AVAILABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS TO EARTHWORMS
(EISENIA ANDREI, OLGOCHAETA) IN FIELD-POLLUTED SOILS AND
SOIL–SEDIMENT MIXTURES

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Abstract—The bioavailability of polycyclic aromatic hydrocarbons (PAHs) for earthworms (Eisenia andrei) was experimentally
determined in seven field-polluted soils and 15 soil–sediment mixtures. The pore-water concentration of most PAHs was higher
than predicted. However, most of the compound was associated with dissolved organic carbon (DOC) and not directly available
for uptake by earthworms. The apparent sorption could be reasonably predicted on the basis of interactions with DOC; however,
the biota-soil accumulation factors (BSAFs) for earthworms were up to two orders of magnitude lower than predicted by equilibrium
partitioning. The large variability between sites was not fully explained by differences in sorption. Experimental results indicate
that the pool of freely dissolved PAHs in the pore water became partially depleted because of uptake by the earthworms and that
bioaccumulation is thus also influenced by the kinetics of PAH desorption and mass transport. A pilot study with Lumbricus rubellus
showed that steady-state body residues were well correlated to E. andrei. Current results show that depositing dredge spoil on land
may lead to increased bioavailability of the lower-molecular-weight PAHs. However, risk assessment can conservatively rely on
equilibrium partitioning, but accurate prediction requires quantification of the kinetics of bioavailability.

Keywords—Earthworms Polycyclic aromatic hydrocarbons Bioaccumulation Toxicokinetics Bioavailability

INTRODUCTION

Polycyclic aromatic hydrocarbons are common pollutants
in soils and typically tend to be persistent in soils because of
their relatively low mobility and high resistance to degradation.
Over the past 100 years, levels of PAHs in soils have been
steadily increasing, primarily as a result of combustion of fossil
fuels, followed by atmospheric deposition [1]. An additional
input to soils in The Netherlands follows from dredging prac-
tices. Sediments in regional waters are dredged on a regular
basis to ensure sufficient water depth for navigation and water
discharge. The main part of these dredged sediments is placed
on soils where they can contribute to the local PAH contamina-
tion [2]. To prevent unacceptable levels in soil, environ-
mental quality criteria for sediment are in place, based on the
total level of PAHs in the sediment. However, quality standards
and risk assessment should preferably be based on actual bio-
available concentrations in soil, which appears to be the chem-
ical freely dissolved in pore water [3].

Earthworms are common representatives of the soil mac-
rofauna, which live in close contact with the soil. Uptake of
organic chemicals in earthworms is assumed to occur through
passive diffusion, driven by the fugacity difference between
pore water and the organism’s tissues. This equilibrium par-
titioning (EP) approach generally provides a satisfactory de-
scription for earthworms [4], although it seems likely that in

many situations limited diffusion or other transport processes
prevent establishment of true equilibrium [5,6]. Bioavailability
and uptake of PAHs is also quite predictable based on pore-
water concentrations, at least under laboratory conditions when
using spiked artificial soil [7]. However, under field conditions,
bioavailability of PAHs is generally much more difficult to
predict, as a multitude of factors play a role, including se-
qustration or aging [8], mass-transport limitations [9], strong
binding to soot [10], and variability in the polarity of organic

The purpose of this study was to examine the current prac-
tice of depositing dredge spoil on soils. This was done by
experimentally determining the bioavailability of PAHs in typ-
ical field-polluted soils and soil–sediment mixtures. The mea-
sure of availability that is used here is the dynamic accumu-
lation pattern in the compost worm (Eisenia andrei), expressed
by the steady-state BSAF and the elimination rate constant.
Bioavailability may differ between species, and although the
compost worm is not a typical soil dweller, it is used as stan-
dard test organism for soil [12]. The BSAFs are compared to
EP estimates to determine the predictive power of the EP ap-
proach for bioavailability of PAHs in typical field-polluted
media. Furthermore, we attempt to explain the observed bio-
availability from the dynamic sorption and uptake processes
that determine it.

MATERIALS AND METHODS

Sampling of soils and sediments

Sediment samples were taken on 45 contaminated sites in
The Netherlands. From these, 15 sites were selected for further
use on the basis of elevated levels of either metals (not discussed in this paper) or PAHs and representing sediments with different predominant constituents (sand, clay, and peat). Furthermore, seven soils with the same predominant constituents were sampled. After removal of the vegetation, soil was taken from the upper soil horizon (0–20 cm). Soil and sediments were stored in closed containers at 5°C, and within two weeks the samples were sieved (4 mm) and homogenized in a baker’s mill. For several samples, the high clay content prohibited this procedure. For these soils, large objects (e.g., stones and roots) were removed, after which the samples were homogenized manually. Mixtures of soil and sediment were created by adding a sediment to one of the seven soils in a fixed proportion: sand and clay 1:4, peat 1:2 (sediment:soil on dry-wt basis). This mimics realistic practices in The Netherlands. In total, 22 media (seven soils and 15 mixtures) were used for subsequent testing (see Table 1).

Physical and chemical analyses

In the 22 exposure media, the following parameters were determined: pH (CaCl₂), organic carbon content (elemental analyzer [Model EA 1108], Fisons Instruments, Rodana, Italy), clay content (≤2 μm, gravimetric analysis), water-holding capacity, and DOC content of pore water (Dohrmann Division DC-190, Santa Clara, CA, USA). The media were analyzed for selected PAHs (Table 2) as described earlier [7] after cryogenic grinding. For the determination of PAHs in pore water, soils were brought to 85% of the water-holding capacity using 2-mmol Ca(NO₃)₂. After at least two weeks of equilibration, pore water was collected using a centrifugation method in

Table 1. Soil properties for the soils (S) and soil-sediment mixtures (M) used in this study, including fraction organic carbon (fOC), clay content, pH, dissolved organic carbon (DOC), and total content of polycyclic aromatic hydrocarbons (ΣPAHs)

<table>
<thead>
<tr>
<th>Soil/mixture code</th>
<th>Mixed with</th>
<th>Character</th>
<th>fOC (%)</th>
<th>Clay (%)</th>
<th>pH (CaCl₂)</th>
<th>DOC (mg/L)</th>
<th>ΣPAHs (mg/kg dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Peat</td>
<td></td>
<td>10</td>
<td>14</td>
<td>7.0</td>
<td>76</td>
<td>10</td>
</tr>
<tr>
<td>S2</td>
<td>Sand</td>
<td></td>
<td>2.4</td>
<td>6.1</td>
<td>4.7</td>
<td>315</td>
<td>0.25</td>
</tr>
<tr>
<td>S3</td>
<td>Clay</td>
<td></td>
<td>4.5</td>
<td>12</td>
<td>6.8</td>
<td>57</td>
<td>2.8</td>
</tr>
<tr>
<td>S4</td>
<td>Peat</td>
<td></td>
<td>14</td>
<td>8.8</td>
<td>7.2</td>
<td>77</td>
<td>25</td>
</tr>
<tr>
<td>S5</td>
<td>Clay</td>
<td></td>
<td>3.7</td>
<td>21</td>
<td>7.4</td>
<td>47</td>
<td>1.8</td>
</tr>
<tr>
<td>S6</td>
<td>Sand</td>
<td></td>
<td>1.3</td>
<td>0.69</td>
<td>7.1</td>
<td>91</td>
<td>17</td>
</tr>
<tr>
<td>S7</td>
<td>Clay</td>
<td></td>
<td>6.4</td>
<td>35</td>
<td>6.1</td>
<td>108</td>
<td>1.2</td>
</tr>
<tr>
<td>M1</td>
<td>S5</td>
<td>Clay</td>
<td>4.4</td>
<td>20</td>
<td>7.5</td>
<td>54</td>
<td>2.3</td>
</tr>
<tr>
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<td>S4</td>
<td>Peat</td>
<td>11</td>
<td>14</td>
<td>6.8</td>
<td>95</td>
<td>13</td>
</tr>
<tr>
<td>M3</td>
<td>S6</td>
<td>Sand</td>
<td>1.9</td>
<td>0.61</td>
<td>7.1</td>
<td>152</td>
<td>16</td>
</tr>
<tr>
<td>M4</td>
<td>S4</td>
<td>Peat</td>
<td>9.8</td>
<td>11</td>
<td>7.0</td>
<td>121</td>
<td>11</td>
</tr>
<tr>
<td>M5</td>
<td>S2</td>
<td>Sand</td>
<td>2.4</td>
<td>4.7</td>
<td>5.5</td>
<td>311</td>
<td>4.6</td>
</tr>
<tr>
<td>M6</td>
<td>S3</td>
<td>Clay</td>
<td>4.6</td>
<td>11</td>
<td>7.1</td>
<td>116</td>
<td>3.8</td>
</tr>
<tr>
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<td>Sand</td>
<td>2.2</td>
<td>2.4</td>
<td>7.3</td>
<td>156</td>
<td>19</td>
</tr>
<tr>
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<td>Clay</td>
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<td>9.8</td>
<td>6.8</td>
<td>78</td>
<td>3.9</td>
</tr>
<tr>
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<td>S2</td>
<td>Sand</td>
<td>3.6</td>
<td>7.9</td>
<td>6.6</td>
<td>298</td>
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</tr>
<tr>
<td>M10</td>
<td>S1</td>
<td>Peat</td>
<td>10</td>
<td>48</td>
<td>7.3</td>
<td>76</td>
<td>12</td>
</tr>
<tr>
<td>M11</td>
<td>S5</td>
<td>Clay</td>
<td>4.7</td>
<td>23</td>
<td>7.3</td>
<td>62</td>
<td>1.3</td>
</tr>
<tr>
<td>M12</td>
<td>S4</td>
<td>Peat</td>
<td>14</td>
<td>14</td>
<td>7.3</td>
<td>102</td>
<td>23</td>
</tr>
<tr>
<td>M13</td>
<td>S7</td>
<td>Clay</td>
<td>7.0</td>
<td>24</td>
<td>6.2</td>
<td>113</td>
<td>1.1</td>
</tr>
<tr>
<td>M14</td>
<td>S1</td>
<td>Peat</td>
<td>9.8</td>
<td>16</td>
<td>7.4</td>
<td>33</td>
<td>14</td>
</tr>
<tr>
<td>M15</td>
<td>S7</td>
<td>Clay</td>
<td>6.3</td>
<td>39</td>
<td>6.7</td>
<td>84</td>
<td>1.8</td>
</tr>
<tr>
<td>Min.</td>
<td></td>
<td></td>
<td>1.3</td>
<td>0.61</td>
<td>4.7</td>
<td>33</td>
<td>0.25</td>
</tr>
<tr>
<td>Max.</td>
<td></td>
<td></td>
<td>14</td>
<td>48</td>
<td>7.5</td>
<td>315</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 2. Summary of the polycyclic aromatic hydrocarbons (PAHs) included in this study, their log Kow values [27], and the percentage of the particular PAH in the total concentration (mean and range). The last three columns give the number of steady-state body residues (Cw (∞)), elimination rates (k) and sorption coefficients (Koc-wp) that could be determined

<table>
<thead>
<tr>
<th>PAH</th>
<th>Log Kow</th>
<th>Percentage of ΣPAHs</th>
<th>No. Cw (∞)</th>
<th>No. k</th>
<th>No. Koc-wp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>3.37</td>
<td>1.8 (0.46–5.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>3.92</td>
<td>0.75 (0.40–1.4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fluorene</td>
<td>4.18</td>
<td>1.0 (0.50–1.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>4.57</td>
<td>8.6 (4.1–14)</td>
<td>4</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Anthracene</td>
<td>4.54</td>
<td>2.0 (0.58–3.2)</td>
<td>7</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>5.22</td>
<td>18 (10–23)</td>
<td>12</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Pyrene</td>
<td>5.18</td>
<td>13 (8–16)</td>
<td>12</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>5.91</td>
<td>9.0 (6.6–10)</td>
<td>18</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Chrysene</td>
<td>5.86</td>
<td>9.0 (6.9–10)</td>
<td>19</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>5.80</td>
<td>12 (8.7–18)</td>
<td>18</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>6.00</td>
<td>11 (8.5–14)</td>
<td>17</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>6.04</td>
<td>4.7 (3.4–5.7)</td>
<td>17</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>6.75</td>
<td>1.4 (0.81–2.6)</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>6.50</td>
<td>3.7 (2.1–5.7)</td>
<td>17</td>
<td>6</td>
<td>16</td>
</tr>
</tbody>
</table>
which the water is pushed upward and can be collected with a Pasteur pipette. This procedure resulted in a clear supernatant, and filtering was judged unnecessary (pilot experiments showed that a 0.45-μm filter also removed large amounts of dissolved PAHs). Total PAH contents in pore water were determined after exhaustive extraction employing acetone and petroleum ether, drying over sodium sulfate, and evaporation on a Kuderna Danish apparatus (custom made at our institute). Residues were dissolved in 1 ml acetone/water (2:3), and 100 μl were injected into a liquid chromatography system with fluorescence detection.

Freely dissolved concentrations of benzo[a]pyrene were determined by leading 600 μl pore water through a capillary chemical-bonded siloxane gas chromatography column (55-cm-0.32-mm i.d., film thickness 1.2 μm) at 0.3 ml/min. The capillary was rinsed with 600 μl high-performance liquid chromatography water with d12-labeled PAH isotopes as internal standards. Nitrogen was led through the capillary, after which it was connected to a gas chromatography with high-resolution mass spectrometry detection. Using a cold trap, the analytes were concentrated at the top of the analytical column and subsequently separated and determined.

The PAHs were extracted from earthworm tissues by means of an Ultra-Turrax (Janke & Kunkel, Staufen, Germany) extraction with petroleum ether. After drying over sodium sulfate, the samples were concentrated to 1 ml and transferred to preweighed autosampler bottles. Part of the sample was injected into a gel-permeation-chromatography system (Model 305 liquid chromatography pump, Model 321 autosampler, fraction collector Model FC 204 from Gislon, Villier-le-Bel, France) for cleanup (lipid separation). The fraction with analytes was evaporated and dissolved in 1 ml acetone-water (2:3), and 100 μl were injected into the liquid chromatography system.

**Bioassays**

Earthworms (*E. andrei*) were obtained from mass cultures at our institute and kept under climatized conditions (temperature 20 ± 2°C). Juvenile worms were selected to ensure that no loss of accumulated chemicals would occur due to reproduction. The worms were weighed, placed in plastic containers (10 worms per container, total wet wt ~3 g) with 600 to 900 g (wet wt) of medium, and incubated at 20°C in full light (to prevent escape). Instead of maximizing replication, we chose to use a large number of exposure periods: 0.25, 1, 2, 3, 4, 7, 10, 12, 14, and 21 d. Worms were recaptured and depurated (48 h on moist filter paper, which was changed after 24 h). Subsequently, the worms were divided into two groups (five for PAH analysis and five for metal analysis; metals not discussed in this paper) and frozen before analysis. Four groups of five worms, taken directly from the culture, were used for the test of validity of *E. andrei* as a model for other species, a similar but limited set of assays was performed with *Lumbricus rubellus*, a species that occurs in a range of Dutch field soils. Adult or subadult specimens were obtained from a low-intensity culture, and six worms (total wet wt ~3 g) were placed in containers with 750 to 900 g of medium. Only two soil–sediment mixtures were tested, with five exposure periods (1, 3, 7, 14, and 21 d). Three worms from each container were frozen for PAH analysis, and four groups of three worms were used for the test of validity.

To test the validity of *E. andrei* as a model for other species, a similar but limited set of assays was performed with *Lumbricus rubellus*, a species that occurs in a range of Dutch field soils. Adult or subadult specimens were obtained from a low-intensity culture, and six worms (total wet wt ~3 g) were placed in containers with 750 to 900 g of medium. Only two soil–sediment mixtures were tested, with five exposure periods (1, 3, 7, 14, and 21 d). Three worms from each container were frozen for PAH analysis, and four groups of three worms were used for the test of validity.

**Data analysis**

Apparent sorption coefficients (*K*<sub>oc-app</sub>) were calculated from PAH concentrations in the solid phase (*C*<sub>s</sub>) and the total PAH concentration in pore water (*C*<sub>p-tot</sub>) normalized to the fraction of organic carbon (*f*<sub>oc</sub>) in the solid test medium:

\[
K_{oc-app} = \frac{C_s}{C_{p-tot} f_{oc}} \text{ (L/kg oc)}
\] (1)

In case of “significant accumulation” (body residues at least 2 standard deviations above the level in the culture and above the detection limit), the measured body residues in earthworms (*C*<sub>W</sub>) were fitted with a standard one-compartment model:

\[
C_w(t) = C_w(0)e^{-kt} + C_w(\infty)(1 - e^{-kt}) \text{ (μg/kg wet wt)}
\] (2)

resulting in estimates of the steady-state concentration, *C*<sub>W(∞)</sub>, and the overall elimination rate constant (*k*, 1/d). Note that the calculation of the elimination rate from an accumulation experiment is valid only when the bioavailable exposure concentration is constant during the bioassay. The *C*<sub>W(0)</sub> is the initial concentration in the organisms from the culture, which was close to the detection limit for all PAHs. The initial concentration was fitted on the data unless negative or unrealistically high values were obtained; in that case, *C*<sub>W(0)</sub> was fixed to the initial value measured in the worms. Regression analyses were performed with the software package Graphpad Prism® 2.01 (San Diego, CA, USA).

The BSAFs were calculated from the steady-state levels in earthworm, normalized to lipids (*F*<sub>lip</sub>, taken as 1% on wet-weight basis [4]), and the total soil concentrations, normalized to the organic carbon fraction in the soil (*f*<sub>oc</sub>):

\[
BSAF = \frac{C_w(\infty) f_{lip}}{C_{lip}} \text{ (kg oc/kg lipid)}
\] (3)

Steady-state body residues were also converted to bioconcentration factors (BCF) on a soil-solution basis by using both measured and estimated pore-water concentrations (*C*:<sub>p</sub>):

\[
BCF = \frac{C_w(\infty)}{C_p} \text{ (L water/kg wet wt)}
\] (4)

**Correlations and differences between sites and PAHs**

Pearson correlation coefficients were calculated between the estimates of *K*<sub>oc-app</sub>, BSAF, and *k*. Furthermore, these parameters were correlated to *K*<sub>s</sub> and soil properties (after log transformation using Graphpad Prism 2.01). Only significant correlations (*p* < 0.05) are reported.

Not all parameters could be determined for all PAHs in all soils. The missing data hinder a proper comparison between the different PAHs and between mixtures and soils from different sites. To obtain a stronger data set, five PAHs were selected for which most parameters could be determined at most sites (see Table 2): benzo[a]anthracene, chrysene, benzo[k]fluoranthene, benzo[k]fluoranthene, and benzo[a]pyrene. For these PAHs, completely filled matrices of PAH × site could be obtained for *K*<sub>oc-app</sub>, BSAF, and *k*. These data matrices were log transformed and analyzed with analysis of variance ([ANOVA], one-way, repeated measures) using Graphpad Prism 2.01 to test for differences between sites and between PAHs.

**RESULTS AND DISCUSSION**

The ranges of the properties of the soils and soil–sediment mixtures are shown in Table 1. Total PAH levels were hardly
influenced by the addition of sediment; the ΣPAHs in the mixtures was between a factor of 0.4 and 1.4 of the level in the original soil. Only in the least contaminated soil (S2) was a decrease in PAH levels observed after adding sediment (a factor of 10–18 on ΣPAHs). The individual PAHs included in this study are shown in Table 2 along with their concentration range in soil. Even though the total level of PAHs varies between soils by two orders of magnitude (Table 1), the proportion of individual PAHs is quite similar in all soils (Table 2). This indicates that the contamination of these soils is likely due to non-point source contamination, without substantial impact from specific local sources. Table 2 also shows the number of steady-state concentrations and elimination rates determined from the earthworm bioassays and the number of K_{oc-app} values derived.

**Sorption**

The apparent sorption coefficient (K_{oc-app}, Eqn. 1) was calculated after normalization with respect to the OC content in each soil. No difference was observed in K_{oc-app} values that could be determined in soil–sediment mixtures and soils alone. However, the low-molecular-weight PAHs (naphthalene and fluorene) were not detected in pore water from most terrestrial soils. As the total levels of PAHs did not differ between soils and mixtures, it is conceivable that PAHs and mixtures, it is conceivable that soils. As the total levels of PAHs did not differ between soils and mixtures, it is conceivable that the true sorption coefficient (K_{oc-free}) and the fraction of the chemical that is freely dissolved in the soil solution determine the measured apparent sorption coefficient (K_{oc-app}). The dissolved fraction can be calculated from the concentration of DOC ([DOC] in kg/L) and the partition coefficient with DOC (K_{doc} in L/kg), leading to [16]:

\[
K_{oc-app} = \frac{K_{oc-free}}{1 + [DOC]K_{doc}} \quad \text{(L/kg oc)}
\]

Only for benzo[a]pyrene (BaP), the freely dissolved concentration was directly measured using an experimental method (see Materials and Methods). The method was not extensively validated yet, so results must be interpreted with care. The measured K_{oc-free} for this compound is also shown in Figure 1. The average value corresponds quite nicely to the QSAR of Gerstl [15], although the variation is still considerable. Using Equation 6, K_{doc} can be calculated for BaP. The resulting K_{doc} was on average a factor of three lower than K_{oc-free}, which is well in line with the factor of two found for humic acid [16]. The total deviation was almost a factor of 10 around this value. This deviation is caused by differences in organic matter composition but may also reflect technical difficulties in determining freely dissolved fractions [17]. It is expected that K_{doc} is related to the hydrophobicity of the compound [17], which allows for a prediction of K_{oc-app} for PAHs, based on the following assumptions. First, the QSAR of Equation 5 is a valid estimation of K_{oc-free}. This assumption is supported by the correspondence of the QSAR with K_{oc-app} for the less hydrophobic PAHs and the measured K_{oc-free} for BaP. Second, K_{doc} is a factor of three lower than K_{oc-free} for all PAHs, and, finally, we assume an average DOC concentration of 100 mg/L in all soils (see Table 1).

With these assumptions, the trend in the data is well described (solid line in Fig. 1), indicating that sorption to DOC can indeed explain the loss of linearity in Figure 1. The observed variation in K_{doc} for BaP and the DOC content between soils, is largely sufficient to cover the observed variation in this figure. However, several of the individual K_{oc-app} and K_{oc-free} values exceed the line of the QSAR by Gerstl [15] (indicated by the standard deviations exceeding the broken line in Fig. 1). These values must represent higher values of K_{oc-free} than predicted, indicating that not all variation between soils is explained by the action of DOC. As stated in the introduction, several processes are known to influence the observed sorption and partitioning of PAHs in soils.

**Biota-soil accumulation factors**

The one-compartment model of Equation 2 agreed reasonably well with most of the data, although the variation in

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**Fig. 1.** Measured apparent sorption data (K_{oc-app}, geometric mean and standard deviation) in relation to several model predictions. Furthermore, K_{oc} based on measured freely dissolved concentrations of benzo[a]pyrene is shown. ○ apparent sorption; ■ BaP freely dissolved; ● = predicted [13]; — = predicted [15]; — = predicted ([15] + DOC); OC = organic carbon; DOC = dissolved organic carbon.
goodness of fit was considerable. Most $r^2$ values ranged from 0.80 to 0.98, but several fits were poor ($r^2 \approx 0.50$). Several typical examples of accumulation curves are shown in Figure 2. Most data showed a smooth increase in time, but several PAH-soil combinations showed a very rapid steady state (within 1 d), precluding a firm estimation of $k$. This pattern was most clear for dibenzo[a,h]anthracene and benzo[qhi]pyrene, the most hydrophobic PAHs examined. It is unlikely that true equilibrium is reached so rapidly for these PAHs, and this pattern may therefore be related to an experimental artifact. This could be depletion of the bioavailable phase, although the BSAFs for these PAHs are not particularly low when a rapid steady state is evident.

A few curves showed the distinct pattern of an apparent maximum in the body residues. This behavior was found in only three soil–sediment mixtures (M4, M5, and M7) and was limited to one or more of the lighter PAHs (anthracene, fluoranthene, and pyrene). In the few cases where this pattern or a rapid steady state was observed, only the estimated BSAF derived from the one-compartment model was used. The same pattern of an apparent maximum and subsequent decrease in the accumulation was previously observed in studies of PAHs spiked into soils [7,18]. A likely cause for this pattern is a substantial decrease in bioavailability during the bioassay, possibly mediated by biodegradation in pore water [7], or resulting from a very rapid aging or sequestration of PAHs in soil [8] during the course of exposure, thus decreasing the concentration of PAHs in the soil solution. Induced biotransformation could also lead to such a peak. Although earthworms are able to transform pyrene, the amount of metabolites formed is very low [7], and the P450 system is apparently not induced by exposure to PAHs [19]. Furthermore, if biotransformation in the earthworm is the cause of this result, the pattern is expected to be evident in more than just a few soils.

Overall, soil–sediment mixtures yielded similar BSAFs as soils alone, but on careful observation, a difference can be observed. For the low-molecular-weight PAHs (up to pyrene in Table 2), few BSAFs could be calculated, and most of them are for soil–sediment mixtures. In fact, only one soil yielded significant body residues in the earthworms (S6, which has the highest level of PAHs and the lowest $f_{oc}$; see Table 1). The BSAFs for soil-only and soil–sediment mixtures are shown in Figure 3A and B, respectively. As little difference was observed in total levels between soils and mixtures, it appears that the low-molecular-weight PAHs are hardly available for uptake in the soils but are more bioavailable in the sediments.

The calculated BSAFs are quite variable and show little relationship with $K_{ow}$ (Fig. 3A and B). The BSAFs are on average 0.23 kg$_{oc}$/kg$_{lip}$, but the variation is large. The lack of influence of hydrophobicity on BSAF is to be expected, as both sorption and accumulation from pore-water increase with increasing $K_{ow}$. The EP estimate of BSAF in Figure 3 is taken as the BCF from an equilibrium partitioning estimate [4] divided by the QSAR from Equation 5.

The observed BSAFs are much lower than the EP estimate (up to two orders of magnitude) and also lower than the max-
imium observed in spiked artificial soil medium [7] (values of 2–8 kg oc/kg lip), although body residues in artificial soil decreased after reaching this maximum. The large variation in BSAFs may partly reflect sequestration in these field-polluted media; however, in the previous section we argued that Equation 5 was likely to be a reasonable estimate of the average \(K_{oc-free}\) in our soils. These consistently lower BSAFs must therefore also have other explanations.

Generally, the experimental BSAFs are quite comparable to similar bioassays with Lumbricus terrestris [20] (BSAFs on average between 0.13 and 0.41 for PAHs, with large variation between sites). Ma et al. [21] reported BSAFs for PAHs in Lumbricus rubellus sampled from floodplain sites. Their BSAFs also show a substantial variation between sites, but their values are on average a factor of four lower than the present results. However, the actual concentration that these field-collected earthworms had been exposed to is not easily reconstructed (the soil concentration was taken as a bulk sample from the top 20 cm).

**BCFs**

The BCFs (Eqn. 4) calculated on the basis of the measured total concentrations in pore water were low (around 50 L/kg wet wt) and showed little variation between the different PAHs. This effect was predicted earlier [4] in case BCF is estimated on the basis of the total concentration in pore water (thus including DOC). This supports the findings by other authors who claim that only the freely dissolved concentration in pore water is available for uptake by organisms [22,23]. The BCFs on the basis of estimated freely dissolved concentrations (using Eqn. 5 as estimate of \(K_{oc-free}\)) show a linear increase with \(K_{ow}\) as expected from the theoretical relationship (Fig. 3C). The data are generally lower than the theoretical estimate (on average a factor of 11), which is consistent with the low BSAFs, discussed in the previous section. This seems to be a general trend, as BCFs for earthworms tested in a soil medium are on average a factor of six lower than expected on the basis of EP theory [4].

The BCF of BaP can also be expressed on the basis of the measured freely dissolved concentration in pore water. It is interesting to see that this BCF is similar to the data based on the QSAR for \(K_{oc-free}\) and that they have a similar variation. Although these measurements must be interpreted carefully, it appears that free concentrations do not fully determine BCFs. It is possible that the free pool is partially depleted by the earthworms and that differences between soils (e.g., the rate of desorption) determine the final body residues. A simple mass-balance calculation for BaP shows that the amount of chemical accumulated in earthworms during the bioassay exceeds the amount freely dissolved in pore water by an order of magnitude on average. This implies that the freely dissolved pool of PAHs in soil solution is concurrently being partially depleted and replenished before a steady state is achieved. The total amount of BaP in pore water (including DOC) is in the same order of magnitude as the amount accumulated by the worms. Therefore, it is likely that the kinetics of depletion of pore-water PAHs and the kinetics of their replenishment have influenced the accumulation patterns and the steady-state body residues.

The calculation of a BCF implies that pore water is considered to be the primary bioavailable phase for earthworms. The earthworms were feeding on soil, too, and a large part of the body burden may actually be derived from the gut contents. Nevertheless, we believe the focus on exposure via pore water to be appropriate, as body residues were in virtually all cases lower than expected on the basis of EP. Furthermore, uptake from the gut contents is not fundamentally different from uptake through the external skin, as both are likely mediated through a dissolved phase and driven by passive diffusion [24]. However, processes in the gut may release residual PAH fractions that are otherwise not participating in EP. Thus, equilibrium partitioning can be considered to estimate the maximum amount that can be taken up, but the total variation in body residues and uptake kinetics may be driven by differences in assimilation efficiencies between soils as well as differences in desorption kinetics of PAHs from soils.

**Elimination rate constants**

An estimate of the elimination rate constant (\(k\)) follows from fitting Equation 2 to the accumulation data. The rate constant was generally poorly identified by the data, and these results must therefore be interpreted with care. Even though the relationship between log \(k\) and log \(K_{ow}\) is significant \((p < 0.05)\), the slope of the regression is only –0.16 (Fig. 4). These findings are in contrast with those of Belfroid et al. [25], who derived a slope of –0.66 for PCBs and chlorobenzenes. Our \(k\) values are in fact quite similar for all PAHs (95% are within 0.72/d ± a factor of three). The rate constants for fluoranthene and pyrene are in the same range as values reported from earthworms in artificial soil (Paris, France) [7], but the values for benzo[a]pyrene are generally larger in the present study. It is, however, very well possible that these apparent elimi-
nation rate constants are artifacts. As discussed in the previous section, desorption of PAHs from organic carbon phases is necessary to establish the observed body residues in the worms. If these desorption rates are relatively slow, Equation 2 is not a valid description of the accumulation process anymore, as the exposure concentration (the dissolved pool) is not constant. Any \( k \) estimate will then be a compound parameter, including the kinetics of depletion and replenishment of the freely dissolved pool. Furthermore, the estimate of \( k \) will include uptake from the gut as well as across the skin.

Correlations and differences between PAHs and between sites

Correlations for the complete data set are listed in Table 3. Because so many significant correlations exist between the estimated parameters and with the soil properties (which are also correlated among themselves), it is difficult to make inferences about causal relationships. The BSAF and \( k \) are negatively correlated, which implies that when a rapid steady state is achieved, the BSAF is lower than expected on the basis of EP. This finding is consistent with the hypothesis of partial depletion and replenishment. The BSAF correlates negatively to \( K_{oc-app} \) indicating low total pore-water levels when BSAF is low. This correlation supports the assumption that uptake is driven by the pore-water concentration, but care must be taken, as \( K_{oc-app} \) is not necessarily representative for freely dissolved concentrations.

The correlations with \( K_{ow} \) are significant but not very large. The correlations with the soil properties are difficult to interpret, although a few conclusions seem justified. The large differences in \( K_{oc-app} \) between sites (Fig. 1) may be related to differences in the quality of the soil organic matter between sites (and thus differences in \( K_{oc-free} \)), but they may also reflect differences in DOC. The correlation with the quantity of DOC is not significant, but the apparent sorption may be determined largely by DOC composition (which is also indicated by the correlation with pH) [17,22,23]. This in turn would help explain the correlation between apparent sorption and the PAH concentration in soil. The DOC-bound chemicals are potentially available for leaching, and it is conceivable that a low value of \( K_{oc-app} \) at a site may contribute to leaching of PAHs from the top soil layer. The highest BSAFs are found in soils with a high organic matter and clay content, a low pH, and low PAH levels. Although these correlations may be practical for predicting potential problems in the field, it is likely that the causal relationship is through the action of these properties on PAH sorption.

The differences between PAHs and between sites are calculated using data for five PAHs only, as explained in Materials and Methods. Even though the selected PAHs have very similar \( K_{ow} \) values (5.80–6.04), significant (ANOVA, \( p < 0.0001 \)) differences exist between them in \( K_{oc-app} \), BSAF, and \( k \). This shows that the behavior of the PAHs is determined not only by their hydrophobicity. The mean difference between the PAHs is, however, not very large (less than a factor of seven for \( K_{oc-app} \) and BSAF and less than a factor of two for \( k \)). Significant (ANOVA, \( p < 0.0001 \)) differences also exist in \( K_{oc-app} \), BSAF, and \( k \) between the sites for the five selected PAHs. The mean differences are much larger than between PAHs (~ a factor of 30 for \( K_{oc-app} \) and BSAF and a factor of nine for \( k \)), indicating that \( f_{oc} \) is not the only soil property governing sorption and bioaccumulation. No clear difference was observed between soil–sediment mixtures and soils alone and no new relations with soil properties (the correlations are similar to those shown in Table 3).

Pilot study with L. rubellus

A pilot study was performed with two soil–sediment mixtures (M3 and M15) and a different species of earthworm: L.

![Fig. 4. The rate constant for elimination (k) as estimated from the accumulation bioassays, shown against log K_{ow}.](image)

Table 3. Significant correlations (\( p < 0.05 \)) for the estimated sorption (\( K_{oc-app} \)), biota-soil accumulation factor (BSAF), and elimination rates (\( k \)) after log transformation (NS = not significant). Also correlations between these parameters and hydrophobicity (\( K_{ow} \)), fraction organic carbon (\( f_{oc} \)), clay content, pH, dissolved organic carbon (DOC), concentration in soil of individual compounds (\( C_i \)) and the total level of polycyclic aromatic hydrocarbons (\( \Sigma PAHs \)).

<table>
<thead>
<tr>
<th>( K_{oc-app} )</th>
<th>BSAF</th>
<th>( k )</th>
<th>( K_{ow} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_{oc-app} )</td>
<td>1</td>
<td></td>
<td>0.31</td>
</tr>
<tr>
<td>BSAF</td>
<td>−0.53</td>
<td>1</td>
<td>0.33</td>
</tr>
<tr>
<td>( k )</td>
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<td>−0.48</td>
<td>1</td>
</tr>
<tr>
<td>( K_{oc-app} )</td>
<td></td>
<td></td>
<td>−0.24</td>
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<tr>
<td>BSAF</td>
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<tr>
<td>( f_{oc} )</td>
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<tr>
<td>Clay</td>
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<td>pH</td>
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<td>DOC</td>
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<td></td>
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<tr>
<td>( C_i )</td>
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<td></td>
<td></td>
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<tr>
<td>( \Sigma PAHs )</td>
<td>0.46</td>
<td>−0.61</td>
<td>NS</td>
</tr>
</tbody>
</table>

Other correlations

- \( f_{oc} \) and clay correlated 0.70
- pH and DOC correlated −0.69
- Correlation to pH 0.62
rubellus. In contrast to E. andrei, this species is common in Dutch field soils. As shown in Figure 5, the steady-state body residues are clearly correlated between both species \( (r = 0.91) \), but body residues of PAHs in L. rubellus are on average a factor of two lower than in E. andrei. It is unclear what has caused the difference between these species, but differences in lipid content (not measured) or in body size may contribute.

The weight ratio of worm:soil was nearly similar for both species, but the individuals of L. rubellus were much larger, causing differences in uptake kinetics (through the ratio of the surface area to body volume). Especially deviating is a value for benzo[ghi]perylene, where levels in L. rubellus were 17 times lower. For this compound, a rapid steady state was in many cases observed for E. andrei (also in the same soil), so accumulation of this compound may be especially influenced by kinetic constraints.

**CONCLUSIONS**

In this study, bioassays were performed with the earthworm E. andrei in seven soils and 15 soil–sediment mixtures. The soil–sediment mixtures tested do not apparently differ in sorption or accumulation of the high-molecular-weight PAHs from the terrestrial soils tested. However, the low-molecular-weight PAHs (up to pyrene) have a very low bioavailability in soil but are more readily taken up from the soil–sediment mixtures. In several of the mixtures, bioavailability was already declining during the course of the bioaccumulation experiment. Risk assessment for dredged materials thus has to be aware that the low-molecular-weight PAHs can be more bioavailable than in the terrestrial soil found on-site directly after depositing dredge spoil.

Total levels of PAHs in pore water were higher than predicted, but most of the dissolved compound is associated with DOC. The current data set supports earlier assumptions that DOC-bound chemicals are not directly available for uptake by earthworms. Prediction of \( K_{\infty,\text{free}} \) can be done on the basis of Equation 5 and is probably accurate within an order of magnitude. In most soils, the earthworms do not reach the body residue expected by EP (on average, one order of magnitude lower). Furthermore, the variability is very high for BSAF in these field-contaminated soils, which points at large differences in bioavailability that are not fully explained by differences in sorption. Even though E. andrei is not a typical soil species, a pilot study with L. rubellus indicates that the two species are well correlated with regard to steady-state body residues. The different PAHs are quite similar in their behavior, although between PAHs with similar \( K_{\infty,\text{app}} \), consistent differences exist up to a factor of seven in BSAF and \( K_{\infty,\text{app}} \). The differences between sites are larger and are consistent for all PAHs. Soils with a high content of clay and organic carbon and a low pH and PAH level lead to higher BSAFs (closer to EP predictions).

Indications exist that the freely dissolved pool of PAHs in soil pore water is partially depleted and that bioaccumulation is influenced by the kinetics of PAH desorption and mass transport in soil, and equilibrium partitioning is not achieved. Similar conclusions were drawn for biodegradation of PAHs in soils [9] and accumulation in sediment amphipods [26]. It is therefore likely that the apparent elimination rate constant also reflects these processes and that Equation 2 is not a valid representation of bioaccumulation. Furthermore, differences in feeding rates and assimilation efficiencies may have contributed to the total variation between soils. Nevertheless, risk assessment for PAHs in soils and soil–sediment mixtures may rely on QSARs for \( K_{\infty} \) [15] and BCF [4]. Combined with the \( f_{\infty} \) of the site and the total content of PAHs, this generally seems to provide a worst-case estimate of body residues in earthworms. Nevertheless, the applicability of this approach is limited, as body residues in bioassays may be overestimated by two orders of magnitude (although this does not necessarily reflect levels in earthworms under field conditions). For a better explanation of the toxicokinetics, methods need to be applied that measure or estimate the freely dissolved concentration in pore water with greater precision, but this alone is not sufficient to predict bioavailability of PAHs. Bioavailability of PAHs appears to be a highly dynamic problem that additionally requires quantification of the kinetics of desorption and mass transfer as well as the influence of uptake from the gut contents.

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