication on contaminant cycling in marine benthic systems. *Ambio* 29: 252-259.
87. Gobas, FAPC, Zhang, X, and Wells, R. 1993. Gastrointestinal magnification: The mechanism of biomagnification and food chain accumulation of organic chemicals. *Environ Sci Technol* 27: 2855-2863.
88. Gobas, FAPC, Wilcockson, JB, Russell, RW, and Haffner, GD. 1999. Mechanism of biomagnification in fish under laboratory and field conditions. *Environ Sci Technol* 33: 133-141.

Publications of this thesis

Kraaij, R, Ciarelli, S, Tolls, J, Kater, B, and Belfroid, A. 2001. Bioavailability of lab-contaminated and native PAHs to the amphipod *Corophium volutator* relates to chemical desorption. *Environ. Toxicol. Chem.* 20. In press. **(Chapter 2)**

Kraaij, R, Tolls, J, Sijm, D, Cornelissen, G, Heikens, A, and Belfroid, A. The effect of contact time on the sequestration and bioavailability of different classes of hydrophobic organic chemicals to benthic oligochaetes (*Tubificidae*). *Submitted.* **(Chapter 3)**

Kraaij, R, Belfroid, A, Cornelissen, G, Seinen, W, and Tolls, J. Direct evidence of sequestration in sediments affecting the bioavailability of hydrophobic organic chemicals to benthic deposit-feeders. *In preparation.* **(Chapter 4)**

Kraaij, R, Mayer, P, Busser, F, van het Bolscher, M, Seinen, W, Belfroid, A, Tolls, J. Equilibrium partitioning of non-sequestered fractions of hydrophobic organic chemicals between sediment, pore water and benthic deposit-feeders (*Tubificidae*). *In preparation.* **(Chapter 5)**

Samenvatting voor niet-ingewijden

Overal in de wereld, zelfs op de meest afgelegen plekken, zijn bodems van wateren of sedimenten vervuild met hydrofobe organische chemicaliën (HOCs). Dit zijn organische stoffen die erg slecht in water oplossen (hydrofobie = 'watervrees'). HOCs zijn veelal te herleiden tot menselijke activiteiten zoals de verbranding van fossiele brandstoffen (verkeer, energieopwekking), bestrijdingsmiddelengebruik en chemische produktieprocessen. Een klein aandeel van HOCs komt door natuurlijke processen als spontane bosbranden in het milieu terecht. Voorbeelden van HOCs zijn PCBs (polychloorbifenylen), PAKs (polyaromatische koolwaterstoffen), chloorbenzenen en bestrijdingsmiddelen.

Sediment bestaat uit vaste bodemdeeltjes en water, dat poriewater wordt genoemd. De vaste bodemdeeltjes bestaan weer uit een organisch, humusachtig, gedeelte en een minerale fractie. Vanwege de slechte wateroplosbaarheid hecht (sorbeert) een zeer groot deel van de HOCs zich aan de vaste bodemdeeltjes, met name aan de organische fractie. Sinds een aantal jaren is bekend dat een gedeelte van de gehechte HOCs zo sterk gebonden wordt dat het relatief moeizaam weer vrijkomt. Ook kan een gedeelte op plaatsen terechtkomen, waarvandaan ze maar langzaam kunnen ontsnappen, bijvoorbeeld in nauwe poriën. De vorming van al deze traag vrijkomende fracties wordt 'sequestratie' genoemd. Dit is een belangrijke term in het proefschrift. Soms neemt de mate van sequestratie toe in de tijd.

Het probleem van HOCs is dat het behalve in sedimentdeeltjes ook sterk kan ophopen (accumuleren) in levend weefsel, zoals dat van organismen die in sediment voorkomen (benthische organismen). Eenmaal opgehoopt kunnen toxische effecten optreden, variërend van kaakmisvorming tot verlaagde reproductie en sterfte. De aanwezigheid van HOCs in sedimenten vormt daarmee een potentieel risico voor de plaatselijke ecosystemen. Ook kan een gedeelte van de HOCs in voedselketens terechtkomen die zich boven de waterbodem uitstrekken, zoals bij de consumptie van wormen door platvissen.

Om het risico van HOCs voor sediment organismen te kunnen inschatten, en daar verstandig beleid op af te stemmen, moeten twee vragen beantwoord worden: - hoe schadelijk is het als sediment organismen een bepaalde hoeveelheid HOCs ophopen, en – hoeveel HOCs hoopt zich op in sediment organismen. Dit proefschrift gaat in op de tweede vraag. Om te weten hoeveel HOCs worden opgenomen door deposit-feeders op een bepaalde locatie, kunnen organismen worden gevangen en geanalyseerd. Echter, deze aanpak is kostbaar en tijdrovend en leent zich niet voor frequente toepassing. Er is daarom behoefte aan een methode om te voorspellen hoeveel HOCs door de plaatselijke fauna worden opgehoopt. In de praktijk blijkt dit erg lastig. Op de ene locatie kan veel meer worden opgenomen dan op de andere locatie, terwijl de concentraties in het organische materiaal van het sediment gelijk zijn. Dit betekent dat je niet kunt voorspellen hoeveel een sediment organisme, bijvoorbeeld een slijkgarnaal, binnen zal krijgen door alleen maar naar de concentratie in het sediment te kijken. Er is meer informatie nodig, in het bijzonder over de interactie tussen organisme,

sediment en HOC. Als over deze interactie wordt gesproken, wordt vaak de term biobeschikbaarheid gebruikt: Biobeschikbaarheid staat voor de totale concentratie van chemicaliën die wordt opgenomen, of potentieel kan worden opgenomen ('beschikbaar is') door een organisme. Een verschil in ophoping bij gelijke concentraties duidt dus op een verschil in biobeschikbaarheid.

In dit proefschrift wordt onderzoek beschreven waarmee de biobeschikbaarheid van HOCs door sediment organismen beter ingeschat kan worden. Speciale aandacht is besteedt aan het verschijnsel van sequestratie. Mogelijk zijn verschillen in biobeschikbaarheid tussen sedimenten en chemicaliën op een of ander manier gerelateerd aan verschillen in sequestratiegedrag.

Als proefdieren hebben we gekozen voor twee soorten zogenaamde deposit-feeders: tubifex wormen (mengsel van *Limnodrilus hoffmeisteri* en *Tubifex tubifex*) en slijkgarnaaltjes (*Corophium volutator*) (zie plaatjes inleiding). Deposit-feeders zijn organismen die sediment deeltjes inslikken en benutten als voedselbron. We verwachtten dat een risico-inschatting voor deze groep organismen een goede conservatieve voorspelling oplevert voor de hele gemeenschap van sediment organismen, omdat deposit-feeders in intensief contact staan met het sediment. Sediment organismen kunnen HOCs binnenkrijgen door contact met poriewater waarin kleine hoeveelheden HOCs zijn opgelost. Een tweede route van opname speelt mogelijke een rol bij de deposit-feeders. Bij de passage van sedimentdeeltjes in het maag-darmkanaal kunnen gehechte HOCs vrijkomen en opgenomen worden in het weefsel van deze organismen. Is de situatie safe voor deposit-feeders, dan is dat waarschijnlijk ook zo voor andere sediment bewoners.

Het proefschrift is opgebouwd uit een inleiding (Hoofdstuk 1), een experimenteel gedeelte (Hoofdstuk 2, 3, 4 en 5) en een discussie. In de inleiding wordt het kader van het onderzoek beschreven, de vraagsteling gepreciseerd en sleutelwoorden uitgelegd. De experimentele sectie is grofweg verdeeld in twee stukken. In hoofdstuk 2 en 3 is onderzocht of de ophoping van HOCs door deposit-feeders afhankelijk is van sequestratie en of de factor contact tijd tussen HOCs en sediment hierbij een rol speelt (empirisch gedeelte). In hoofdstuk 4 en 5 worden speciale experimenten beschreven om het mechanisme, het waarom, van de relatie tussen sequestratie en ophoping bloot te leggen (mechanistisch gedeelte). In de discussie wordt de samenhang tussen de experimenten besproken en de consequenties voor de risico-analyse bediscussieerd.

In een eerste experiment (Hoofdstuk 2) zijn slijkgarnaaltjes onder gecontroleerde lab-omstandigheden uitgezet in verschillende sedimenten: vervuild sediment uit de Westerschelde, en porties van hetzelfde sediment waaraan PAKs waren toegevoegd. Groot verschil tussen deze sedimenten was de verblijfstijd van de PAKs: De PAKs die al in het sediment zaten, waren daar

al vele jaren geleden in terechtgekomen. De PAKs die in het lab waren toegevoegd, waren op het moment van uitzetting van de garnaaltjes maar enkele dagen in contact geweest met het sediment. De biobeschikbaarheid van deze 'verse' PAKs was een factor 1-3 hoger dan de 'oude' vervuiling. De verschillen in biobeschikbaarheid tussen verschillende PAKs en de 'oude' en 'verse' vervuiling waren in overeenstemming met de mate van sequestratie. De ‘oude’ PAKs in de Westerschelde hadden een factor 1-2 hogere mate van sequestratie dan de lab additieven (de 'verse PAKs').

In een tweede experiment (Hoofdstuk 3) werd schoon sediment in het lab vervuild met een mengsel van HOCs en over lange tijd, bijna drie jaar, gevolgd. Op verschillende tijdstippen na toevoeging van de HOCs werden sediment wormen uitgezet in het sediment. Voor de meeste chemicaliën veranderde er niet zoveel in drie jaar: De ophoping van HOCs in de wormen daalde hooguit met een factor 2, en ook de mate van sequestratie was nauwelijks aan verandering onderhevig. Opvallende uitzondering waren de chloorbenzenen. Voor deze chemicaliën daalde de biobeschikbaarheid met een factor 5 tot 18, en nam de mate van sequestratie sterker toe. Dit verschijnsel kon voor een groot deel worden verklaard doordat chloorbenzenen door vervluchtiging en afbraak uit het sediment verdwenen. Deze verdwijnprocessen hebben een relatief grote impact op de snel vrijkomende (desorberende), niet gesequestreerde fractie. Hierdoor neemt het aandeel van gesequestreerde stoffen toe en neemt de biobeschikbaarheid van de resterende chloorbenzenen af. Verschillen in biobeschikbaarheid tussen verschillende chemicaliën op verschillende tijdstippen na toevoeging van de HOCs waren weer goed te verklaren met verschillen in sequestratie.

In de tweede fase van het onderzoek werd een uitgebalanceerd experiment uitgevoerd om meer inzicht in het verband tussen sequestratie en ophoping te verkrijgen. Bij dit experiment werd op nog meer fronten gemeten: Naast ophoping en sequestratie, werden nu ook concentraties in het poriewater bepaald.

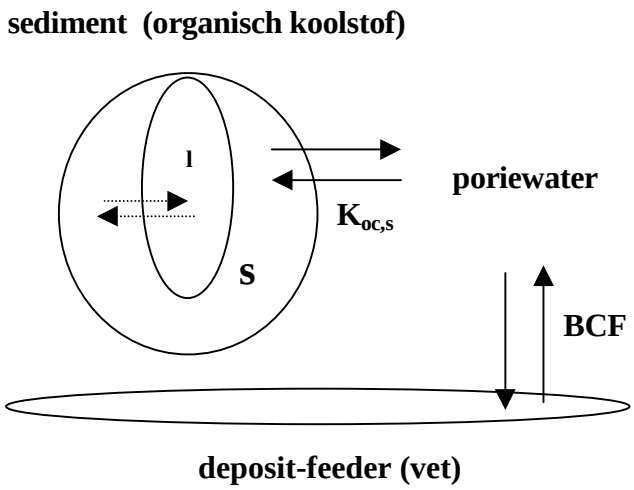
Voor het experiment werd het met HOCs vervuilde sediment uit hoofdstuk 3 gebruikt. Een gedeelte van dit sediment werd behandeld door het tijdelijk in water te brengen waaraan Tenax korreltjes waren toegevoegd. De korreltjes absorbeerden de snel vrijkomende HOCs, waardoor de resterende HOCs grotendeels bestonden uit langzaam vrijkomend (gesequestreerd) materiaal. In het niet behandelde sediment waren nog vrij veel snel vrijkomend (niet gesequestreerde) HOCs aanwezig. In beide sedimenten, behandeld en niet behandeld, werd de ophoping in tubifex wormen en de sequestratie van HOCs gemeten. Omdat de twee sedimenten, behalve de mate van sequestratie, verder geheel identiek waren, kon precies worden onderzocht wat de factor sequestratie voor uitwerking had op de biobeschikbaarheid. Resultaat: de biobeschikbaarheid nam navenant af met de afname in de snel vrijkomende fractie. Dit resultaat was belangrijk om een causaal verband tussen sequestratie en biobeschikbaarheid, en daarmee een algemene geldigheid, aan te tonen.

De poriewatermetingen waren het laatste puzzelstukje van het onderzoek. De poriewatermetingen vormden de verbinding tussen de metingen aan het sediment

(sequestratiemetingen) en de organismen (ophopingsmetingen). Het blijkt dat sequestratie leidt tot proportioneel lagere concentraties in het poriewater en daarmee tot een lagere ophoping in deposit-feeders. We vonden dat HOCs zich volgens een vaste verhouding verdelen tussen het snel vrijkomende compartiment van het organische materiaal in het sediment en het poriewater, en tussen het poriewater en het organisme (zie figuur). Deze laatste verhouding was gelijk aan de verhouding in concentraties van HOCs in organismen en water (bio-concentratiefactor) die werd gevonden in experimenten zonder sediment deeltjes. De uitkomst is redelijk verrassend omdat deposit-feeders niet alleen via poriewater, maar ook via het inslikken van sediment-deeltjes worden blootgesteld aan HOCs. Het verschijnsel van de vaste verhoudingen wordt equilibrium partitie genoemd. Het nieuwe concept is een verbetering van een al bestaand model, de EqP (Equilibrium partitie theorie). In dit model wordt verondersteld dat HOCs zich constant verdelen over het totale organische materiaal en het poriewater, en wordt geen rekening gehouden met sequestratie.

De nieuwe inzichten kunnen worden toegepast in de risico-analyse van HOCs in sediment. De ophoping van HOCs in sediment deposit-feeders kan worden voorspeld uit twee metingen aan het sediment: de concentratie van HOCs in het sediment en de mate van sequestratie. We raden af om de ophoping in sediment organismen te voorspellen uit louter concentratiemetingen. Daarmee kan de ophoping flink onder- of overschat worden. In plaats van metingen aan het sediment, kan ook worden volstaan met een nauwkeurige bepaling van de vrij opgeloste concentratie HOCs in het poriewater. De ophoping van HOCs in sediment organismen is dan te voorspellen uit vermenigvuldiging van de poriewaterconcentratie met de bio-concentratiefactor. De inschatting uit poriewaterconcentraties heeft als voordeel dat verschillen tussen sedimenten wegvallen: Poriewater is poriewater, ongeacht het type sediment. Beide methoden zijn naar verwachting breed toepasbaar in de risico-analyse van HOCs in sedimenten omdat ze gebaseerd zijn op algemeen geldende processen die zich overal ter wereld afspelen.

Samenvattend: Het onderzoek dat in dit proefschrift wordt beschreven geeft inzicht in de samenhang tussen sequestratie van HOCs in bodemdeeltjes en ophoping in sediment organismen. Sequestratie leidt tot een lagere ophoping van HOCs in sediment organismen. Dit komt doordat de concentraties van HOCs in het poriewater verlaagd worden, en HOCs zich volgens een vaste verhouding verdelen over het poriewater en de organismen. De nieuwe inzichten hebben geleid tot een verbeterde methode om de ophoping van HOCs in sediment-organismen te voorspellen. Bij deze methode worden concentraties in het poriewater, in plaats van sedimentconcentraties, gebruikt om de ophoping in sediment organismen in te schatten.



Figuur. Model van de verdeling van HOCs in sediment. l = langzaam vrijlatend (langzaam desorberend) compartiment; s = snel vrijlatend compartiment; $K_{oc,s}$ = partitiecoëfficiënt tussen snel vrijlatend compartiment en poriewater (L/kg organisch koolstof); BCF = bio-concentratie factor (BCF) (L/kg vet)

Curriculum vitae

De auteur werd op 23 november 1965 geboren in Eindhoven. In 1984 behaalde hij het diploma VWO-B aan het Eindhovens Protestants Lyceum. In dat jaar startte hij de studie Milieuhygiëne aan de Landbouwuniversiteit Wageningen. Afstudeervakken werden gevolgd in Wageningen bij de vakgroep Toxicologie en in Amsterdam aan de Vrije Universiteit bij de werkgroep Theoretische Biologie. Na afronding van de studie in 1991 liep hij stage bij Akzo, CRL-ecotoxicologie. Na een korte periode als vrijwilliger bij het Research Institute of Toxicology, was hij projectmedewerker bij het Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling (RIZA). In de periode 1994-1995 ontving hij een Australische beurs (Australian-European Award) voor onderzoek aan de Griffith University in Brisbane, Australië. Op 1 juni 1996 startte het promotieonderzoek aan het Institute for Risk Assessment Sciences (voorheen Research Institute of Toxicology) in Utrecht.