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BIOACCUMULATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY EARTHWORMS: ASSESSMENT OF EQUILIBRIUM PARTITIONING THEORY IN IN SITU STUDIES AND WATER EXPERIMENTS

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Abstract—The purpose of this study was to assess the suitability of applying equilibrium partitioning (EqP) theory to predict the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) by earthworms when these are exposed to contaminated soils in the field. Studies carried out in situ in various contaminated floodplain sites showed the presence of linear relationships with intercept zero between the lipid-normalized concentration of different PAHs in the earthworm *Lumbricus rubellus* and the organic-matter-normalized concentration of the compounds in soil. The demonstration of such an isometric relationship is in agreement with the prediction of EqP theory that the biota–soil accumulation factor (BSAF) should be independent of the octanol/water partition coefficient, log K_{ow}. The average BSAF of PAH compounds in the sampled 20-cm top layer of soil was 0.10 (range, 0.03–0.26). The present study also investigated the route of uptake of PAHs for earthworms in soil. The bioconcentration factor of low-molecular-weight PAHs, such as phenanthrene, fluoranthene, and pyrene, was derived from bioconcentration kinetic modeling of water-only experiments and found to be of the same order of magnitude as the bioaccumulation factor in the field when the latter was normalized to calculated concentrations in soil pore water. The results indicated that the exposure of earthworms to PAHs in soil is mediated through direct contact of the worms with the dissolved interstitial soil–water phase, further supporting the applicability of EqP theory to PAHs. Our experimental data on the biotransformation of PAHs suggest that earthworms possess some capacity of metabolization, although this does not seem to be a major factor in the total elimination of these compounds. Even though the EqP approach was found to be applicable to low-molecular-weight PAHs with respect to the prediction of bioaccumulation by earthworms in the field, the results were less conclusive for high-molecular-weight compounds, such as benzo[*a*]pyrene.

Keywords—Polycyclic aromatic hydrocarbons Bioaccumulation Soil Earthworms Equilibrium partitioning theory

INTRODUCTION

Oligochaete earthworms are functionally important in terrestrial ecosystems as food organisms for avian and mammalian wildlife and as intermediates in nutrient cycling processes. The bioaccumulation potential of toxic chemicals in earthworms provides a useful parameter when attempting to derive adequate soil quality criteria for ecological risk assessment of contaminated areas. In calculating the uptake of contaminants by organisms present in soil or sediment, the equilibrium partitioning (EqP) theory has been developed [1]. This theory predicts, for instance, that the biota–soil accumulation factor (BSAF) of organic chemicals is independent of the octanol/water partition coefficient (log K_{ow}) [2]. One of the basic assumptions underlying EqP theory is that the uptake of contaminants takes place through passive diffusion of the freely dissolved phase present in the soil interstitial pore water. Previous studies on earthworms have confirmed the validity of this assumption for a number of organic and inorganic chemicals [3–6]. However, several possible factors can interfere with the general applicability of EqP theory. First, for hydrophobic organic compounds of log $K_{ow} > 5$, the ingestion of contaminated soil particles may provide an additional route of uptake [7]. Second, the capacity to accumulate in organisms would remain limited in case of rapid biotransformation of the compounds. The application of EqP theory therefore needs to be evaluated for different compounds and different organisms.

Polycyclic aromatic hydrocarbons (PAHs) are a widespread group of multiring aromatic compounds. They are of great environmental concern because of their biotransformation in animals, which may produce metabolites with mutagenic and carcinogenic properties [8]. Some evidence indicates that PAHs may accumulate to some extent in the body tissues of earthworms [9,10]. However, the extent to which biotransformation is important as an elimination mechanism in earthworms and whether EqP theory is unconditionally applicable to predict the in situ bioaccumulation of PAHs in these animals in contaminated areas remain unclear. The purpose of the study reported here was to answer these questions using an approach involving both an experimental and a field investigation.

MATERIALS AND METHODS

Field study

The field study was carried out in the Rhine Delta river floodplains of the Netherlands. The soils of these areas are contaminated with PAHs over a depth of about 85 cm because of the deposition of polluted river sediments during periodic inundation events over the past 150 years [11]. Samples of soil and earthworms were collected from sites in the floodplains along the River Waal near Ochten and Gelderse Poort. The two localities are separated from each other by a distance

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Table 1. Soil parameters of field sites (20-cm top layer)[a]

Site	pH (KCl)	$CaCO_3$	SOM	<2 μm	<16 μm	16–50 μm	50–105 μm	>105 μm	Soil type
OT1	7.3	10.1	7.5	27.8	45.3	15.1	16.5	4.0	Silty clay loam
OT2	7.3	4.1	2.4	8.9	12.9	16.1	5.2	71.3	Light sandy loam
OT3	7.2	9.4	9.1	33.3	55.8	13.6	7.1	4.3	Silty clay loam
OT4	7.6	11.7	2.9	29.4	56.3	26.4	2.9	1.4	Silty clay loam
OT5	7.5	11.6	3.3	25.2	50.4	30.3	4.5	1.0	Silty clay loam
OT6	7.4	6.2	4.0	22.4	41.0	43.9	4.7	1.4	Silty clay loam
GP1	7.1	7.6	9.0	39.3	65.2	13.6	4.1	1.4	Silty clay loam
GP2	7.2	8.0	8.7	30.9	51.9	13.4	11.7	6.6	Silty clay loam
GP3	7.3	8.1	8.2	27.1	45.0	16.3	17.0	6.0	Silty clay loam
GP4	7.3	5.1	10.7	42.7	72.1	11.2	1.0	1.0	Silty clay
GP5	7.1	4.9	10.2	42.3	73.2	9.6	1.3	1.0	Silty clay
GP6	7.1	5.0	9.9	43.0	73.1	9.8	1.1	0.9	Silty clay

SOM = soil organic matter.
[a] Grain-size fractions, SOM, and $CaCO_3$ content (in g/100 g oven-dried soil).

of about 10 km. Variations in the level of pollution within the floodplains are correlated with differences in inundation frequency [11]. Six sites (OT1–OT6) were investigated in the Ochten area, and another six sites (GP1–GP6) were studied in the area of Gelderse Poort. All sites contained rough vegetation dominated by grasses such as *Glyceria maxima*, except site GP2, where other grasses, such as *Molinia caerulea* and *Phalaris* sp., were dominant. Soil properties of the sites are summarized in Table 1.

As shown in Table 1, the textural class of the soil at the different sites varied widely, ranging from a light sandy loam to a heavy silty clay. The soil at site OT2 consisted predominantly of sand (>50 μm), whereas clay (<2 μm) and silt (2–50 μm) were major fractions in soil at the other sites. All soils were calcareous, had a pH slightly higher than 7.0, and had a low to moderate amount of organic matter (Table 1).

Samples of *Lumbricus rubellus* worms were extracted from the upper 20 cm of soil by digging and hand sorting. *Lumbricus rubellus* is a commonly occurring epigeic species that burrows mainly in the upper 20 cm of soil. Only adult worms were used for the investigation. The worms were incubated for 48 h in total darkness at 15°C in large glass petri dishes lined with moistened filter paper so that they would eliminate their gut contents before residue analysis. Previous studies have shown that such treatment conditions are sufficient to allow the total concentration of PAHs in *L. rubellus* to decrease to a stable level [9]. Cleaned worm samples were stored at −20°C until chemical analysis. Soil samples were collected with a stainless-steel auger from the same soil layer as the earthworms. The samples were sieved (2-mm sieve), homogenized, and stored at −20°C until chemical analysis.

Experimental study

Adult specimens of *L. rubellus* with emptied guts were exposed to 200 ml of aqueous solution in glass vessels. The experiments were conducted in total darkness to avoid photolytic degradation or phototoxic effects of the test compounds. One animal was used per experimental vessel, and three replicates were performed for each exposure time. Test concentrations in water were chosen on the premise that they were sufficiently low to avoid overt toxic effects on the exposed animals within the period of exposure applied. As judged from physical appearance and motility, all exposed worms remained in an apparently healthy condition. The worms were killed by immersion in liquid nitrogen and stored at −20°C until chemical analysis. Solutions were made using reconstituted groundwater prepared by dissolving analytical-grade $NaHCO_3$ (100 mg/L), $KHCO_3$ (20 mg/L), $CaCl_2$ (200 mg/L), and $MgSO_4$ (180 mg/L) (Merck-Schuchardt, Darmstadt, Germany) in demineralized water. This aqueous solution is a suitable medium for subchronic exposure of earthworms in water [5]. Phenanthrene (Merck-Schuhardt), fluoranthene (Sigma-Aldrich Fine Chemicals, Milwaukee, WI, USA), pyrene (Riedel-de-Haën, Hannover, Germany), and benzo[*a*]pyrene (Sigma-Aldrich) were selected for experimental study. The compounds were dissolved in the reconstituted groundwater using a generator column made from a Teflon®-sealed glass tube filled with PAH-loaded Chromosorb W HP (Merck, Darmstadt, Germany; 80 to 100 mesh) [12]. Final checks of the dissolved PAH concentration were made by high-performance liquid chromatography (HPLC). Stock solutions were diluted as required for experimentation. Recoveries of spiked samples of worm tissue were 96% for phenanthrene, 98% for pyrene, 95% for fluoranthene, and 87% for benzo[*a*]pyrene.

The uptake experiments continued as long as the concentration in water stayed above the analytical detection limits, which was usually shorter than 7 days. Aliquots (25 ml) of solution were extracted with 5 ml of hexane. The hexane extract was added to 1 ml of acetonitrile, and the hexane fraction was evaporated in a nitrogen stream to 1 ml. The samples were analyzed with HPLC as described above. The rate constant k_v, which describes the loss of compound due to physical disappearance such as volatilization, physical degradation, and glass wall adsorption, was determined in vessels containing solution only. Mass balances were established for each vessel.

First-order, one-compartment kinetic constants of uptake and elimination were derived from concentration changes in water and the exposed organism [13]. To distinguish between passive elimination and biotransformation, a modification was used in which rate constants were determined according to the following model equations using small time steps ($t < 0.1$ h) [14]:

$$C_w t = \{C_w(t-1) \times V_w - [k_1 \times \Delta t \times C_w(t-1) \times M_{lip}] + [k_{pe} \times \Delta t \times C_{lip}(t-1) \times M_{lip}]\}/V_w \quad (1)$$

$$C_{lip} t = C_{lip}(t-1) + \{k_1 \times \Delta t \times C_w(t-1) - [(k_{pe} + k_m) \times \Delta t \times C_{lip}(t-1)]\} \quad (2)$$

where

C_w = concentration in water (g/L)

C_{lip} = concentration in worm lipid (g/kg)

M_{lip} = mass of lipid per worm (kg)

V_w = volume of water (L)

k_1 = first-order uptake rate constant (L/kg·d^{-1})

k_{pe} = rate constant for passive elimination (d^{-1})

k_m = rate constant for biotransformation (d^{-1})

Rate constants were determined from nonlinear curve fitting by applying an iterative least-squares method to both model equations and using data on C_{lip} and C_w versus *t*. The value of k_{pe} was estimated from Equation 1, whereas the best fit for Equation 2 was subsequently obtained from model simulation by varying the value of k_m. Because worm sizes could vary among exposure vessels while the volume of water remained constant, bioconcentration factors (BCFs) were calculated separately for each individual.

Chemical analysis

Samples (4–9 g) of dry-blotted worms were cut into small pieces and ground in a glass mortar in the presence of anhydrous sodium sulfate. Soxhlet extraction was performed with 100 ml of hexane, and extracts were concentrated in a Kuderna-Danish evaporator to 10 ml. Soil samples were homogenized in a mortar, and 20-g subsamples were shaken in 20 ml of acetone. The mixture was shaken in 40 ml of petroleum ether and filtered through quartz wool. The residue was shaken in 30 ml of acetone/petroleum ether (1:2 v/v). The combined extracts (about 100 ml) were shaken twice with 100 ml of Milli-Q® water. The petroleum ether was filtered through quartz wool with sodium sulfate and concentrated in a Kuderna-Danish evaporator to 10 ml.

Cleanup was performed by column chromatography using aluminum oxide (14% deactivated) and anhydrous sodium sulfate. Petroleum ether and hexane (10 ml) were used for elution of soil and worm extracts, respectively. Eluates were collected in a calibrated tube and reduced in a stream of nitrogen gas to a volume of about 2 ml. After adding 1 ml of acetonitrile, the petroleum ether was evaporated, and acetonitrile was added to obtain a volume of 1 ml. The exact volume was determined by weighing and recalculating using the density of acetonitrile.

Components of PAHs were identified using a high-performance liquid chromatograph (Spectra-Physics) equipped with a Chromspher PAH column (Chromspher, Bergen op Zoom, Holland; 100 × 3 mm, 5 μm particles). A certified solution of 16 PAHs in acetonitrile (SRM 1647b, U.S. National Institute of Standards and Technology, Gaithersburg, Maryland) was used for calibration. An internal standard of 6-methylchrysene (code BCR82, Community Bureau of Reference, Brussels, Belgium) was added to both the calibration solution and the sample extracts (final concentration, 15 ng/ml) to check retention times and injection volumes (10 μl). Fluorescence detection was programmed at the following optimum excitation/emission wavelengths: 254/358 nm for naphthalene, acenaphthene, and fluorene; 254/386 nm for phenanthrene and anthracene; 254/430 nm for fluoranthene and pyrene; 254/386 nm for benzo[*a*]anthracene, chrysene, and the internal standard; 300/420 nm for benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and benzo[*a*]pyrene; and 300/490 nm for dibenzo[*b*]anthracene and benzo[*ghi*]perylene. Detection limits were set at six times the standard deviation of the background noise level. All analyses were made in duplicate.

Soil organic matter was determined by loss on ignition using oven-dried samples. Soil organic matter on average was assumed to consist of 58% organic carbon [15]. Total lipid contents of worms were determined gravimetrically from residues of Soxhlet-extracted samples and are expressed as fraction of wet-weight mass. The worms had an average lipid content of 1.21 ± 0.061% (mean ± SD, n = 12) on a wet-weight basis.

RESULTS

Biota–soil accumulation factor

Assuming steady-state conditions, the bioaccumulation of PAHs in *L. rubellus* in the river floodplains can be described by the BSAF:

$$\text{BSAF} = \frac{C_w \times f_{om}}{C_s \times f_{lip}} \quad (3)$$

where

C_w = concentration in worm (mg/kg fresh weight)

C_s = concentration in soil solid phase (mg/kg)

f_{lip} = weight fraction of lipid (kg/kg)

f_{om} = weight fraction of organic matter (kg/kg)

In Figure 1, the lipid-normalized internal concentration in *L. rubellus* of 11 priority PAHs is plotted against the organic-matter-normalized concentration in soil. The plots represent floodplain sites with a high, intermediate, and low level of pollution. Concentrations of PAHs in either soil or worms below detection limits were not included in the figure. In worms, the concentration of naphthalene (<30 μg/kg fresh weight), acenaphthene (<20 μg/kg), and fluorene (<10 μg/kg) was below the detection limit at all sites. In soil, only the concentration of acenaphthene was below the detection limit (0.01 μg/kg dry matter) at all sites. Fluoranthene and pyrene were the predominant compounds in the river floodplains.

As illustrated in Figure 1, the partitioning of PAHs between the worm lipid phase and soil organic carbon conformed to a linear relationship with intercept zero. The presence of such an isometric relationship indicates that the BSAF, as represented by the slope coefficient of the regression, can be considered a constant for compounds with varying log K_{ow} values.

The BSAF values determined at the different sites are summarized in Table 2 and range from 0.03 to 0.26. The average BSAF value was 0.10 (±0.06 SD, n = 12). No significant correlations were present between the BSAF and any of the soil properties shown in Table 1, including organic matter content and grain-size fractions.

Bioaccumulation factor

The bioaccumulation factor (BAF) was established from the concentration in worm lipid and the concentration in interstitial soil water assuming equilibrium conditions as follows:

$$\text{BAF} = \frac{C_w \times K_p}{f_{lip} \times C_s} \quad (4)$$

where the equilibrium soil sorption constant, K_p (L/kg), can be estimated from

$$K_p = 0.58 \times f_{om} \times K_{oc} \quad (5)$$

where the organic carbon/water partition coefficient, K_{oc} (ml/g), for hydrophobic organic chemicals with log K_{ow} values

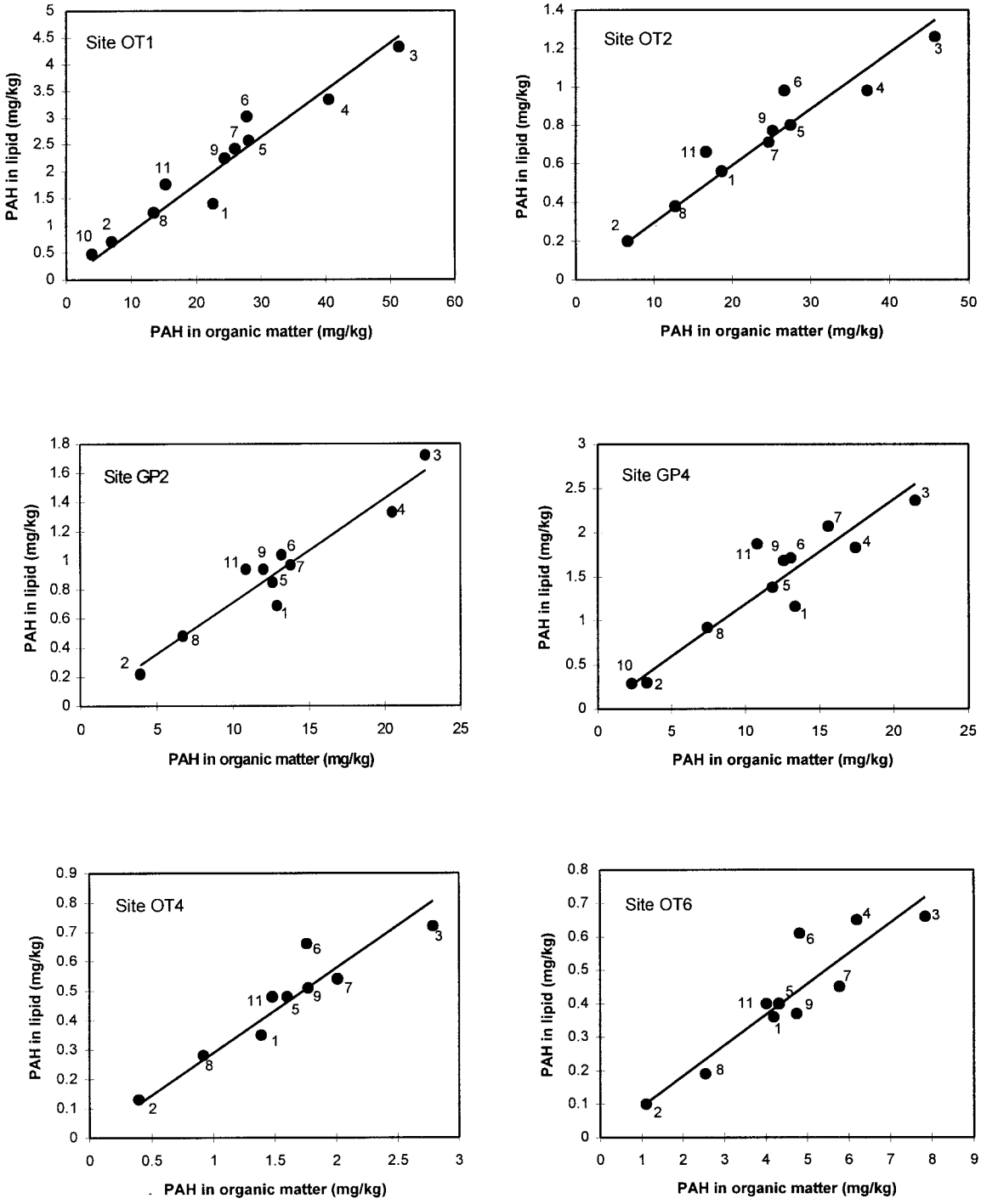

Fig. 1. Lipid-normalized concentration of polycyclic aromatic hydrocarbons (PAHs) in *Lumbricus rubellus* plotted against the organic-carbon-normalized concentration in soil for a number of selected field sites. The sites were selected to represent different levels of soil contamination with PAHs (high level, OT1 and OT2; intermediate level, GP2 and GP4; and low level, OT4 and OT6). 1 = phenanthrene; 2 = acenaphthene; 3 = fluorene; 4 = pyrene; 5 = benzo[*a*]anthracene; 6 = chrysene; 7 = benzo[*b*]fluoranthene; 8 = benzo[*k*]fluoranthene; 9 = benzo[*a*]pyrene; 10 = dibenzo[*b*]anthracene; 11 = benzo[*ghi*]perylene.

between 1.5 and 6.5, can be derived from the following approximation [16]:

$$\log K_{oc} = 0.989 \times \log K_{ow} - 0.346 \quad (6)$$

Bioaccumulation factors calculated for the different PAHs in the floodplain sites are summarized in Table 3.

Kinetic modeling of bioconcentration

Mortality was not observed in either of the water-only exposure experiments conducted with phenanthrene, fluoranthene, pyrene, or benzo[*a*]pyrene. The time course of the observed changes in the solution concentration, C_w, is shown for each compound in Figures 2 to 5. These figures show that the decrease in solution concentration followed a first-order rate of decline to a near stable level after 2 to 3 d. The figures also include changes measured in the lipid-normalized concentration, C_{lip}, of the exposed worms. Three compounds (phenanthrene, fluoranthene, and pyrene) showed a rapid initial uptake followed by some temporary loss. According to mass balance

Table 2. Biota–soil accumulation factor for polycyclic aromatic hydrocarbons in *Lumbricus rubellus* in different field sites

Site	BSAF (kg SOM/kg lipid)	r^2	*n*
OT1	0.081	0.935	11
OT2	0.026	0.919	10
OT3	0.105	0.886	11
OT4	0.257	0.875	9
OT5	0.192	0.743	9
OT6	0.091	0.830	10
GP1	0.069	0.893	9
GP2	0.072	0.917	10
GP3	0.062	0.883	10
GP4	0.110	0.849	11
GP5	0.042	0.759	9
GP6	0.062	0.781	10

BSAF = biota–soil accumulation factor; *n* = number of polycyclic aromatic hydrocarbon compounds included in the regression used to calculate BSAF; SOM = soil organic matter.

analysis, this phenomenon could not be ascribed to a physical disappearance and therefore may represent nonequilibrium conditions for PAHs in worms and water.

Rate constants for uptake, elimination, and biotransformation were calculated from fitted values according to Equations 1 and 2. The results are summarized in Table 4, together with data for k_v, the parameter representing the disappearance rate of PAHs from vessels without added worms. In view of the relatively low values observed for k_v (Table 4), losses due to physical disappearance seemed limited. However, the possibility that part of the non-steady-state saturation or partitioning kinetics evident in Figures 2 to 5 is due to possible adsorption of the compounds onto the glass walls of the experimental vessels cannot wholly be excluded. For phenanthrene, fluoranthene, and pyrene, a single value of k_1 gave the best fit for both C_{lip} and C_w. For benzo[*a*]pyrene, however, the best fit for C_w failed to yield an equally good fit for C_{lip}. The value of k_{pe} was obtained from fitting C_w against time, because changes in C_w are due to passive elimination only. An improvement of the fit of C_{lip} against time was obtained for phenanthrene, fluoranthene, and pyrene by introducing k_m into Equation 2. It was not possible to fit a reliable value for k_m for benzo[*a*]pyrene from the model.

Bioconcentration factor

The experimental data did not determine whether a true steady state was reached within the relatively short exposure period applied. However, after $t = 2$ d, changes in C_{lip} and C_w became less apparent, although for benzo[*a*]pyrene the concentration continued to increase. As an approximation of steady state, average values for the experimental BCFs (BCF_{exp}) were calculated for all experiments from 2 d onward. These apparent BCFs were compared with BCFs generated from kinetic modeling (BCF_{fit}). The latter was calculated as

$$BCF_{fit} = \frac{k_1}{k_{pe} + k_m} \quad (7)$$

Table 3. Average values (±95% confidence interval) of the in situ bioaccumulation factor (BAF) of polycyclic aromatic hydrocarbons in *Lumbricus rubellus* in floodplain sites (BAF values × 1,000)

Compound	Log K_{ow}[a]	BAF (L/kg)	*n*
Anthracene	4.54	80 ± 61	12
Phenanthrene	4.57	70 ± 30	12
Pyrene	5.18	330 ± 172	9
Fluoranthene	5.22	350 ± 137	12
Benzo[*b*]fluoranthene	5.80	1,350 ± 552	11
Chrysene	5.86	2,070 ± 980	12
Benzo[*a*]anthracene	5.91	1,880 ± 810	12
Benzo[*k*]fluoranthene	6.00	2,110 ± 845	12
Benzo[*a*]pyrene	6.04	2,470 ± 1,107	12
Benzo[*ghi*]perylene	6.50	9,080 ± 3,778	12
Dibenzo[*a*]anthracene	6.75	18,020 ± 6,962	3

[a] Recommended values [22].

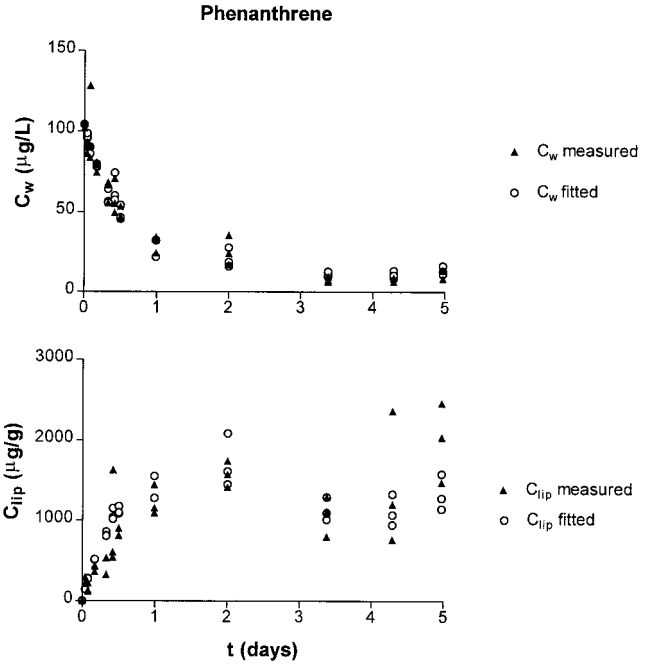

Fig. 2. Time course of concentration changes of phenanthrene in water and worm lipid in experiments with water-only exposures. ▲ = measured; ○ = fitted.

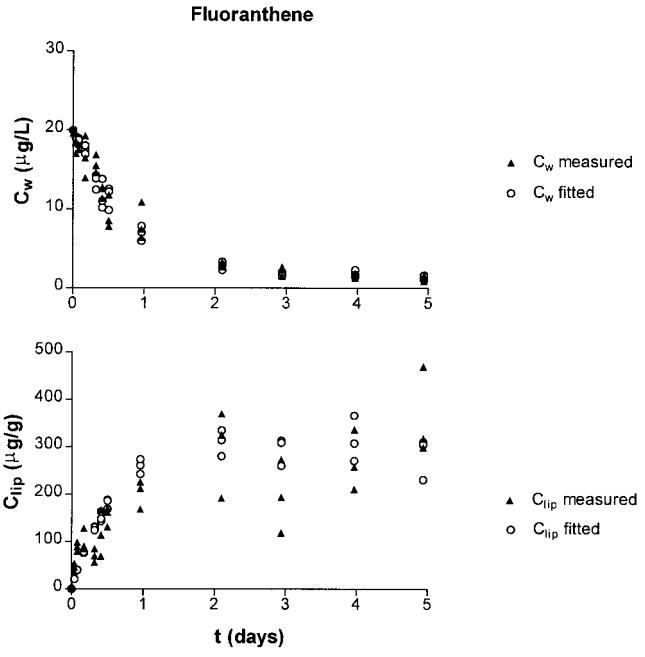

Fig. 3. Time course of concentration changes of fluoranthene in water and worm lipid in experiments with water-only exposures. ▲ = measured; ○ = fitted.

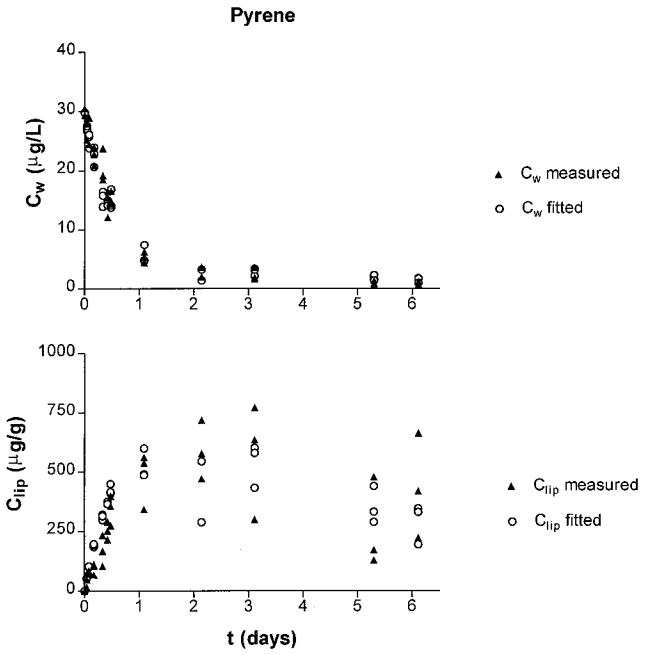

Fig. 4. Time course of concentration changes of pyrene in water and worm lipid in experiments with water-only exposures. ▲ = measured; ○ = fitted.

The results obtained are summarized in Table 5. The fitted BCFs derived from rate constants (BCF_{fit}) were within or close to the 95% confidence interval of the measured values (BCF_{exp}).

DISCUSSION

This study shows that in situ BSAFs of PAHs in earthworms conform to a linear partition coefficient for the equilibrium distribution between the lipid phase and the organic matter phase. This can be concluded from the presence of a linear relationship with intercept zero between the lipid-normalized concentration in worms and the organic-matter-normalized concentration in soil (Fig. 1). The BSAF is hence independent of log K_{ow}, which is in agreement with predictions from EqP theory [2]. The BSAF values determined for 12 floodplain sites ranged from 0.03 to 0.26, with an average value of 0.10. A similar range in BSAF varying between 0.10 and 0.26 has been reported for chrysene in aquatic species of chironomids and oligochaetes [17]. According to EqP theory, the BSAF should be site independent when normalized to lipid and organic carbon. The reason for the observed intersite variation in BSAF is not clear, but it may be due to local differences in the binding properties of the organic matter present. No correlations between BSAF and either the organic matter content or the grain-size fractions of the different soils were observed.

We tested the hypothesis that the uptake of PAHs by earthworms is mediated through direct contact of the organism with the soluble phase. If this is true, then the uptake in water-only tests should result in the same BCF as when the process would occur in the field. Our data tended to support this. Our estimations of interstitial-water-normalized BAFs showed that the in situ BAFs of phenanthrene, fluoranthene, and pyrene are of the same order of magnitude as the BCF determined in water-only experiments (Tables 3 and 5). This suggests that the kinetics of uptake and elimination are essentially similar for worms in soil as in water and that the internal concentration in the worm results from uptake from interstitial water rather than from the ingestion of contaminated soil particles. For benzo[*a*]pyrene, however, the in situ BAF was one order of magnitude greater than the experimental BCF in water. A possible explanation for this discrepancy is that under field conditions, dietary uptake provides an additional route of exposure for hydrophobic compounds of log $K_{ow} > 5$ [7]. Application of EqP theory to such strongly lipophilic compounds would thus lead to an underestimation of the in situ BAF. However, steady state was not reached in our experiments with ben-

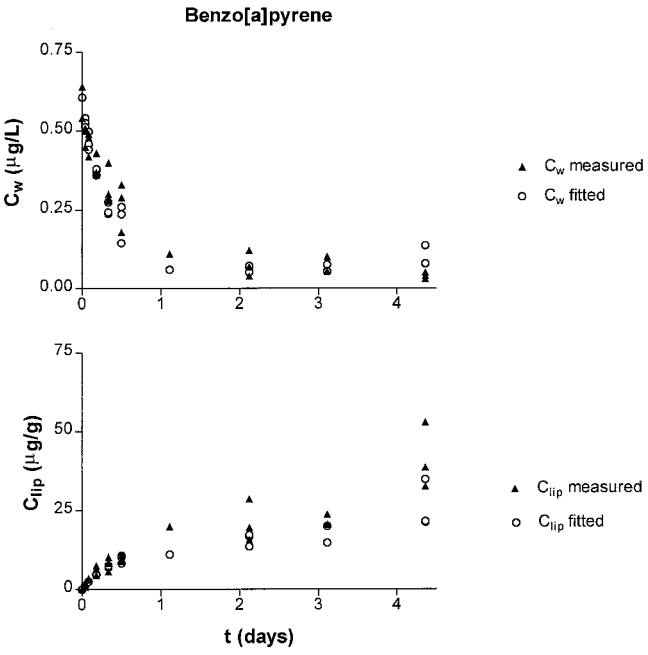

Fig. 5. Time course of concentration changes of benzo[*a*]pyrene in water and worm lipid in experiments with water-only exposures. ▲ = measured; ○ = fitted.

Table 4. Kinetic constants for polycyclic aromatic hydrocarbons calculated for *Lumbricus rubellus* in water-only exposure experiments[a]

Compound	C_w (t = 0) (g/L)	k_1 (L/kg·d)	k_{pe} (d^{-1})	k_m (d^{-1})	k_y (d^{-1})
Phenanthrene	104.2	35,000	0.3	0.1	0.05
Fluoranthene	20.0	25,000	0.1	0.2	0.03
Pyrene	29.8	45,000	0.2	0.2	0.04
Benzo[*a*]pyrene	0.60	55,000	0.2	ND	0.03

ND = not determinable.
[a] See text for definitions of constants.

Table 5. Average experimental bioconcentration factors (BCF_{exp}) (±95% confidence interval) measured for t = 2 d and BCFs generated by kinetic modeling (BCF_{fit}) in water-only exposure experiments (BCF values × 1,000)

Compound	Log K_{ow}[a]	BCF_{exp} (L/kg)	BCF_{fit} (L/kg)
Phenanthrene	4.57	140 ± 39 (n = 12)	90 (n = 12)
Fluoranthene	5.18	165 ± 52 (n = 12)	115 (n = 12)
Pyrene	5.22	300 ± 94 (n = 12)	100 (n = 12)
Benzo[*a*]pyrene	6.04	570 ± 263 (n = 9)	275 (n = 9)

[a] Recommended values [22].

Table 6. Elimination rate constants based on wet-weight values of polycyclic aromatic hydrocarbons in earthworms in comparison to values for chlorobenzenes of comparable log K_{ow}

Compound	Log K_{ow}	k_2 (d^{-1})
Phenanthrene[a]	4.57	0.4
Fluoranthene[a]	5.18	0.3
Pyrene[a]	5.22	0.4
Benzo[*a*]pyrene[a]	6.04	0.2
1,2,3,4-Tetrachlorobenzene[b]	4.63	0.42
Pentachlorobenzene[b]	5.18	0.15
Hexachlorobenzene[b]	5.73	0.02

[a] This study.
[b] [21].

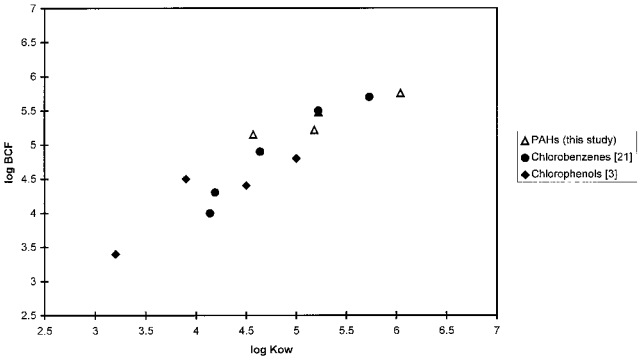

Fig. 6. Log bioconcentration factor (BCF) plotted against log K_{ow} for some hydrophobic compounds in earthworms. The data refer to polycyclic aromatic hydrocarbons (PAHs) in *Lumbricus rubellus* (this study, △), chlorobenzenes in *Eisenia andrei* (●) [21], and chlorophenols in *Eisenia andrei* (♦) [3].

zo[*a*]pyrene, and it is not possible to draw conclusions about the suitability of EqP theory in the case of non-steady-state partitioning kinetics in either water-only or soil solids–pore water systems.

An important issue to realize is that in situ BAFs or in situ BSAFs are highly influenced by the depth and thickness of the soil layer sampled in the field. Concentrations of PAHs in soil often tend to decline rapidly with depth, thus showing a vertical distribution gradient within the habitat layer normally occupied by earthworms. In our study, we took mixed soil samples from the top 20 cm, which covers most of the soil layer inhabited by *L. rubellus*. Other workers have calculated in situ BAFs based on soil layers of variable thickness, such as the humus layer or the mineral layer [10]. It is clear, however, that any comparison of literature data on in situ BAFs or BSAFs is permitted only if the data refer to a standardized soil layer with respect to depth and thickness. To enable such a comparison of literature references, we suggest use of the mixed top 20-cm soil layer as a standard for field studies on earthworms.

The average BSAF value of 0.10 established for PAHs in the present study is smaller than the average value of 0.75 reported for polychlorinated biphenyls (PCBs) in the same floodplain area [18]. Because PCBs are poorly metabolizable, the difference in BSAF may suggest that PAHs are less resistant to biotransformation than PCBs. This is especially true for the low-molecular-weight PAHs, which tended to have smaller BSAFs than the high-molecular-weight PAHs (see also Fig. 1). The significance of biotransformation in the earthworm can be estimated by subtracting k_v from the value of k_m in Table 4. The values obtained for k_m in the water-only exposure experiments suggest that biotransformation does play some role in total PAH elimination. Reports on the detection of DNA adducts in earthworms occurring in heavily contaminated industrial sites seem to support the occurrence of biotransformation [19,20], but what kind of compounds were exactly involved in inducing the DNA adducts in these studies remains open to question. Furthermore, one must distinguish the phenomenon of intrinsic metabolism in worm tissues from the possible contribution made to biodegradation by microorganisms present in the gut or skin surface tissues of the worms.

Other indirect evidence of the occurrence of biotransformation comes from Table 6, where the elimination rate constant established for PAHs, k_2, is compared with available data on elimination constants for chlorobenzenes of corresponding log K_{ow} range [21]. As shown in Table 6, values of k_2 for chlorobenzenes tend to decrease with increasing log K_{ow}, indicating an increased resistance to biotransformation [23], whereas for PAHs, the value of k_2 failed to show a strong decline over a similar range of increasing log K_{ow}. Figure 6, however, shows that the bioconcentration of PAHs in earthworms is of the same order of magnitude as that of chlorinated monoaromatic compounds, such as chlorobenzenes and chlorophenols. Because biotransformation of chlorobenzenes is of relatively minor importance in earthworms [24], this seems applicable to PAHs as well. Taken together, all this information suggests that biotransformation of PAHs does occur in earthworms but probably is not a major factor in the total elimination process. Our results suggest that EqP theory is applicable to low-molecular-weight PAHs, whereas more extensive studies are needed to further elucidate the environmental behavior of the high-molecular-weight components of this group of compounds.

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