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Polycyclic Aromatic Hydrocarbons in Freshwater Isopods and Field-Partitioning Between Abiotic Phases

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Abstract. An assessment was made of the *in situ* bioaccumulation of 13 polycyclic aromatic hydrocarbons (PAHs) in freshwater isopods in relation to their partitioning between sediments, particulate matter (>0.7 μm), and dissolved phases in eight different water systems of The Netherlands. Large differences in total (Γ PAHs) concentrations and in relative abundance of individual PAHs were observed between organisms and abiotic compartments and among sampling stations. Principal component analysis revealed distinct differences between PAH profiles in sediments and water. High molecular weight PAHs dominated in the sediments, fluoranthene and pyrene in the isopods, and naphthalene in water. Apparent lipid-based bioconcentration factors (BCFs) increased with increasing hydrophobicity (n-octanol/water partition coefficient; K_{ow}). The total range of the BCFs varied only one order of magnitude, ranging from $10^{5.1}$ (naphthalene) to $10^{6.1}$ (benzo[a]pyrene). For PAHs with log $K_{ow} > 6.1$ lower BCFs than expected were observed, which was attributed to reduced bioavailability, to the operational definition of the dissolved phase, and to growth dilution preventing equilibrium to be reached within the lifetime of the isopods. Abiotic partitioning coefficients, such as K_{oc} (organic carbon normalized sediment–water partition coefficient) and K_{pm} (particulate matter–water distribution coefficient) increased with hydrophobicity for PAHs having a log $K_{ow} < 6.1$. Sediment-water partition coefficients (K_d) increased with the organic carbon content of the sediments for most PAHs. It is concluded that isopods have a marked ability to accumulate PAHs and that their tissue residues tend to reflect spatial and temporal variations in the bioavailability of PAHs in littoral freshwater environments.

Polycyclic aromatic hydrocarbons (PAHs) are one of the most widely distributed groups of organic pollutants originating from petrogenic, pyrogenic, and natural sources. They have long been of environmental concern because of the mutagenic and carcinogenic properties of the metabolites of several compounds (*e.g.* benzo[a]pyrene and benzo[a]anthracene) (Neff 1979; IARC 1983; Farrington 1991). The partitioning behavior of PAHs between water, sediments, particulate, and dissolved organic material has been documented in several studies (Karickhof *et al.* 1979; Means *et al.* 1980; Readman *et al.* 1984; Landrum *et al.* 1985; McCarthy *et al.* 1985; Di Toro *et al.* 1991). The fraction of freely dissolved PAHs is usually assumed to be readily available for uptake by organisms. This freely dissolved fraction decreases rapidly with increasing hydrophobicity and with increasing concentrations of binding substrates such as particulate and dissolved organic matter. The bioavailability of PAHs in natural systems cannot easily be predicted because seasonal and habitat-specific factors may influence the composition and fluxes of organic material (Farrington 1991).

Cytochrome P450–mediated biotransformation of PAHs is well developed in mammals, birds, and many fish species (Walker 1980; Neff 1984; Varanasi *et al.* 1989). Most invertebrates have a less developed MFO system (Varanasi *et al.* 1985; James 1989; Meador *et al.* 1995; Van Brummelen *et al.* 1998). Large differences may exist in biotransformation rates among and within invertebrate taxa in aquatic (Neff 1984; McLeese and Burridge 1987; Varanasi *et al.* 1989) and terrestrial environments (Van Straalen 1994; Van Brummelen *et al.* 1996). The suitability of the benthic invertebrate *Asellus aquaticus* (L.) as a model species for the monitoring of the bioavailability of sediment-bound trace metals has been previously demonstrated (Van Hattum *et al.* 1991, 1993). Freshwater isopods are widely distributed in littoral habitats of many meso- and eutrophic waters in the northern hemisphere and usually represent an important food source for predatory invertebrates, fish, and waterfowl (Williams 1962; Marcus *et al.* 1978). Previous work of our group on the bioconcentration and toxicokinetics of chlorpyrifos (Cid Montañés *et al.* 1995) and PAHs (Cid Montañés 1994; Van Hattum and Cid Montañés 1998) indicated an absent biotransformation activity in *A. aquaticus* (L.).

This work presents the results of a field survey on PAHs in isopods in relation to concentrations in sediments, particulate matter, and the dissolved water phase. Apparent bioconcentration factors (BCF^{app}), sediment-water distribution coefficients (K_d), and particulate matter–water partition coefficients (K_{pm}) are evaluated within the framework of the equilibrium partition-

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ing theory, which is based on the assumption that partitioning and distribution can be related to compound-specific properties (hydrophobicity), organism lipid content, and sediment and water characteristics (Karickhof *et al.* 1979; Mackay 1982; Di Toro *et al.* 1991).

Materials and Methods

Sampling Stations

During the period November 1990–March 1991 a survey was conducted in eight water systems of The Netherlands. The selected sampling stations (Table 1, Figure 1) were assumed to have a varying degree of PAH contamination from different sources, ranging from atmospheric deposition in rural areas (Lake Zandplas, Loc. 7) to industrial discharges from a coal-tar processing plant (River Amstel, Loc. 6). Three locations situated in the Rhine-Meuse estuary (Lake Ketelmeer, Loc. 4; nature reserve Brabantse Biesbosch, Loc. 2; Lake Hollandsch Diep, Loc. 3) are influenced by PAHs accumulated in the sediments. Location 1, situated near (20 m) Highway A-9, and Lake Brouwerskolk (Loc. 8), situated in a recreational area near the North Sea coast, are influenced by traffic emissions. Lake Nieuwe Meer (Loc. 5) is a small lake that was used in the past as a dump site for contaminated dredgings from the canals of Amsterdam.

Sampling

Surficial (top layer 10 cm) littoral sediments were sampled with polycarbonate corers and stored in hexane-acetone washed glass jars at 4°C until analysis (within 48 h). At each location composite samples were prepared from at least 10 subsamples of a plot along 20 m of the various water banks. Sediment subsamples were taken for the determination of organic carbon (OC), dry weight, $CaCO_3$ (volumetric method), and grain size distribution (laser diffraction). The OC fraction was determined with an elemental analyzer (Carlo Erba Elemental Analyzer, Model 1106, Milan, Italy) and corrected for carbonate-carbon, similar as in Van Hattum *et al.* (1991) and (1993).

One-liter samples of surface water were collected in brown glass bottles and processed at the laboratory within 4 h. Water samples were filtered over Whatman GF/F™ glass fiber filters of 0.7 µm nominal pore size (Whatman Scientific Limited, Maidstone, UK). Subsamples of each filter were taken for the determinations of suspended matter and PAHs. Dissolved organic carbon (DOC) was determined with a Beckman 914B TOC Analyser; Ca^{2+} and Cl^- were determined according to routine spectrophotometric and flame photometric methods in subsamples of the filtrate as described in APHA (1983).

Samples of litter and benthic filamentous algae (depth: 0.5–1.5 m) with freshwater isopods were collected with a macrofauna net and sorted in the laboratory. A rough estimate of isopod abundance was derived from the area sampled and the number of individuals found. Gut clearance of the isopods was not done because potential elimination of low-molecular-weight PAHs during the defecation period was suspected. Only adult animals (size range 6–20 mm) were included in the study with *A. aquaticus* (L.) being the dominant isopod species at all stations. Pooled samples of the isopods were either directly pretreated for chemical analysis or stored at −20°C until analysis. The lipid contents of the isopods were determined in subsamples of the soxhlet extracts, as hexane-extractable lipids. As known from lipid extraction studies with fish (De Boer 1988; Randall *et al.* 1991), the efficiency of different solvent extraction methods may vary, depending on the nature of the lipid material. Apolar solvents such as hexane may have a limited efficiency for the quantitative extraction of polar bound-lipids compared to mixtures of apolar and polar solvens, such as chloroform/methanol in the standard method of Bligh and Dyer (1959). The amount of sample available was insufficient to apply this widely used standard method and no information is available on the significance of polar lipids in isopods. As stated by Randall *et al.* (1991), differences in lipid extraction methods should be taken in consideration when comparing lipid-based partitioning constants between different studies.

Table 1. Characteristics of sampling stations

Location	Coordinates	Depth[a] (m)	Potential Source of PAHs
1. Highway-A9	52°21′N/4°45′E	0.2	Traffic
2. Brabantse Biesbosch	51°45′N/4°45′E	0.5	Mixed emission sources Rhine/ Meuse
3. Lake Hollandsch Diep	51°43′N/4°34′E	1	Mixed emission sources Rhine/ Meuse
4. Lake Ketelmeer	52°37′N/5°50′E	1	Mixed emission sources Rhine
5. Lake Nieuwe Meer	52°20′N/4°50′E	1	Recreation, various sources
6. River Amstel	52°18′N/4°53′E	1	Coal-tar processing plant
7. Lake Zandplas	52°42′N/4°50′E	1	Atmospheric background deposition
8. Lake Brouwerskolk	52°23′N/4°36′E	1	Recreation, traffic

[a] Depth of sampling

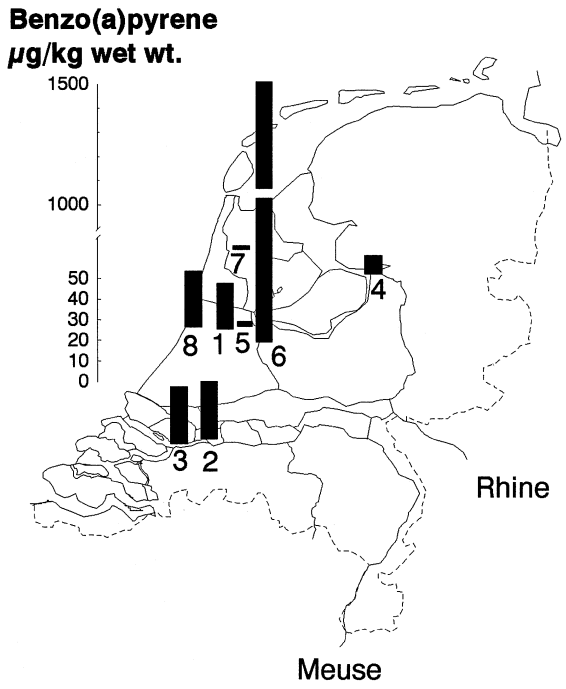

Fig. 1. Benzo[a]pyrene in isopods (µg kg^{-1} wet weight). Coordinates of sampling stations are given in Table 1

Extraction and Clean-Up

Polycyclic aromatic hydrocarbons were determined by means of solvent extraction, column chromatographic clean-up, and analysis on

a liquid chromatograph with fluorescence detection. Sediment subsamples of 5–10 g (wet weight) and filters were extracted by mechanical shaking with 50 ml acetone (HPLC grade, J.T. Baker, Deventer, Holland) for 20 min and 100 ml petroleum-ether (Merck, Darmstadt, Germany; purified by distillation) were added for another 20-min extraction. The extracts were washed twice with 100 ml of water (HPLC grade, J.T. Baker) and concentrated to 1 ml in a Kuderna-Danish apparatus. Pooled samples of freshwater isopods (0.2–1 g wet weight) were blotted dry, homogenized with anhydrous Na_2SO_4 (Merck), and extracted with 30 ml of n-hexane (HPLC grade, J.T. Baker) in a homemade micro-Soxhlet apparatus for 4 hs. The extracts were concentrated to 1 ml under a gentle stream of nitrogen gas. Water filtrates (1 L) were extracted twice with 50 ml petroleum-ether in a separatory funnel. The organic phase was dried with anhydrous Na_2SO_4 and transferred quantitatively into a Kuderna Danish apparatus. The extracts were concentrated to between 3 and 6 ml and further reduced to 1 ml under a gentle stream of nitrogen gas.

For the clean-up, aluminum oxide (Super I, ICN Biomedicals, Eschwege, FRG) was activated at 200°C for 24 h and deactivated with 15% water. The concentrated extracts were eluted with 15 ml of petroleum-ether from a column filled with 2 g of deactivated alumina, previously washed with 4 ml petroleum-ether. Eluates were concentrated to 1 ml under nitrogen gas, taken up in 1 ml of acetonitrile (HPLC grade ACN; J.T. Baker), and stored in crimp top vials until analysis.

Analysis

An HP1090 liquid chromatograph equipped with a programmable fluorescence detector and a Vydac 201 TP54 reversed-phase column (250 × 0.46 mm; 5 Tm; Chrompack, Middelburg, Holland) was used for the determination of PAHs. A 40-min gradient elution program was applied: 10 min isocratic at 55:45 ACN:H_2O, increase to 100:0 during 25 min and 5 min isocratic at 100:0 (flow rate of 1.0 ml/min). The column was thermostatted at 30°C and automatic injections (20 µl) were performed with an autosampler. External standards were prepared daily from a certified mixture of PAH (SRM 1747b—Priority Pollutants PAH, NIST, Gaithersburg, MD, US).

Quality Control

Recoveries (mean ± SD) of spiked samples (n = 3) ranged from 78 ± 1% (naphthalene) to 97 ± 4% (benzo[b]fluoranthene) for sediments, from 70 ± 17% (naphthalene) to 103 ± 13% (indeno[123-c,d]pyrene) for organisms, and from 86 ± 3% (naphthalene) to 103 ± 3% (dibenz[a]anthracene) for filtered water samples, respectively. Measured values (n = 3) of a reference material (SRM 1941—organics in marine sediment, NIST) ranged from 72% (phenanthrene) to 106% (benzo[b]fluoranthene) of the certified values and averaged 92 ± 11% for the 11 PAHs for which certified concentrations were available. When sufficient material was available, sediment and isopods were analyzed in duplicate. Detection limits ranged from 0.18 to 10 µg kg^{-1} (wet weight) in sediments, from 0.20 to 15 µg kg^{-1} (wet weight) in isopods and from 0.1 to 8 ng L^{-1} in filtered water samples. Reported final concentrations were corrected only for procedural blanks.

Statistical Analysis

Analysis of variance (ANOVA) and Tukey's multiple range test, as described by Sokal and Rohlf (1987) were applied to investigate significant differences between locations and compartments ($p < 0.05$ level). The relationships of apparent bioconcentration factors and abiotic partition coefficients with measured variables and K_{ow} were evaluated by linear regression techniques. Significance of regressions was tested with ANOVA on model explained variance. Statistical calculations were performed with the software packages Statgraphics 2.6™ and SPSS-PC 5.1™. The variation of PAH patterns was investigated with the principal component analysis (PCA) routine included in the software package Sirius 2.3™ (Karstang and Kvalheim 1990). The data transformation procedure applied in the PCA analysis was similar to methods applied in Leonards *et al.* (1997) and included normalization (with respect to total PAH concentrations) and standardization (with respect to total variance of individual PAH) in order to rule out confounding effects of concentration differences and to give all PAHs equal weight.

Results

Station Characteristics

Lipid content of isopods ranged from 1.4 to 3.8% (fresh weight) and were slightly higher compared to values observed in a previous study (Cid Montañés *et al.* 1995). The organic carbon content of the sediments (f_{oc}) ranged from 0.9% (Lake Zandplas, Highway A9) to 22% (Lake Nieuwe Meer). The lutum fraction (particles < 2 µm) varied between < 0.1% (Lake Brouwerskolk) and 22% (Lake Hollandsch Diep) and suspended matter concentrations varied between 5 and 39 mg L^{-1} (dry weight). Dissolved organic carbon (DOC) concentrations ranged from 3.2 to 18 mg L^{-1}. Additional site characteristics are presented in Table A-1 in the Appendix.

PAH Concentrations in the Environment

Ranges of concentrations of PAHs observed at the different sampling stations are summarized in Table A-2 in the Appendix. The full data set is available on request. Large differences in PAH concentrations were found between sampling stations, biotic and abiotic compartments, and individual PAHs. As an example, Figure 1 shows the broad range of benzo[a]pyrene concentrations found in isopods, which are three to six orders of magnitude higher than corresponding concentrations in water. Extremely high concentrations of PAHs were found for all compartments near the coal-tar processing plant (Loc. 6). The lowest concentrations were observed at the stations Lake Zandplas (Loc. 7) and Lake Nieuwe Meer (Loc. 5). Within specific compartments, individual PAH concentrations relative to each other varied over a narrower range: one to two orders of magnitude, indicating the existence of compartment-specific PAH patterns.

Except for naphthalene, strong correlations were present between PAH concentrations in organisms (lipid based) or sediments (normalized to organic carbon) and PAH concentrations in water (filtered) with correlation coefficients (r) ranging from 0.89 to 1.00 ($0.001 < p < 0.01$). For particulate matter (dry weight), less strong but significant correlations ($0.76 < r < 0.97$ with $0.001 < p < 0.05$) with PAHs concentrations in water were observed for most individual compounds, except for dibenzo[ah]anthracene. As an example, the relationships with aqueous concentrations have been indicated for fluoranthene in Figure 2. The existence of strong correlations between concentrations in abiotic or biotic compartments and dissolved water concentrations is one of the basic assumptions of the equilib-

rium partitioning theory (Di Toro *et al.* 1991), which seems to be partly supported by our sediment and isopod data.

PAHs Patterns

Figure 3 shows the patterns of individual PAHs (as fraction of PAHs) in each compartment for location 3 (Lake Hollandsch Diep). PAHs with two, three, and four ring systems (naphthalene, phenanthrene, fluoranthene, and pyrene) were dominant in filtered water. Pyrene, naphthalene, and fluoranthene dominated in the PAH profiles of the isopods, followed by naphthalene, chrysene, and phenanthrene. PAHs with four to six rings were more abundant in sediments. The particulate matter profile seems to occupy an intermediate position between the sediment and water patterns. The unexpectedly high contribution of dibenzo[ah]anthracene, benzo[ghi]perylene, and indeno[123-cd]pyrene in the water samples is attributed to cosampling of colloidal and dissolved organic matter, due to the cut-off diameter (0.7 μm) of the filter material.

Further examination of the PAH profiles was conducted with PCA. Normalization and standardization of concentrations were applied to rule out effects of differences in concentrations within and between categories of samples and to give equal weight to all PAHs. The component score plots for the first two principal components are indicated in Figure 4, together with the contribution (component loading) of each PAH. The biplot shows that the first component, which explains about 37% of the total variance, is mainly determined by high concentrations of benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[a]anthracene, and low concentrations of naphthalene. This component is mainly responsible for the separation of water, isopod, and sediment profiles. The second component, explaining almost 23% of the total variance, is mainly determined (*i.e.* factor loadings > 0.3 or < -0.3) by high relative concentrations of indeno[123-cd]pyrene, benzo[ghi]perylene, and naphthalene, and relatively low concentrations of pyrene and fluoranthene. This component contributes to the separation of most water and isopod samples. In summary, high naphthalene concentrations in water and of pyrene and fluoranthene in isopods, and relatively high levels of four intercorrelated PAHs (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, and benzo[k]fluoranthene) in sediments contribute to the different PAH profiles observed.

Bioconcentration

Apparent *in situ* bioconcentration factors (log BCF^{app}, fresh weight, and lipid-based) were calculated from measured isopod and water concentrations for each location, and are listed in Table 2. Regression estimates for the log BCF–log K_{ow} relationship of PAHs in freshwater isopods are presented in Table 3. Apparent lipid-based bioconcentration factors do increase with hydrophobicity, but their total range covers only one order of magnitude, from $10^{5.12}$ (naphthalene) to $10^{6.1}$ (benzo[a]pyrene). Lower values for PAHs with log $K_{ow} > 6.1$ were observed. For individual PAHs, observed BCF^{app}s varied sometimes up to several orders of magnitude between different locations. Analysis of the covariation of BCF^{app} with factors such as sediment organic carbon, dissolved organic carbon, or suspended matter content did not reveal any significant relationship (not shown).

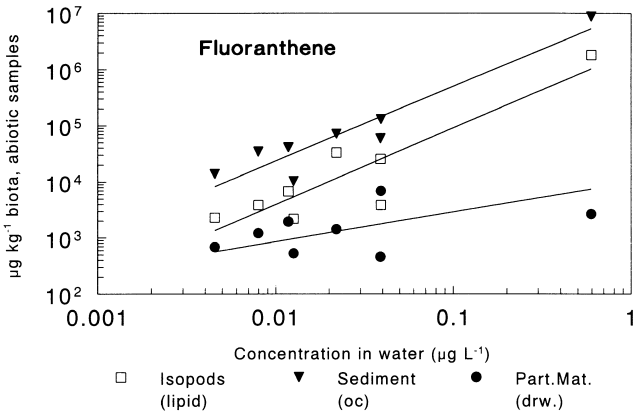

Fig. 2. Variability of concentrations of fluoranthene in sediments (μg kg^{-1} organic carbon normalized), isopods (μg kg^{-1} lipid based), and particulate matter (μg kg^{-1} dry wt.) between sampling stations in relation to concentrations in filtered water (μg L^{-1}; <0.7 μm). The solid lines represent the double logarithmic regression relationships with filtered water

Sediment-Water Partitioning

The mean dry weight sediment-water partition coefficients (K_d; L kg^{-1}), presented in Table 4, show an increase with hydrophobicity for PAHs with $3 <$ log $K_{ow} < 6.1$. The K_d values of individual PAHs varied by two to three orders of magnitude between sampling stations. The dependence of K_d of organic contaminants on their physicochemical characteristics (K_{ow}) and the organic carbon content (f_{oc}) of the sediments is well known (Karickhof *et al.* 1979; Means *et al.* 1980; Farrington 1991). For the sampling stations with f_{oc} below 10%, the K_d variability could partly be attributed to covariation with f_{oc}, while for some stations with f_{oc} of approximately 1% a large variation of K_d values was observed (*e.g.,* benzo[a]pyrene). The weak double logarithmic relationships found between K_d and f_{oc} are shown for some PAHs in Figure 5. Organic carbon–normalized sediment–water distribution coefficients (K_{oc}) of PAHs increased with hydrophobicity (Table 4), with benzo[a]pyrene, benzo[b]fluoranthene, and benzo[k]fluoranthene showing the highest values. An apparent cut-off of log K_d and log K_{oc} for PAHs with log $K_{ow} > 6.1$ (*e.g.,* dibenzo[ah]anthracene, benzo[ghi]perylene, and indeno[123-cd]pyrene) was found.

Regression estimates for the relationships of K_d and K_{oc} with K_{ow} are presented in Table 3. Compared to the relationship reported by Karickhof *et al.* (1979), the slope of the log K_{oc} − log K_{ow} relationship is similar, whereas the intercept for the field relationship is higher by almost two log units.

Particulate Matter–Water Partitioning

Particulate matter–water distribution coefficients (K_{pm}; L kg^{-1} dry weight) are listed in Table 4, and seem to be similar to corresponding values of K_d (dry weight). An increase of K_{pm} with increasing hydrophobicity was found for PAHs with $3 <$ log $K_{ow} < 6.1$, similar to what was observed for the sediment-water partition coefficients described in the previous section. Maximum values of K_{pm} were observed for benzo[a]pyrene, benzo[b]fluoranthene, and benzo[k]fluoranthene. Regression

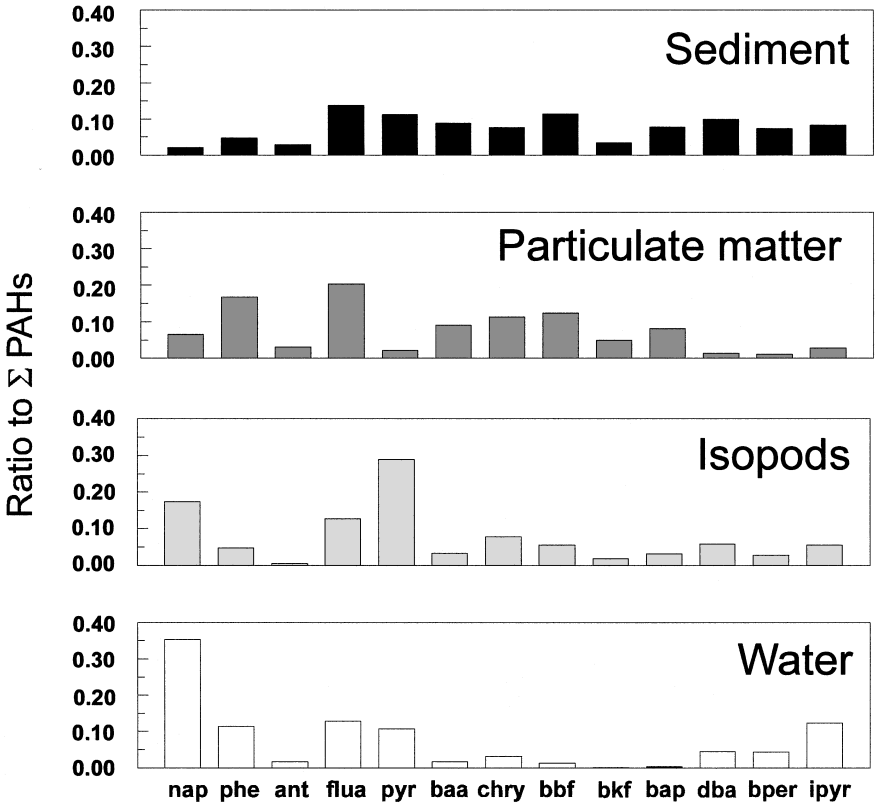

Fig. 3. Patterns of relative concentrations (ratio to Σ PAHs) in sediment, particulate matter, isopods, and filtered water from Lake Hollandsch Diep

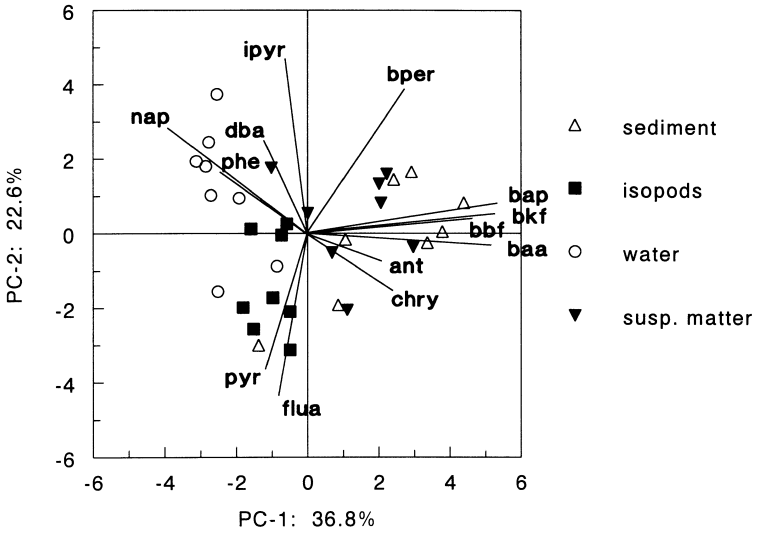

Fig. 4. Principal component analysis. Biplot with component loadings for individual PAHs and component scores for normalized and standardized concentrations of isopods (filled squares), sediments (open triangles), water (open dots), and particulate matter (filled triangles). The variance explained by each principal component is indicated along the axes and the abbreviated names of PAHs correspond to those indicated in Table 2

estimates of the relationship log K_{pm} − log K_{ow} are also included in Table 3. For individual PAHs a considerable variation in K_{pm} values was found between different locations, with relatively low K_{pm} values for all PAHs in the River Amstel (Loc. 6). At this location relatively high DOC, suspended matter and dissolved water concentrations were found (f_{oc} of particulate matter was not determined in this study).

Organism-Sediment Accumulation Factors

Biota-sediment accumulation factors (BSAFs), normalized to the sediment organic carbon and isopod lipid content, are plotted against log K_{ow} in Figure 6. For seven PAHs, significant correlations were observed between lipid-based concentrations in isopods and organic carbon normalized concentrations in sediments. Correlation coefficients ranged from 0.74 (benzo-[a]pyrene) to 0.95 (phenanthrene). No significant correlations were found for benzo[b]fluoranthene, benzo[k]fluoranthene, dibenzo[ah]anthracene, benzo[ghi]perylene, or indeno[123-cd]pyrene. For individual PAHs mean values over eight locations ranged between 0.1 (anthracene) and 4.7 (naphthalene). In agreement with other studies (Connor 1984; Neff 1984), no apparent relationship between BSAFs and K_{ow} was observed. Between different locations one to three orders of magnitude differences in BSAFs for individual PAHs were observed.

Table 2. Apparent bioconcentration factors (BCF^{app}; L kg^{-1}) of PAHs in freshwater isopods[a]

Compound	Abbreviation	Log K_{ow}[b]	Log BCF^{app} Wet Wt. x ± SD (range)	Log BCF^{app} Lipid Wt. x ± SD (range)
Naphthalene	nap	3.35	3.4 ± 0.6 (2.5–4.3)	5.1 ± 0.5 (4.3–5.9)
Phenanthrene	phe	4.57	3.6 ± 0.5 (2.9–4.5)	5.4 ± 0.5 (4.6–6.3)
Anthracene	ant	4.54	3.8 ± 0.2 (3.5–4.2)	5.5 ± 0.3 (5.1–6.0)
Fluoranthene	flua	5.22	4.0 ± 0.4 (3.3–4.6)	5.7 ± 0.4 (5.0–6.5)
Pyrene	pyr	5.18	4.2 ± 0.4 (3.6–4.8)	6.0 ± 0.4 (4.2–6.9)
Benzo[a]anthracene	baa	5.61	4.1 ± 0.8 (2.5–5.1)	5.9 ± 0.8 (5.1–6.4)
Chrysene	chry	5.61	4.3 ± 0.6 (3.4–5.4)	6.0 ± 0.6 (4.2–6.9)
Benzo[b]fluoranthene	bbf	5.98	4.3 ± 0.8 (2.7–5.4)	6.0 ± 0.8 (5.0–7.0)
Benzo[k]fluoranthene	bbk	6.04	4.4 ± 0.9 (2.7–5.4)	6.1 ± 0.9 (4.4–7.2)
Benzo[a]pyrene	bap	6.04	4.4 ± 0.8 (2.8–5.5)	6.1 ± 0.8 (4.4–7.2)
Dibenzo[ah]anthracene	dba	7.11	3.7 ± 0.5 (2.6–4.2)	5.5 ± 0.4 (4.2–5.8)
Benzo[ghi]perylene	bgp	7.04	3.7 ± 0.4 (3.0–4.5)	5.4 ± 0.5 (4.7–6.3)
Indeno[123-cd]pyrene	ipyr	7.04	3.4 ± 0.4 (2.3–3.8)	5.1 ± 0.4 (4.1–5.7)

[a] Indicated are mean, standard deviation, and minimum to maximum ranges (between parentheses) of values determined at eight locations
[b] K_{ow} values taken from Miller *et al.* (1985), Radding *et al.* (1976), and Rekker and De Kort (1979)

Table 3. Relationships between apparent bioconcentration factors (BCF^{app}, L kg^{-1}), apparent organic-carbon adsorption coefficients (K_{oc}; L kg^{-1} dry weight), particulate matter–water partition coefficients (K_{pm}; L kg^{-1} dry weight), and hydrophobicity (n-octanol-water partition coefficient, K_{ow}). Regression coefficients for the relationship: $\log Y = a \log K_{ow} + b$[a]

Log Y =	a	b	R^2	F	p	s_y
Log BCF^{app} wet wt.	0.38 ± 0.04	2.1 ± 0.2	0.92	97	<0.00001	0.1
Log BCF^{app} lipid wt.	0.38 ± 0.04	3.8 ± 0.2	0.93	95	<0.00001	0.1
Log K_{oc}	0.86 ± 0.11	2.2 ± 0.6	0.89	63	<0.00005	0.3
Log K_{pm}	0.66 ± 0.06	1.5 ± 0.3	0.93	106	<0.00001	0.2

Abbreviations: R^2: coefficient of determination; F: value for F test on significance of regression; p: significance of F; s_y: standard error of regression estimate
[a] Mean values (n = 8) of BCF, K_{oc}, and K_{pm}. K_{ow} values taken from Table 2. PAHs (n = 10) with log $K_{ow} < 6.1$ were included in the regression analysis

Discussion

Concentrations in Isopods

The results of the present study demonstrated a significant bioaccumulation of PAHs by freshwater isopods in various waters of The Netherlands, which was in line with results from toxicokinetic experiments (Van Hattum and Cid Montañés 1998). Large differences for individual PAH concentrations in isopods were observed between the different sampling stations. When location 6 is excluded from the analysis, the range of benzo[a]pyrene concentrations in *A. aquaticus* at the other sampling stations (1–50 µg kg^{-1} wet weight) is comparable to levels reported by Eadie *et al.* (1983) for oligochaetes (10–30 µg kg^{-1}) and chironomid larvae (40–150 µg kg^{-1}) from Lake Erie (US), and lower than levels reported for amphipods (250–1,000 µg kg^{-1}) from the same area. In a comparative study in the Rhine-Meuse estuary (Van Brummelen *et al.* 1998) we observed the highest (lipid-based) concentrations of PAHs in aquatic plants (*Elodea* spp., *Potamogeton* spp.; 1,300–3,000 µg kg^{-1}), oligochaetes (2,600 µg kg^{-1}), and freshwater isopods (1,300–1,400 µg kg^{-1}). Lower concentrations were found in bivalves (zebra mussels, freshwater clams; 500–1,100 µg kg^{-1}), chironomids (200–600 µg kg^{-1}), and fish (<10 µg kg^{-1}). For the latter two taxa, metabolism has been documented in the literature (Varanasi *et al.* 1989; Van Brummelen *et al.* 1998). Compared to the levels of benzo[a]pyrene reported for marine invertebrates (<0.4–750 µg kg^{-1} dry weight) in the comprehensive review by Neff (1979), the values observed in *Asellus* at sampling stations without direct industrial emissions (5–250 µg kg^{-1} dry weight) are well within this range. However it should be noted that a true comparison between species cannot be made without additional information on *e.g.*, variation in microhabitat bioavailability of PAHs, differences in lifestage, age, and exposure pathways, and species-specific differences in toxicokinetics and biotransformation of PAHs.

Bioavailability of Aqueous PAHs

The mean wet weight bioconcentration factors (log BCF^{app}; Table 2) of PAHs with $3 < \log K_{ow} < 6.1$ increased from 3.4 for naphthalene) to 4.4 for benzo[a]pyrene and benzo[k]fluoranthene (Figure 7). In laboratory bioconcentration studies with fish (Mackay 1982; indicated in Figure 7) or invertebrates (Southworth *et al.* 1978; Eastmond *et al.* 1984) the log BCF for steady-state conditions covaried with log K_{ow} with a regression slope (0.66–1.1) approaching unity. In a 21-day laboratory study on the toxicokinetics of six PAHs in *A. aquaticus* a similar slope value (1.1) was found (Van Hattum and Cid Montañés 1998) that is much higher than the values shown in Table 3. Reduced bioavailability of PAHs in the field, due to sorption onto sediments, suspended or colloidal matter, and dissolved organic carbon has been reported by many authors (McCarthy and Jimenez 1985; McCarthy *et al.* 1985; Landrum *et al.* 1985, 1994). The binding affinity of PAHs toward these substrates is known to increase with K_{ow} (Karickhof *et al.* 1979; Means *et al.* 1980; McCarthy *et al.* 1985), which is confirmed by the observed sediment and particulate matter partition coefficients obtained in this study (Table 4).

The dimensions of the filter material used (0.7 µm) allow the passage of fine colloidal material and dissolved organic matter containing sorbed PAHs that may not be fully bioavailable to

Table 4. Sediment-water distribution coefficients (K_d; L kg^{-1} dry weight), organic carbon normalized sediment-water partitioning coefficients (K_{oc}; L kg^{-1}), and particulate matter–water distribution coefficients (K_{pm}; L kg^{-1} dry weight)

Compound	Log K_d Dry Weight [x ± SD (range)]	Log K_{oc} [x ± SD (range)]	Log K_{pm} Dry Weight [x ± SD (range)]
Naphthalene	3.3 ± 0.9 (2.0–4.5)	4.8 ± 0.8 (3.1–5.6)	3.8 ± 0.9 (2.0–4.9)
Phenanthrene	4.4 ± 0.9 (3.6–6.3)	5.9 ± 0.8 (4.5–7.5)	4.6 ± 0.6 (3.4–5.2)
Anthracene	5.2 ± 0.7 (3.7–6.5)	6.7 ± 0.6 (5.8–7.6)	4.7 ± 0.9 (3.2–5.9)
Fluoranthene	5.0 ± 0.6 (4.1–6.0)	6.5 ± 0.3 (5.9–7.2)	4.8 ± 0.6 (3.6–5.2)
Pyrene	5.0 ± 0.6 (4.4–6.2)	6.6 ± 0.4 (5.8–7.3)	4.8 ± 0.5 (3.8–5.5)
Benzo[a]anthracene	5.6 ± 0.7 (4.5–6.9)	7.2 ± 0.5 (6.3–8.0)	5.3 ± 0.5 (4.4–5.8)
Chrysene	5.2 ± 0.8 (4.2–6.7)	6.8 ± 0.5 (6.1–7.8)	5.1 ± 0.4 (4.4–5.6)
Benzo[b]fluoranthene	5.8 ± 0.8 (4.4–7.1)	7.3 ± 0.6 (6.4–8.2)	5.5 ± 0.5 (4.8–6.4)
Benzo[k]fluoranthene	5.9 ± 0.8 (4.5–7.2)	7.4 ± 0.6 (6.5–8.3)	5.8 ± 0.5 (4.9–6.5)
Benzo[a]pyrene	5.8 ± 0.9 (4.0–7.3)	7.4 ± 0.7 (6.0–8.4)	5.6 ± 0.5 (4.8–6.3)
Dibenzo[ah]anthracene	4.6 ± 0.8 (3.2–5.6)	6.2 ± 0.6 (5.3–6.9)	4.6 ± 0.7 (3.5–5.9)
Benzo[ghi]perylene	5.1 ± 0.9 (3.3–6.4)	6.6 ± 0.6 (5.3–7.6)	4.9 ± 0.6 (4.0–5.6)
Indeno[123-cd]pyrene	4.7 ± 0.8 (3.2–6.0)	6.3 ± 0.5 (5.2–7.2)	4.6 ± 0.6 (3.5–5.3)

[a] Indicated are mean values (n = 8), standard deviations, and ranges

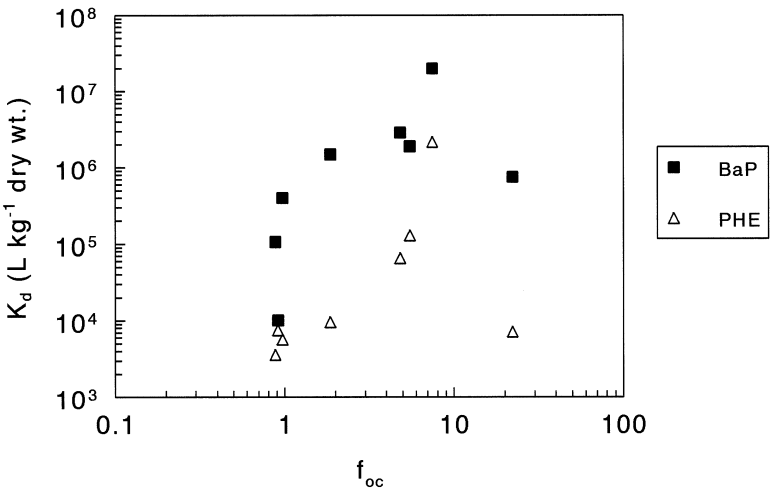

Fig. 5. Variation of dry weight sediment-water distribution coefficients (K_d; L kg^{-1}) of phenanthrene (triangles) and benzo[a]pyrene (squares) among sampling stations with the sediment organic carbon content (f_{oc} dry weight)

isopods. The binding affinity of PAHs for fine colloidal material increases with K_{ow} (Gschwend and Wu 1985; Landrum *et al.* 1985). This is the most likely explanation for the relatively high concentrations of dibenzo[ah]anthracene, benzo[ghi]perylene, and indeno[123-cd]pyrene in the filtered water samples (Figure 3). Consequently, the low apparent BCFs observed for more hydrophobic PAHs may be the result of an overestimation of the freely dissolved fraction. The systematically reduced values of BCF^{app}, K_{oc}, and K_{pm} for these compounds must be attributed at least partly to artifacts caused by the operationally defined (<0.7 µm) phase separation of the PAHs.

Estimation of Aqueous Partitioning

The relationship of Landrum *et al.* (1985) for dissolved organic matter sorption coefficients (K_{dom}) and K_{ow} (log K_{dom} = 0.59. log K_{ow} + 1.89) was used to approximate the distribution of PAHs between the dissolved aqueous phases (''free'' dissolved and DOC-bound). Combined with measured DOC, total suspended matter, and PAHs concentrations in filtered water and particulate matter, the aqueous partitioning was estimated for each of PAHs at the eight different locations. Except for naphthalene, particulate matter was the dominant aqueous fraction. Approximately 43 to 85% of the PAHs was bound to particulate material (>0.7 µm). The DOC-bound fraction varied between 6 and 50% for most PAHs. The estimated freely dissolved fraction decreased rapidly with K_{ow}, from 76% for naphthalene to 4% for benzo[a]pyrene. Using the estimates of the free dissolved fraction, new lipid based BCF^{app} (in L kg^{-1}; results not presented) were derived. These corrected BCFs were approximately 0.5–1.1 log units higher than the values presented in Table 2 (for PAHs with log K_{ow} 6–7). The need to correct field-derived bioconcentration data for binding to DOC has been noted previously by various authors (Landrum 1989; Farrington 1991). Our results are in line with these observations and further demonstrate that for highly hydrophobic PAHs present in the water column, only a small fraction may be actually available for uptake by organisms.

Kinetic Limitations

Another explanation of the limited range of mean isopod BCFs among different PAHs may be derived from kinetic considerations. Most of the water systems included in this study are eutrophic with relatively high annual turnover rates of organic carbon. If desorption rates are small in comparison to organic

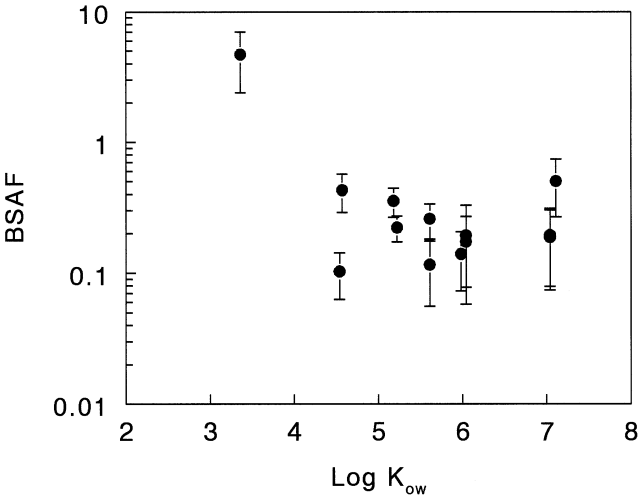

Fig. 6. Biota-sediment accumulation factors (BSAF; normalized to organism lipids and sediment organic carbon; mean ± SE; n = 8) for individual PAHs in relation to log K_{ow}

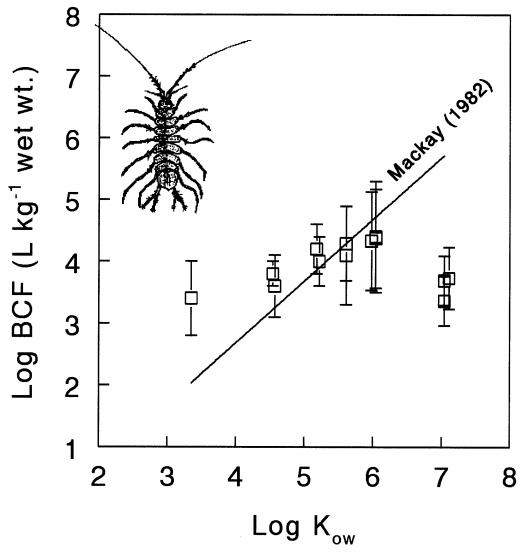

Fig. 7. Apparent wet weight bioconcentrations factors (log BCF^{app}; L kg^{-1}) of 13 PAHs in freshwater isopods from field locations (mean ± SE, n = 8). BCFs are plotted versus K_{ow} for comparison with the laboratory-based benchmark relationship of Mackay (1982)

carbon degradation rates, then deviations from steady-state conditions may be expected and low apparent bioavailability of the more hydrophobic PAHs will be observed. As a result, more PAHs remain bound to organic carbon substrates than would be predicted by partition coefficients. A decrease of sediment and particulate matter desorption rates with K_{ow} has been reported previously (Karickhof and Morris 1985).

Biological Factors

Animal growth can result in a decrease of apparent bioconcentration factors, especially for compounds with elimination rate constants in the order of magnitude of relative body growth rates (Spacie and Hamelink 1985). In toxicokinetic experiments with PAHs in *A. aquaticus* elimination rate constants decreased with increasing K_{ow} (Van Hattum and Cid Montañés 1998). Using relative growth rate values for freshwater isopods (0.01–0.05 day^{-1}) reported by Marcus *et al.* (1978), it can be demonstrated that growth dilution is likely to restrain the maximum apparent bioconcentration. This may have been the case for compounds with elimination rate constants less than 0.01–0.05 day^{-1}, which were observed for PAHs with $5 < \log K_{ow} < 6$. The apparent BCF of naphthalene and some of the BCFs corrected for sorption to DOC (phenanthrene and anthracene) are higher than predicted from laboratory results and may indicate either an increased uptake or a selective retention of decreased elimination of these compounds. Potential additional uptake in the field from dietary sources is not likely for compounds with relatively low hydrophobicity (Thomann *et al.* 1992; Landrum *et al.* 1994). In previous studies on the toxicokinetics of trace metals, direct uptake from water via the gill-pleopod system was assumed to be the dominant uptake mechanism.

Bioavailability of Sediment-Bound PAHs

The results of this study further indicated that the bioavailability of sediment-bound PAHs may be one to two orders of magnitude lower than what would be predicted from the log $k_{oc} - K_{ow}$ benchmark relationships (Karickhof *et al.* 1979), commonly used in equilibrium partitioning–based risk assessment procedures (Di Toro *et al.* 1991; Van der Meent and De Bruijn 1995). Similar deviations of PAH partitioning from benchmark relationship have been reported from an increasing number of field studies, such as reported by Kayal and Connell (1990), Readman *et al.* (1984), Broman *et al.* (1991), and McGroddy *et al.* (1996), and have been attributed to aging of sediments and increased incorporation of PAHs with time in slowly exchanging phases (Harkey *et al.* 1995; Pignatello and Xing 1995; Belfroid and Sijm 1996) or to the specific binding of PAHs to soot particles in the sediments. Gustafsson *et al.* (1997) recently demonstrated that especially combustion source–derived PAHs have a much higher affinity to soot particles than to natural organic matter in sediments.

The low bioavailability of sediment-bound PAHs is further corroborated by the relatively low lipid-organic carbon normalized isopod-sediment BSAF values of PAHs with three or more ring systems (mean value over eight locations: 0.1–0.8). Similar values have been reported for PAHs in various aquatic species of molluscs (0.2–2.2) and annelids (0.02–1.8) from laboratory and field studies (reviewed in Meador *et al.* 1995; Van Brummelen *et al.* 1998). According to predictions from equilibrium partitioning, BSAF values for neutral hydrophobic contaminants that are not subject to biotransformation are expected to range from 0.8–10 (Lee *et al.* 1993; Thomann *et al.* 1992).

As the biotransformation potential of *A. aquaticus* is expected to be low (Cid Montañés *et al.* 1995), the observed BSAF values probably must be attributed to a low bioavailability of PAHs. In studies on the terrestrial isopod (*Porcelio scaber*) Van Brummelen *et al.* (1996) reported field-derived BSAF values ranging from 0.01 to 0.2. The existence of biotransformation in this terrestrial species has recently been demonstrated for pyrene (Stroomberg *et al.* 1996).

The relatively low *in situ* bioavailability of sediment-bound PAHs to freshwater isopods cannot be explained from biota-sediment-water partitioning in the framework of the equilibrium partitioning theory, as currently applied in environmental policy for the derivation of quality objectives (Di Toro *et al.* 1991; Van der Meent and De Bruijn 1995).

Conclusions

The present study confirmed the marked ability of freshwater isopods to accumulate PAHs. Apparent lipid-based bioconcentration factors (BCFs) showed an increase with increasing hydrophobicity and ranged from $10^{5.12}$ to $10^{6.1}$. For PAHs with log K_{ow} values > 6.1, lower BCFs were observed, which was attributed to reduced bioavailability, to potential cosampling of colloidal and dissolved organic material, and to growth dilution preventing equilibrium to be reached within the lifetime of the isopods. Apparent BCFs, BSAFs, and abiotic distribution coefficients (sediment-water K_d, K_{oc}; particulate matter–water K_{pm}) exhibited differences of one to three orders of magnitude between locations, which could not be attributed to the lipid content of the isopods, the aqueous dissolved organic carbon, or the sediment organic carbon content. This unexplained variability points to the inadequacy of laboratory derived equilibrium-partitioning concepts to fully account for the complex and dynamic phenomena in the field.

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Appendix

Appendix 1. Water constituents, sediment characteristics, and isopod parameters for the sampling stations

	1 Highway	2 Biesbosch	3 H. Diep	4 Ketelmeer	5 N. Meer	6 R. Amstel	7 Zandplas	8 Brouwerskolk
Water								
Ca^{2+} (mg L^{-1})	204	75	78	90	124	181	105	84
Cl^- (mg L^{-1})	896	41	84	138	277	159	168	53
DOC (mg L^{-1})	18	3.2	3.5	5.6	12	17	10	4.9
Particulate matter (mg L^{-1} dry wt.)	37	16	31	26	5	39	5	18
Sediment								
Organic carbon (%)	0.9	5.5	4.8	1.8	22	7.5	0.9	1.0
$CaCO_3$ (%)	10	7.9	13	8.4	7.1	5.5	16	<0.1
Grain size (cumul. %)								
<2 mm	4	12	22	1	5	8	7	<0.1
<63 mm	19	48	90	17	57	37	61	1
Isopods								
Abundance class (m^{-2})	1–10	10–100	1–10	>100	10–100	10–100	10–100	10–100
Sample size (g)	0.089	0.229	0.145	1.770	0.720	0.741	1.054	0.7870
Lipid content (%)	3.8	2.2	2.1	2.2	2.1	1.4	1.5	1.6

Appendix 2. Range (minimum to maximum) of concentrations of PAHs in sediments, water, particulate matter, and isopods at the eight sampling stations

PAH	Sediment (mg kg^{-1} dry wt.)	Water (ng L^{-1})	Particulate Matter (µg kg^{-1} dry wt.)	Isopods (µg kg^{-1} wet wt.)
Naphthalene	0.001–6	6–210	19–630	11–360
Phenanthrene	0.03–300	5–140	115–2,200	4–4,100
Anthracene	0.009–130	2–42	14–300	1–630
Fluoranthene	0.12–660	4–590	460–6,900	32–26,000
Pyrene	0.12–510	4–300	210–3,500	19–10,000
Benzo[a]anthracene	0.03–14	0.3–19	77–870	1–2,200
Chrysene	0.04–130	2–27	160–2,500	9–3,500
Benzo[b]fluoranthene	0.03–110	0.4–8	80–2,000	1– 2,000
Benzo[k]fluoranthene	0.006–48	0.1–3	34–740	0.4–710
Benzo[a]pyrene	0.006–91	0.2–5	37–780	1–1,300
Dibenzo[ah]anthracene	0.02–11	1–55	122–920	4–270
Benzo[ghi]perylene	0.01–48	1–18	108–1,100	3–520
Indeno[123-cd]pyrene	0.03–61	3–52	195–1,600	1–360

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