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# Toxicokinetics and Bioconcentration of Polycyclic Aromatic Hydrocarbons in Freshwater Isopods

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A novel method based on a first-order two-compartment model was used to determine the bioconcentration and toxicokinetic rate constants of six different polycyclic aromatic hydrocarbons (PAHs) in the freshwater isopod *Asellus aquaticus,* a common species in most European freshwater systems. Numerical integration and iterative parameter estimation techniques were applied to account for time-varying aqueous exposure concentrations. All PAHs exhibited a rapid uptake. Monophasic elimination patterns were observed for benzo[*e*]pyrene and benzo[*a*]-pyrene (biological half-life $t_{0.5}$: 7−8 days). For the other PAHs (anthracene, phenanthrene, pyrene, benzo[*ghi*]perylene) typical biphasic patterns were encountered with rapidly ($t_{0.5}$: 0.6−2 days) and slowly ($t_{0.5}$: 99 to >1000 days) exchanging compartments. Some PAHs (pyrene and benzo[*ghi*]-perylene) will hardly be eliminated during the normal life span of the organism. Bioconcentration factors (log BCF, wet weight; derived from rate constants) increased with molecular weight and $K_{ow}$ (*n*-octanol/water partition coefficient) from 2.6 L kg$^{-1}$ (anthracene) to 5.7 L kg$^{-1}$ (benzo-[*ghi*]perylene). A linear relationship was observed between log BCF and log $K_{ow}$. It is argued that biotransformation of PAHs in freshwater isopods seems to play a minor role and that this species may be suitable for the assessment of the bioavailability of PAHs in littoral freshwater environments.

## Introduction

Carcinogenic and mutagenic activities of a number of polycyclic aromatic hydrocarbons (PAHs) have been documented extensively (*1*). Reported effects of PAHs in aquatic systems include increased incidence of liver tumors and skin diseases in bottom dwelling fish (*2*−*6*), induction of biochemical and physiological responses, e.g., mixed function oxidases (MFO), and DNA−adduct formation in teleosts and invertebrates (*5, 7, 8*). The class of PAHs commonly encountered in aquatic environments comprises a broad array of compounds ranging from two to eight condensed ring systems (*7*) without or with varying alkyl substitution patterns, depending on the type of emission sources (combustion processes or petrogenic). Both physicochemical characteristics (e.g., solubility, $K_{ow}$, sorption characteristics, vapor pressure, photodegradation rate) and biological activity (e.g., microbial biodegradation, toxicokinetics, carcinogenicity, mutagenicity) may vary several orders of magnitude among the different homologues (*3, 9*−*10*).

In aquatic ecosystems PAHs usually are present predominantly in the abiotic compartments (sediments, water, suspended matter) and the lower trophic levels (plankton, macrophytes, invertebrates). The relatively low levels of PAHs encountered in vertebrates (fish, mammals, birds) are attributed to MFO-mediated biotransformation (*11*). Hydroxylated metabolites in excretion products of fish (bile) have been used as a reflection of current exposure to PAHs and as a predictor of effects in teleosts (*8, 12*). For the biological monitoring of bioavailable PAHs in aquatic systems, invertebrates seem to be more suitable model species than teleosts. For several species of bivalves (*13*−*15*) and crustaceans (*16*−*18*), sufficient toxicokinetic data has been documented, which would allow meaningful interpretation of field concentrations of selected PAHs.

In earlier studies, we investigated the toxicokinetics of several trace metals and the potential suitability of freshwater isopods for the monitoring of trace metal bioavailability (*19*−*21*). We further observed the capacity of freshwater isopods to accumulate PAHs and organophosphorus compounds (*22, 23*). These studies provided indications that biotransformation of these compounds seemed to be negligible in *Asellus aquaticus,* suggesting that freshwater isopods could be a suitable model species for biomonitoring studies. The objectives of the present study were to verify the ability of freshwater isopods to bioconcentrate aqueous PAHs under controlled laboratory conditions, to determine several toxicokinetic parameters (BCF, uptake and elimination rate constants, biological half-lives), and to evaluate the applicability of first-order compartment models to describe uptake and elimination patterns.

## Experimental Section

Freshwater isopods were exposed in static systems during 7 days to six different PAHs with three to six condensed ring systems: anthracene (ANT), phenanthrene (PHE), pyrene (PYR), benzo[*e*]pyrene (BeP), benzo[*a*]pyrene (BaP), and benzo[*ghi*]perylene (BgP). This selection included two pairs of structural isomers (ANT−PHE and BaP−BeP), each pair with matching $K_{ow}$ but different molecular conformation. It was expected that this could provide information on the potential influence of structural characteristics in addition to lipophilicity on the toxicokinetics of PAHs in freshwater isopods. Physicochemical characteristics of the compounds and full experimental details of the sections described below are indicated in the Supporting Information (SI).

Freshwater isopods (*A. aquaticus*) originated from a nearby field location (Lake Brouwerskolk) and were acclimated during a 3-month period. Only adult animals (size range 4−20 mm), without apparent sexual or reproductional activity (precopula position, ovigerous), were used for the experiments.

**Test Solutions.** Individual PAH standards were obtained from Supelco Inc. (Bellefonte, PA) as part of a set (610-N) of 16 homologues recommended by the EPA. Benzo[*e*]pyrene (CRM-50) was purchased from the EC Community Bureau of Reference (BCR, Brussels, Belgium). Aqueous solutions of the different PAHs were prepared by the generator column method (*24*). Final PAH concentrations for the bioconcentration experiments were prepared by appropriate dilution just before the start of the experiments. Initial concentrations in the static exposure experiments (indicated in Table 1) were chosen as a compromise between the acute toxicity (96 h $LC_{50}$) to *Asellus*, as observed in preceding experiments (*25*)

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**TABLE 1. Physicochemical Data of PAHs Studied and Initial Exposure Concentrations at the Start of the Experiments**

| compound | mol wt | log $K_{ow}$[a] used for calculation | initial concn ($\mu$g L$^{-1}$) |
|---|---|---|---|
| anthracene | 178.2 | 4.54 | 12 |
| phenanthrene | 178.2 | 4.57 | 25 |
| pyrene | 202.3 | 5.18 | 15.7 |
| benzo[*a*]pyrene | 252.3 | 6.04 | 0.54 |
| benzo[*e*]pyrene | 252.3 | 6.04 | 2.4 |
| benzo[*ghi*]perylene | 276.3 | 7.04 | 0.70 |

[a] $K_{ow}$ and solubility data summarized in Table A-1, SI. Values used for calculations in this study are from Miller et al. (*70*) and De Voogt et al. (*26*).

(Table A-1, SI), the expected concentrations in isopods, and the analytical detection limits.

**Experimental Design.** Exposure systems (conical 500-mL Pyrex flasks with two stoppered openings with i.d. of 30 and 8 mm) were adapted from a system used in previous studies (*26*). Each exposure flask contained 10−20 isopods (biomass loading of 0.1−0.3 g L$^{-1}$; (see Table A-3, SI). Oxygen saturation levels were maintained above 80%. Animals were fed with several leaves of *Elodea*. Throughout the experiments a photoperiod of 16/8 h light/dark was maintained. Yellow fluorescent light ($\lambda$ > 500 nm) was applied to avoid the influence of photodegradation of PAHs and to avoid potential photoinduced toxicity of PAHs. Experiments were started by transferring the isopods to the exposure flasks with ($n$ = 8−18 for the different compounds), which had been filled previously with the test solutions and kept in a thermostatic bath at 21 ± 1 °C. Two control flasks (PAH solution without isopods) for each compound were added to each series for the monitoring of losses of PAHs due to abiotic processes (e.g., adsorption, evaporation) or microbial degradation. One flask at a time was removed at regular intervals during the exposure phase (first 7 days), and both water and isopods were sampled subsequently. Isopods were split in two subsamples (5−10 isopods each) in order to determine the variability of the residue analysis. Water samples were also taken in duplicate. At the termination of the exposure period, isopods were removed from remaining flasks and transferred to new flasks containing only water and some shoots of *Elodea*. The elimination period lasted from 12 to 21 days with water being renewed every 48 h. Isopods were sampled at regular intervals during this period. No detectable amounts of PAHs were found in wastewater.

**Analysis.** Water samples (1 mL) were mixed with 1 mL of acetonitrile (HPLC grade; Baker, Deventer, The Netherlands) in 2-mL vials, sealed, and kept in the dark at −18 °C until analysis. Pooled samples of isopods (0.05−2 g) were blotted dry, weighed, and homogenized in a mortar with anhydrous sodium sulfate (Baker, Deventer, The Netherlands). Soxhlet extracts (4 h with 30 mL of *n*-hexane, HPLC grade; Baker, Deventer, The Netherlands) of the homogenates were purified (deactivated basic alumina; Woelm, Eschwege, FRG) and taken over in acetonitile (1 mL). PAHs in the water and isopod extracts were analyzed with RP-HPLC with fluorescence detection. Quality control procedures included recovery determinations, analysis of certified reference material, and monitoring of blanks, response, precision, and detection limits. Detection limits were sufficiently low to quantitate the relatively low concentrations in water and isopods during the elimination phase. Full analytical details are provided in the SI. Isopod concentrations are reported on the basis of fresh weight. The lipid content of the isopods was determined in subsamples (2 mL) of the Soxhlet extracts as hexane-extractable lipids. The amount of sample was not sufficient for separate determinations with standard lipid determination methods or the determination of dry weights. A mean lipid content of 0.34 ± 0.07% (mean ± SE, $n$ = 91) was found. It should be noted that differences in lipid extraction methods should be taken in consideration when comparing lipid-based partitioning constants between different studies (*27*). Hexane-extractable lipids (as applied in this study) may not include sufficiently the polar-bound lipid fractions (*22, 27*). For conversions to dry weight-based concentrations we recommend to use a factor of 4.8 ± 1.9 (mean ± SE, $n$ = 184), as obtained from previous studies (*19, 21*).

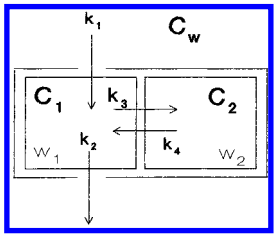


**FIGURE 1. First-order rate constant two-compartment model applied in this study. Symbols and units are explained in the text.**

**Toxicokinetic Model.** First-order compartment models were used to describe the time-dependent tissue residues in *Asellus*. Examination of the elimination patterns revealed monophasic elimination for benzo[*a*]pyrene and benzo[*e*]-pyrene. For these compounds a one-compartment model was used. For the other PAHs the results indicated biphasic elimination patterns. A two-compartment model was assumed to give a better description. The two-compartment model assumes a central compartment and a slowly exchanging peripheral compartment (Figure 1). The traditional approach to two-compartment models in ecotoxicological studies, as described by Spacie and Hamelink (*28*), was deemed unsuitable, because of the implicit hypothesis with respect to the virtual dimensions (volume) of the compartments, which does not allow extrapolations to biological compartments (*29, 30*). In that approach the set of differential equations for the elimination phase is solved by approximation with a closely resembling model, which does not account for mutual contaminant fluxes between both compartments. Apparent elimination rate constants and relative initial concentrations of both compartments are estimated with the so-called 'peeling' or 'back-stripping' method. As discussed by various authors (*29−31*) the traditional approach is only suitable for the comparison of dimension-independent biological half-lives. Therefore, we chose to use a slightly different parametrization of the two-compartment model with dimensions and time-dependent variables and rate constants as indicated in Figure 1. The weights of the first and second compartment and of the whole organism were defined as $w_1$, $w_2$, and $w_1 + w_2$ (in kg) with an assumed equal specific gravity. We further defined the relative weight of the first compartment as $f_{w_1}$ (dimensionless ratio):

$$f_{w_1} = w_1/(w_1 + w_2) \quad (1)$$

The concentrations of the PAHs were defined as $C_w(t)$ representing the external aqueous concentration in $\mu$g L$^{-1}$ and $C_1(t)$ and $C_2(t)$ representing the internal concentrations in the isopod in the first and second compartments in $\mu$g kg$^{-1}$ (fresh weight). The total concentration in the isopod $C_{tot}(t)$, analyzed as whole body residue ($\mu$g kg$^{-1}$ fresh weight), is defined as:

$$C_{tot}(t) = (w_1C_1(t) + w_2C_2(t))/(w_1 + w_2)$$
$$= f_{w_1}C_1(t) + (1 - f_{w_1})C_2(t) \quad (2)$$

The first-order rate constants defined in the two-compartment model are the uptake rate constants of the

central compartment $k_1$ (in L kg$^{-1}$ day$^{-1}$) and the peripheral compartment $k_3$ (day$^{-1}$) and the elimination rate constants of both compartments $k_2$ and $k_4$ (day$^{-1}$). The differential equations for both compartments can be described as:

$$dC_1(t)/dt = k_1C_w(t) - k_2C_1(t) - k_3C_1(t) + k_4C_2(t) \quad (3)$$

$$dC_2(t)/dt = k_3C_1(t) - k_4C_2(t) \quad (4)$$

For theoretical steady-state conditions at constant aqueous exposure concentrations, the net transport between adjacent compartments equals zero and the following concentration factors can be defined:

$$BCF_{C_1} = k_1/k_2 \quad (5)$$

$$C_2(t)/C_1(t) = k_3/k_4 \quad (6)$$

$$BCF_{tot} = C_{tot}(t)/C_w(t) = f_{w_1}(k_1/k_2) + (1 - f_{w_1})(k_3/k_4)(k_1/k_2) \quad (7)$$

in which $BCF_{C_1}$ is the bioconcentration factor (wet weight, L kg$^{-1}$) of the first compartment and $BCF_{tot}$ is the whole body bioconcentration factor (wet weight, L kg$^{-1}$). When the peripheral compartment is absent, i.e., $f_{w_1} = 1$, the model description reduces to the well-known equations of the first-order one-compartment model.

**Parameter Estimation and Statistical Analysis.** For the well-known one-compartment model the differential equations can be solved for constant water concentrations (*28, 32*) or first-order decaying water concentrations (*16−17, 22, 26, 33*). Rate constants are usually estimated from fitting measured concentrations or bioconcentration factors to the integrated equations. When subsequent depuration studies have been conducted, elimination rate constants can be estimated more accurately from the elimination phase (*26, 28, 32*). After introduction of the value of the elimination rate constant in the model equations for the exposure phase, the uptake rate constant can be estimated separately. Simultaneous estimation of rate constants from consecutive exposure and elimination studies has been used only by a limited number of authors for the one-compartment model (*34*). For two-compartment model studies (*30, 35−36*) such an approach has not yet been used. The recent availability of user-friendly computer programs with numerical techniques for the solution of the differential equations enables simultaneous parameter estimation for compartment models even with time-variable water concentrations (*23, 37−38*).

In this study, uptake and elimination rate constants were estimated with the STEM 2.1 program (*39*) by numeric integration (fourth-order Runge−Kutta−Fehlberg integration) of eqs 3 and 4. A simplex-optimization procedure (*40*) was used to obtain the best possible fit (least-squares criterion) of measured whole-body concentrations to eq 2. The minimization criterion was applied to log-transformed data in order to avoid a dominant influence of the high concentrations during the exposure phase on the fitting procedure. The relative weight ratio of the first compartment $f_{w_1}$ was chosen at 0.5, assuming equal weights and volumes of peripheral and central compartments. The value of $f_{w_1}$ does not affect the elimination rate constants ($k_2$ and $k_4$) or $BCF_{tot}$ (whole body bioconcentration factor). As no experimental data was available about the physical dimensions of the different compartments, the value of 0.5 was considered to be a reasonable default value. Water concentrations at any time were approximated by linear interpolation (0.1-day steps) between measured values. For benzo[*a*]pyrene and benzo[*e*]pyrene, the one-compartment model was used and $f_{w_1}$ was set at 1. Confidence limits of the parameter estimates (reported as standard errors) were derived from the covariance estimation for the variables during repeated calibration runs. The significance of fit was evaluated with the *F*-test according to Sokal and Rohlf (*41*). Analysis of variance (ANOVA) and Tukey's multiple range tests (*41*) were applied to investigate significant differences of various variables within and between experiments. Significances were tested at the $p < 0.05$ level. Statistical calculations were performed with the software packages Statgraphics 2.6 and SPSS-PC 5.1.

## Results and Discussion

**Experimental Conditions.** Measurements of aqueous PAH concentrations in control systems without isopods (Table A-2, SI) indicated that, except in phenanthrene-loaded systems, no significant decrease (ANOVA, $p > 0.05$) with time had occurred. For phenanthrene, significant decreases (Tukey's range test, $p < 0.05$) of the initial water concentration in control systems became apparent after 3 days. After this moment the concentrations dropped sharply to values below detection limits. Separately conducted experiments with stoppered control flasks kept in the dark and plastic bottles indicated that the observed decreases could not be attributed to evaporation or photodegradation. On the basis of the gradual disappearance over several days, sorption does not seem a likely explanation. Possibly, microbial degradation could have been of importance. At the time of the experiments, however, no additional tests with sterilized water have been conducted to confirm this assumption. The occurrence of nonexplained disappearance of phenanthrene during experiments has been observed by several other authors (*42, 43*). Nevertheless, the compartment model parametrization and estimation techniques applied in this study can handle time-varying concentrations, and therefore, the observed phenomena for phenanthrene did not prohibit the parameter estimation.

Mass balances were calculated from measured aqueous concentrations and isopod residues during the exposure phase (Table A-3, SI) in order to monitor the fate of the PAHs present in the experimental systems. Except for phenanthrene, the fraction of PAH recovered in the water phase and the organisms accounted for 68−119% of the total amount of PAHs initially present. The recovery ranges reported are comparable to values observed in previous studies (*26*) and seem to be within the limits of the analytical variability of the concentrations analyzed in water and organisms. Mean coefficients of variation of duplicate measurements of PAHs ranged from 4% (pyrene, $n = 8$) to 25% (benzo[*e*]pyrene, $n = 8$) in aqueous samples and from 18% (benzo[*ghi*]perylene, $n = 9$) to 44% (anthracene, $n = 18$) in duplicate isopod samples. The mass balance data indicate that the influence of potentially confounding processes, such as sorption on the walls of the experimental system, sequestration by food items (*Elodea*), evaporation, and degradation, was limited during the experiments. In addition, this indicates that biotransformation of PAHs probably also has been of limited importance.

**Exposure−Elimination Experiments.** Figure 2 depicts mean ($n = 2$) wet weight concentrations ($\mu$g kg$^{-1}$) in isopods and aqueous concentrations ($\mu$g L$^{-1}$) for the PAHs in different exposure systems. For all compounds a rapid accumulation in the isopods was observed, concurrent with a gradual or rapid (phenanthrene) decrease of the water concentration. For benzo[*ghi*]perylene and benzo[*e*]pyrene maximum isopod concentrations occurred after 3 days. For the other PAHs maximum concentrations were reached within the first 2 days. In the phenanthrene experiments isopod concentrations reached maximum values during the first 2 days. After this interval, elimination by the isopods obviously exceeded the uptake from the water phase, due to rapidly decreasing aqueous phenanthrene concentrations. Translocation of the

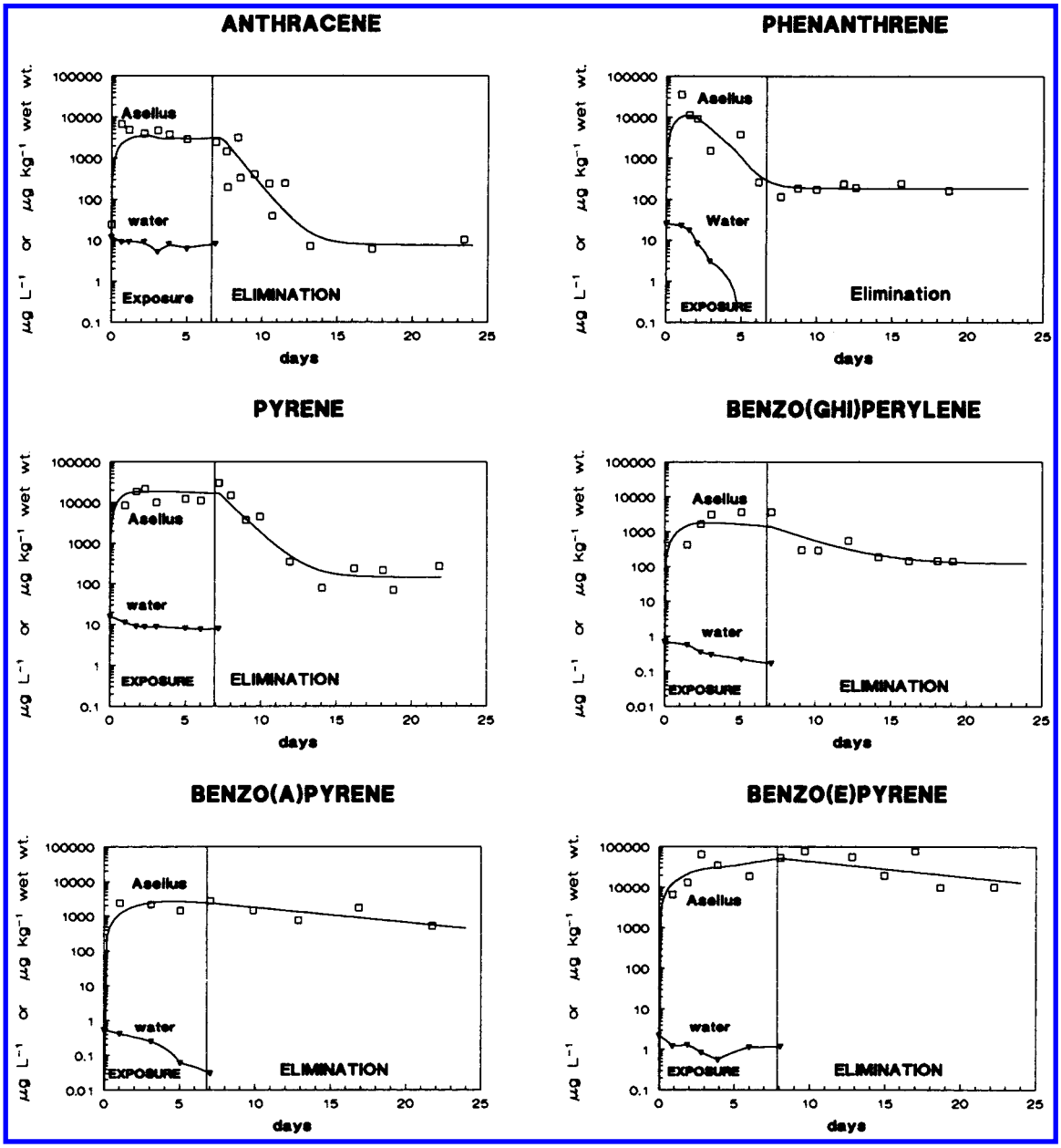


**FIGURE 2. Bioconcentration and elimination of anthracene, phenanthrene, pyrene, benzo[*ghi*]perylene, benzo[*a*]pyrene, and benzo[*e*]-pyrene in freshwater isopods in static exposure and depuration experiments: □, mean wet weight concentrations in isopods ($\mu$g kg$^{-1}$); ▼, aqueous concentrations ($\mu$g L$^{-1}$). The solid lines indicate the model predictions for the whole body concentrations and the linearly interpolated water concentrations.**

exposed isopods to uncontaminated water, which was renewed every 48 h, resulted in a relatively rapid elimination of anthracene and pyrene during the first 5 days and a more slow elimination of benzo[*e*]pyrene, benzo[*a*]pyrene, and benzo[*ghi*]perylene. For phenanthrene the observed decrease in concentrations during the latter part of the exposure period was followed by a slight additional elimination after translocation. Judging from the exponential decrease during the elimination phase, benzo[*a*]pyrene and benzo[*e*]pyrene seem to follow monophasic elimination, while the other compounds seem to exhibit typical biphasic elimination patterns. We have no explanation for the (pseudo) monophasic elimination of benzo[*a*]pyrene and benzo[*e*]pyrene. Maybe incomplete elimination from the central compartment could have obscured the existence of the peripheral compartment within the limited time frame of the experiments. During the elimination phase all aqueous concentrations were below detection limits.

**Apparent BCFs and Uptake Rate Constants.** Apparent bioconcentration factors (BCF, wet weight in L kg$^{-1}$) were derived from measured concentrations in water and isopods and are summarized in Table 2. The time profiles of the apparent BCFs (not indicated) varied among the different PAHs and showed a significant increase with time (ANOVA, $p < 0.05$) for benzo[*a*]pyrene, benzo[*e*]pyrene, and benzo-[*ghi*]perylene. Only values not significantly different from mean plateau levels were used for the calculation of apparent BCFs (log BCF), which ranged from 2.7 for anthracene to 4.4 for benzo[*e*]pyrene. Apparent uptake rate constants for the central compartment ($k_1$, wet weight in L kg$^{-1}$ day$^{-1}$) were derived from the initial uptake of PAHs by the isopods and the interpolated mean water concentration during the first time interval, according to Spacie and Hamelink (*28, 32*). The wet weight BCFs observed in this study for PAHs in *Asellus* are comparable to most of the values observed in experimental studies with freshwater invertebrates (Table A-4, SI).

**TABLE 2. Apparent BCFs (wet weight) and Uptake Rate Constants of the Central Compartment ($k_1$, wet weight) Derived from Concentrations Measured during the Exposure Phase**

| | | apparent BCF[a] | | |
|---|---|---|---|---|
| compound | *n* | BCF ($x \pm$ SE, L kg$^{-1}$) | log BCF ($x \pm$ SE) | $k_1$[b] ($x \pm$ SE, L kg$^{-1}$ day$^{-1}$) |
| anthracene | 7 | 560 ± 70 | 2.7 ± 0.1 | 950 ± 560 |
| phenanthrene | 5 | 1300 ± 330 | 2.8 ± 0.5 | 1500 ± 100 |
| pyrene | 7 | 2050 ± 100 | 3.3 ± 0.1 | 640 ± 110 |
| benzo[*a*]pyrene | 4 | 32400 ± 17400 | 4.3 ± 0.3 | 4800 ± 1800 |
| benzo[*e*]pyrene | 4 | 36500 ± 11300 | 4.4 ± 0.2 | 4300 ± 930 |
| benzo[*ghi*]perylene | 3 | 16000 ± 2600 | 4.2 ± 0.1 | 1380 ± 530 |

[a] Mean ± SE of raw or log transformed BCFs; BCFs calculated as ratio of wet weight organism and aqueous concentration. [b] Determined from initial uptake during the first day (*28*, *32*).

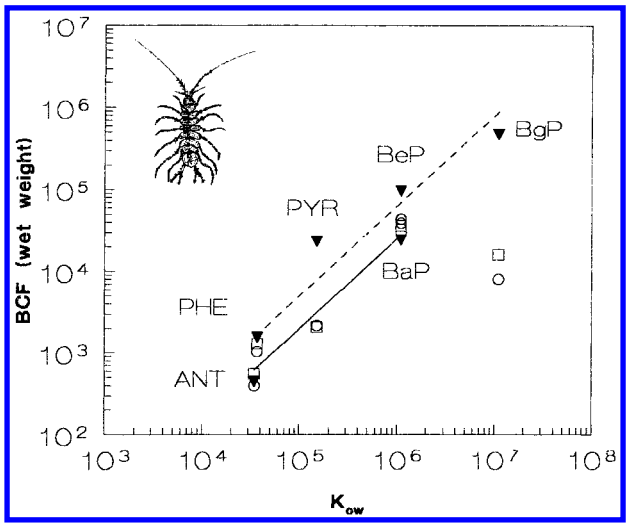


**FIGURE 3. Rate constant-based two-compartment BCFs (wet weight, L kg$^{-1}$; ▼) for theoretical equilibrium conditions ($t = \infty$, according to eq 7), model-predicted BCFs at the end of the exposure period ($t = 7$ days; ○), and measured apparent BCFs (mean values between days 3 and 7; □) in relation to *n*-octanol/water partitioning coefficients ($K_{ow}$; Table 1).**

**Toxicokinetic Parameter Estimates.** The measured isopod residues were fitted to eqs 2−4 of the two-compartment model using measured and interpolated time-variable water concentrations and an assumed value of 0.5 for $f_{w_1}$, the relative weight fraction of the first compartment. For benzo[*e*]pyrene and benzo[*a*]pyrene a one-compartment model was chosen by stating $f_{w_1} = 1$. Parameter estimates for the uptake and elimination rate constants of both compartments and the theoretical equilibrium BCF derived from the rate constants (eq 7) are indicated in Table 3. For the different PAHs weak to strong significant fits (*F*-test, $0.05 > p > 0.0001$) were obtained and explained variance ($R^2$) values ranged from 34% for benzo[*e*]pyrene to 81% for phenanthrene (Table 3).

The standard errors of the rate constants (Table 3) are within reasonable limits for the parameter estimates of the central compartment. For the second compartment the estimated rate constants have large confidence intervals with standard errors approaching or exceeding the parameter estimates. This is a tradeoff of the chosen experimental design, which did not include actual measurements in a physical second compartment and which sometimes had a limited number of replicated treatments (*8*−*18*) during the 21-day experimental period. As the numerical techniques for parameter estimation allowed only approximate estimates for confidence limits, no figures are given for confidence intervals of the rate constant-based BCFs in Table 3.

The rate constant-based log BCFs varied from 2.6 for anthracene to 5.7 for benzo[*ghi*]perylene. A comparison of these steady-state BCFs with the apparent BCFs, derived from the measured whole body residues (Table 2), and with model-predicted 7-day BCFs is made in Figure 3. For anthracene, phenanthrene, benzo[*e*]pyrene, and benzo[*a*]pyrene measured and rate constant-based BCFs were in good agreement. For pyrene and benzo[*ghi*]perylene the rate constant-based BCFs are more than 1 order of magnitude higher than the apparent BCFs. The low rates of uptake and elimination predicted for these compounds in the peripheral compartment (with estimated $k_4$ and $k_3$ values ranging from 0.0001 to 0.01 day$^{-1}$) indicate that especially for extrapolated equilibrium conditions (at infinite time) the compounds present in the second compartment may have a dominant influence on whole body concentrations and BCF values. A comparison of model-predicted 7-day BCFs with measured BCFs shows a much better agreement (see Figure 3). The $k_1$ values estimated with the initial uptake rate method for anthracene and phenanthrene (Table 2) are in reasonable agreement with the corresponding values estimated by the iterative numerical technique (Table 3). About 50% lower values were found for pyrene, benzo[*e*]pyrene, and benzo[*ghi*]perylene and about 50% higher for benzo[*a*]pyrene. Considering the broad and overlapping confidence intervals, these differences probably will not be significant.

The rate constants estimated for benzo[*ghi*]perylene (Table 3) indicate that the low value for the apparent BCF during the 7-day exposure phase seems to be explained mainly by the low elimination from the peripheral compartment, rather than by a decreased uptake. True steady-state conditions can be expected only after a long time. The half-life of benzo[*ghi*]perylene corresponding to the $k_4$ estimated for the peripheral compartment is more than $6 \times 10^3$ days, which is far beyond the life span of short-living small arthropods. The normal generation time of freshwater isopods varies between 0.5 and 1.5 year, depending on climatic zone (*44*). The extremely low elimination rate constants for pyrene and benzo[*ghi*]perylene in the peripheral compartment signify that some PAHs are strongly bound in stable compartments and probably will never leave the organism during its normal life span. In feeding experiments relative growth rates of 0.01−0.05 day$^{-1}$ have been determined for freshwater isopods (*45*). It may be expected that growth dilution is an important factor leading to a reduced apparent bioconcentration of slowly exchanging compounds in the field situation.

**BCF in Relation to $K_{ow}$.** The BCFs observed for PAHs in *A. aquaticus* increase with increasing $K_{ow}$ (Figures 3 and 4). The double logarithmic regression relationships of measured or rate constant-based BCFs in *Asellus* with $K_{ow}$ were highly significant (*F*-test, $p < 0.004$):

measured BCF:

$$\log \text{BCF} = (1.1 \pm 0.1) \log K_{ow} - (2.2 \pm 0.9) \quad (8)$$

$$n = 5;\ R^2 = 0.96;\ S_y = 0.2$$

rate constant-based BCF:

$$\log \text{BCF} = (1.1 \pm 0.2) \log K_{ow} - (1.8 \pm 0.9) \quad (9)$$

$$n = 6;\ R^2 = 0.88;\ S_y = 0.4$$

The measured BCF for benzo[*ghi*]perylene was not included in the regression, because this value deviated strongly from the double logarithmic relationship. Similar relationships reported in the literature were summarized recently (*3*) and are indicated in Table A-5 (SI).

**TABLE 3. Toxicokinetic Parameter Estimates Obtained with an Iterative Numerical Integration Technique: Uptake, Elimination Rate Constants for Central and Peripheral Compartments,[a] Rate Constant-Based Wet Weight BCFs According to Eq 7, and Statistics of Fit[c]**

| | | central compartment | | peripheral compartment | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| compound | *n* | $k_1$ ($x \pm$ SE, L kg$^{-1}$ day$^{-1}$) | $k_2$ ($x \pm$ SE, day$^{-1}$) | $k_3$ ($x \pm$ SE, day$^{-1}$) | $k_4$ ($x \pm$ SE, day$^{-1}$) | $R^2$ (%) | *F*-test *p* | BCF (L kg$^{-1}$) | log BCF |
| anthracene | 18 | 890 ± 1530 | 1.0 ± 0.8 | 0.0003 ± 0.004 | 0.007 ± 0.8 | 58 | <0.0002 | 450 | 2.6 |
| phenanthrene | 14 | 1580 ± 390 | 1.2 ± 0.3 | 0.007 ± 0.007 | 0.004 ± 0.09 | 81 | <0.0001 | 1590 | 3.2 |
| pyrene | 17 | 3490 ± 1420 | 0.82 ± 0.17 | 0.001 ± 0.002 | 0.0001 ± 0.2 | 64 | <0.0001 | 23500 | 4.4 |
| benzo[*a*]pyrene[b] | 8 | 2410 ± 1040 | 0.10 ± 0.04 | | | 37 | <0.05 | 24400 | 4.4 |
| benzo[*e*]pyrene[b] | 12 | 8310 ± 3560 | 0.09 ± 0.05 | | | 34 | <0.02 | 43700 | 4.6 |
| benzo[*ghi*]perylene | 13 | 4030 ± 3000 | 0.36 ± 0.23 | 0.009 ± 0.01 | 0.0001 ± 0.004 | 43 | <0.01 | 486000 | 5.7 |

[a] First-order rate constants for compartment model described in Figure 1. [b] Monophasic elimination, only one-compartment model rate constants were estimated. [c] Statistics of fit: $R^2$ is explained variance (corrected for degrees of freedom); *p* is significance of *F*-test for significance of regression.

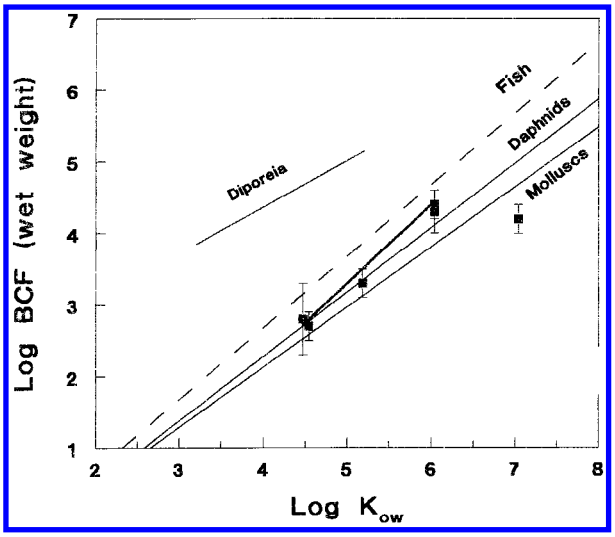


**FIGURE 4. Apparent BCFs (log BCF; L kg$^{-1}$, wet weight; mean ± SE) for PAHs in freshwater isopods in relation to *n*-octanol/water partitioning coefficients (log $K_{ow}$) and similar linear relationships (solid lines; see Table 2) documented for fish (*48*), daphnids, mollusks (*49*), and a lipid-rich amphipod species (*16, 65*).**

Bioconcentration factors and first-order elimination rate constants for persistent neutral organic compounds in aquatic organisms usually exhibit strong (double logarithmic) relationships with $K_{ow}$ (*46*−*52*). The relationship proposed by Mackay (*48*) for (wet weight) BCFs in fish commonly is considered a benchmark for the equilibrium partitioning theory (*50, 52*−*53*). The slopes of the wet weight bioconcentration QSARs (quantitative structure−activity relationships) calculated for freshwater isopods show a close resemblance to this benchmark relationship and are in good agreement with slopes documented (Table A-5, SI) for the bioconcentration of PAHs in daphnids and marine mollusks (*13, 49*). The different regression constants among organisms probably reflect variations in lipid content. The QSAR for fish species in the study of Mackay (*48*), with an estimated regression constant of −1.32, is based on different species of fish, with a mean lipid content of around 5% (*52*). The mean lipid contents of the daphnids and mollusks in the study of Hawker and Connell (*49*) were 4% and 1−2%, respectively (*50*). *Diporeia* spp. has a relatively high lipid content; figures ranging from 4% to 12% have been reported (*16*). Taking into account the relatively low lipid content of *Asellus* (0.34 ± 0.07%), the lower intercepts (−1.8 to −2.2) seem to be in reasonable agreement with lipid equilibrium partitioning concepts.

The apparent BCF for benzo[*ghi*]perylene in *Asellus* deviated from the value predicted by the double logarithmic relationship observed for the other five PAHs. This phenomenon has been reported for many other hydrophobic compounds with log $K_{ow}$ exceeding 6−7 and attributed to various factors, such as reduced membrane permeability in relation to molecular size (*54*), decreased lipid solubility and limited predictive capacity of $K_{ow}$ (*55, 56*), biotransformation (*51*), insufficient duration of exposure in relation to the time required to attain effective equilibrium conditions (*50*), limited bioavailability, and combinations of these factors (*57*). Connell and Hawker (*58*) reanalyzed published bioconcentration data for fish and proposed a fourth-order polynomial relationship with a maximum wet weight log BCF value of 4.6 for compounds with a log $K_{ow}$ value of 6.7. Related parabolic (second order) and bilinear relationships have been proposed by Bintein et al. (*59*). The apparent BCFs determined for *Asellus* are in agreement with these curvilinear relationships.

**Rate Constants versus $K_{ow}$.** The parameter estimates for the elimination rate constants of the six PAHs in *Asellus* show a decreasing trend with increasing $K_{ow}$. Elimination rate constants of many organic compounds decrease with $K_{ow}$, and double logarithmic regression relationships have been reported for different classes of compounds in various groups of organisms (*16, 32, 35, 49, 51*). Relationships reported for PAHs in mollusks, daphnids (*49*), fish (*48*), amphipods (*16*), and other species have been summarized in Van Brummelen et al. (*3*). The elimination rate constants for the central compartment in *Asellus* are within the range of values reported for fish and other invertebrates and seem to decrease with increasing $K_{ow}$ according to a common slope. In general, the elimination rate constants for *Asellus* tend to decrease with increasing $K_{ow}$ up to log $K_{ow}$ values of 6. The value for $k_2$ of benzo[*ghi*]perylene seems to be 1 order of magnitude higher than expected from the slope of similar relationships for other invertebrates. The regression equation for the elimination rate constants of five PAHs (benzo[*ghi*]perylene excluded) in the freshwater isopod has an explained variance ($R^2$) of 93% ($F = 39.0$; $p < 0.008$), an estimated standard error ($S_y$) of 0.17, and is written as:

$$\log k_2 = (-0.72 \pm 0.11) \log K_{ow} + (3.4 \pm 0.6) \quad (10)$$

Both slope and intercept of eq 10 are in reasonable agreement with results from the other studies previously mentioned (Figure A-1, SI). The estimates for the elimination rate constants of anthracene, phenanthrene, pyrene, and benzo[*ghi*]perylene for the peripheral compartment are much lower, typically 2−3 orders of magnitude. Although the large confidence intervals of the rate constants of the peripheral compartment (Table 3) do not allow firm conclusions, the $k_4$ values seem to follow a decreasing pattern with increasing $K_{ow}$. The uptake rate constants for the central and peripheral compartments are not related to $K_{ow}$ in the freshwater isopod, which is in agreement with similar observations for fish species (*51*).

**Influence of Molecular Structure.** The present study included two pairs of PAHs, each of which had similar $K_{ow}$ values but differed in molecular structure. Anthracene and phenanthrene both have $K_{ow}$ values in the range of 4.4−4.7 (Table A-1, SI) but exhibit orders of magnitude differences in aqueous solubility (anthracene, 40−75 $\mu$g $L^{-1}$; phenanthrene, 1000−1600 $\mu$g $L^{-1}$). The BCFs and toxicokinetic rate constants of both compounds agreed relatively well. The other pair, benzo[*e*]pyrene and benzo[*a*]pyrene, shows large differences in biological activity. Benzo[*a*]pyrene is one of the most potent human carcinogens and mutagens, while benzo[*e*]pyrene is not classified as a carcinogen because of inconclusive evidence (*1*). Both substituted pyrenes showed a remarkable similarity in measured BCFs, their elimination pattern, and their estimated elimination rate constants. The observed differences in uptake rate constants and (consequently) rate constant-based BCFs seem to be within the range of the experimental error. The toxicokinetics of PAHs in freshwater isopods seem to be determined mainly by hydrophobicity-related characteristics. The effects of differences in molecular structure between compounds with similar hydrophobicity probably are of minor importance for the toxicokinetics of PAHs in *Asellus*. The effects of differences in molecular structure and spatial conformation are most pronounced in receptor- or metabolite-mediated ecotoxicological endpoints. Well-known examples of this phenomenon are the order of magnitude differences in biological effects (biochemical and physiological responses, genetic lesions) of PAHs and PHAHs (polyhalogenated aromatic hydrocarbons) in vertebrates (*60*). The results for *Asellus* indicate that biotransformation processes of PAHs probably are of minor importance. The bioconcentration of PAHs in freshwater isopods primarily appears to be a partitioning process. Another interesting observation is that the two PAHs with the slowest release rate from the peripheral compartment, pyrene and benzo[*ghi*]perylene, have the most highly condensed ring structure of the compounds examined. This suggests that the highly condensed ring structure facilitates sequestration.

**Model Conceptualization.** The models provided acceptable descriptions of the bioconcentration and elimination processes in this study. As expected, for the compounds with biphasic elimination patterns, the two-compartment model performed better than the one-compartment model with higher explained variances. Parametrization as fugacity- or clearance volume-based models would not have yielded a better description, as these models are mathematically equivalent to the rate constant-based models (*61, 62*). The numerical integration method allowed the parameter estimation for all four rate constants of the two-compartment model simultaneously from the exposure and elimination phases of the experiments. The direct and simultaneous estimation technique has not yet been used by many authors (*34*) but has the advantage of a mathematically improved processing of the influence of experimental variability on the confidence intervals of the parameter estimates, compared to the classical approach (peeling or backstripping method) (*28*), which is based on an analogy with a bolus-dose model in pharmacokinetic studies (*31, 63*) and which is usually applied in the analysis of multiphasic elimination patterns. In the classical approach, on the basis of substitution of separately estimated parameters, rate constants are derived in an indirect manner, which may yield unrealistically small error terms. The numerical integration method enables direct estimation of the rate constants, instead of indirect calculation. Furthermore, the direct estimation of the two-compartment rate constants facilitates an interpretation beyond the level of analysis of contaminant half-lives in the compartments involved.

For the compounds exhibiting biphasic elimination, the questions related to the physiological interpretation of the central and peripheral compartments are difficult to resolve. The slowly exchanging peripheral compartment may represent a phase, target organ, or cellular compartment in which the PAHs are strongly bound, such as, e.g., the hepatopancreas or the lipid fractions. The central compartment could represent easily exchanging internal tissues or the hemolymph, flushing the gill pleopods and external surface of the hepatopancreas. Except for trace metals (*21, 64*), studies on organ- or tissue-specific distribution of pollutants in isopods or related small aquatic arthropods have hardly been conducted.

The weight fraction ($f_{w_1}$) of the central compartment was arbitrarily chosen as 0.5. Reiterated model runs with ($1 - f_{w_1}$) values, resembling the weight fraction of the hepatopancreas (5%) or the lipid fraction (0.3%), yielded similar central compartment elimination rate constants and BCFs, but different uptake rate constants (results not shown) and increased error terms of the estimates of the peripheral compartment. Apparently, the elimination rate constants are relative insensitive to assumptions with respect to the relative weight or volumes. Without confirmative information on the physical internal distribution of PAHs, there was no need to use a value for $f_{w_1}$ different from 0.5.

The rate constants of the peripheral compartment all had large confidence intervals (Table 3). In a strict sense, the approximated intervals were not significantly different from zero. However, model-predicted tissue residues when one or both peripheral rate constants were set at zero yielded unrealistic values, with low model-explained variance. It is expected that the parameter estimation can be optimized by an improved experimental design with an increased number of measurements with time and with measurements in a physiological second compartment. However, this should be preceded by an analysis of the internal distribution of PAHs in *Asellus*.

**Role of Biotransformation.** Among marine invertebrates, a considerable variation has been reported in biotransformation rates of PAHs (*2, 7*). With respect to freshwater invertebrates, comparative studies have been conducted with organisms from the Great Lakes. Slow but measurable biotransformation has been reported for the amphipod *Hyalella azteca* (*65*) and the mysid *Mysis relicta* (*66*). A marked biotransformation of benzo[*a*]pyrene and anthracene has been observed in larval stages of the midge *Chironomus riparius* (*67*). Biotransformation was absent in amphipod *Diporeia* spp. (*16*) and in the oligochete *Stylodrilus heringianus* (*69*). The close resemblance of the findings from the present study to benchmark QSARs for neutral nondegradable organic compounds (*48*) and the findings from the mass balance studies suggests that biotransformation of PAHs probably is of limited importance in the freshwater isopod. This lack of biotransformation probably is one of the reasons why relatively high BCFs of PAHs were encountered in freshwater isopods in this laboratory study and a previous field study (*22*).

**Comparison with Other Studies.** The results confirm the marked capacity of freshwater isopods to accumulate PAHs, as inferred from a previous field study (*22*). On a lipid basis the apparent field-based BCFs for anthracene, phenanthrene, and pyrene were similar to the laboratory-based BCFs from this study. On a wet weight basis the field-derived BCFs were higher, probably related to the higher lipid concentrations in the field study (1.4−3.8%). For benzo[*a*]pyrene and benzo[*ghi*]perylene lipid and wet weight field BCFs were lower than laboratory values, which may be related to reduced bioavailability of more hydrophobic PAHs (*68*) or the fact that in natural systems equilibrium conditions probably never will be approached. Comparisons between field- and labora-

tory-derived bioconcentration factors are complicated by insufficient insight into the role of environmental and biological factors. Landrum (*16*) demonstrated that uptake, elimination rate constants, and BCFs of PAHs in the freshwater amphipod *Diporeia* spp. (formerly indicated as *Pontoporeia hoyi*) not only varied with lipophilicity but also with season, temperature, age/body weight, and lipid content.

The wet weight BCFs observed in this study for PAHs in *Asellus* are comparable to BCFs reported from experimental studies with fish, mollusks, and daphnids (Tables A-4 and A-5, SI). Compared to other field studies in freshwater systems, higher or similar experimental wet weight BCFs have been reported for amphipods (*D. hoyi*) and oligochaetes (*S. heringianus*) inhabiting the deep sediments of the North American Great Lakes (*16, 69*). Results reported for insect species (*Hexagenia limbata* and *C. riparius*) tended to be lower (*61, 67*). A comparison with lipid-based BCFs from other studies could not be made as in many cases lipid contents were not reported (Table A-4, SI) or different lipid determination methods were used. The method of lipid determination may affect observed lipid-based BCF values. Increased bioaccumulation of PAHs in other aquatic isopods has previously been reported for the marine *Lygia* spp. by Neff *(7)*, in a comprehensive analysis of benzo[*a*]pyrene levels in biotic compartments. For terrestrial isopods low bioaccumulation of PAHs has been reported from field and experimental studies *(3)*.

## Concluding Remarks

Freshwater isopods are abundant in the littoral zones of many West European water systems (*44*) and may constitute an important link in the turnover of PAHs in littoral freshwater ecosystems. As biotransformation seems to be of minor importance, it may be expected that field residues of PAHs in isopods probably are linked to the bioavailable fraction of PAHs in littoral ecosystems. Confirmation of this assumption would require additional validating studies, with respect to uptake pathways and mechanisms, dose dependency of toxicokinetics, and influence of both biotic (sex, weight/age, moulting, lipid content, feeding habits, metabolism) and abiotic (sediment characteristics, turbidity, dissolved organic carbon, temperature) factors on the bioavailability of PAHs. Although these issues remain to be validated, the findings from this study indicate the potential suitability of freshwater isopods for biomonitoring studies.

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## Supporting Information Available

Experimental design details, Tables A-1−A-5, and Figure A-1. This material is available free of charge via the Internet at http://pubs.acs.org.

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