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Predicting PAH Bioaccumulation and Toxicity in Earthworms Exposed to Manufactured Gas Plant Soils with Solid-Phase Microextraction

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Soils from former manufactured gas plant (MGP) sites are often heavily contaminated with polycyclic aromatic hydrocarbons (PAHs). Current risk assessment methods that rely on total PAH concentrations likely overstate adverse effects of such soils since bioavailability is ignored. In this study, solid-phase microextraction (SPME) was applied to estimate bioavailable PAH concentrations and toxicity in earthworms exposed to 15 MGP soils. In addition, PAH sorption to all soils (K_{oc} values) was determined. The results showed a several orders of magnitude variation in K_{oc} values, demonstrating that generic organic carbon-normalized sorption coefficients will typically be overconservative at MGP sites. SPME-predicted bioaccumulation generally was within a factor of 10 of measured bioaccumulation (in earthworm bioassays), in contrast to current risk assessment model estimates that overpredicted bioaccumulation 10−10 000 times. Furthermore, on the basis of estimated total body residues of narcotic PAHs, SPME correctly predicted worm mortality observed during bioassays in the majority of cases. For MGP sites where current risk assessment procedures indicate concerns, SPME thus provides a useful tool for performing a refined, site-specific assessment.

Introduction

In most countries, current risk assessment procedures for hydrophobic organic chemicals in soils and sediments include the quantification of total solid-phase associated concentrations of the chemicals of interest and testing of the results against generic, fixed risk screening values (*1*). Recent studies have demonstrated that this type of assessment may lead to unnecessary remediation, as existing screening values often do not reflect actual risks (*2*−*4*). Depending on the site under investigation, actual ecotoxicological and leaching risks can be up to 3 orders of magnitude lower than anticipated (*3, 4*). The most probable factor causing this discrepancy is the presence of hard carbon materials (e.g., soot, coal, and char), to which hydrophobic organic chemicals, and in particular polycyclic aromatic hydrocarbons (PAHs), are much more strongly bound than to regular soft natural organic matter (i.e., the only phase that is taken into account within current risk assessment) (*3, 4*). Soils and sediments from former manufactured gas plant (MGP) sites often contain high concentrations of PAHs, which were coproduced along with waste (semi-)solid pyrolysis residues (soot and pitch) during the cracking of coal and oil in gas production. The combination of high PAH concentrations and large fractions of these pyrolysis-derived hard carbon materials should be considered as a clear indication of likely enhanced sorption/reduced desorption and thus of reduced risks posed by PAHs. Therefore, the results from prevailing risk assessment procedures applied to MGP sites are questionable.

To improve risk assessment of organic contaminants in soils and sediments, the current generic approach should be replaced with a site-specific alternative, which takes into account any characteristics that influence actual local risks. Site-specific risk assessment should preferably be based on bioassays, as these biological tests incorporate all factors affecting final contaminant levels in organisms. However, bioassays generally are laborious, expensive, and need culturing of test organisms, which may be problematic. In response, several analytical methods have been developed during the past few years that might serve as alternatives for bioassays. These methods try to easily and cost-effectively estimate either the actual chemical concentrations in organisms or the pool of solid-phase associated contaminants that is potentially available for uptake by biota. The most important examples of such bioavailability methods are semipermeable membrane devices (SPMDs) (*5*), solid-phase microextraction (SPME) (*6, 7*), extraction with Tenax (*8*) or cyclodextrin (*9*), and supercritical carbon dioxide extraction (SFE) (*10, 11*).

This paper focuses on PAH bioavailability in 15 MGP soil samples that previously had been subject to thorough chemical characterization (*10*). The sample set consisted of soils with Σ(16 EPA) PAH concentrations between 150 and 40 000 mg/kg and organic carbon contents ranging from 2 to 87%. The set therefore represents a very complex yet interesting test case for the predictive power of chemical methods that aim to assess PAH bioavailability. We applied SPME to measure concentrations of PAHs in soil interstitial water, which were subsequently utilized to (i) determine soil organic carbon−water distribution coefficients (K_{oc} values), (ii) estimate bioaccumulation of PAHs in earthworms, and (iii) assess toxicity to the worms upon exposure to the soils. The last two estimates were related to results from bioassays with the earthworm *Eisenia fetida* that had previously been performed on the same soils (*12*).

Materials and Methods

Chemicals and SPME Fibers. The chemicals used were methanol and acetonitrile (HPLC grade; Lab-Scan, Dublin, Ireland), 2-methylchrysene (99.2%; Community Bureau of Reference, Geel, Belgium), sodium azide (extra pure; Merck, Darmstadt, Germany), and calcium chloride-dihydrate (analytical grade; Merck). The polydimethylsiloxane (PDMS)-coated disposable SPME fiber (glass fiber core diameter 110 μm, PDMS coating thickness 28.5 μm) was obtained from Poly Micro Industries (Phoenix, AZ). Prior to use, the fiber was cut into 5 cm pieces, which were cleaned by thermal desorption at 275 °C for 16 h under a constant flow of helium.

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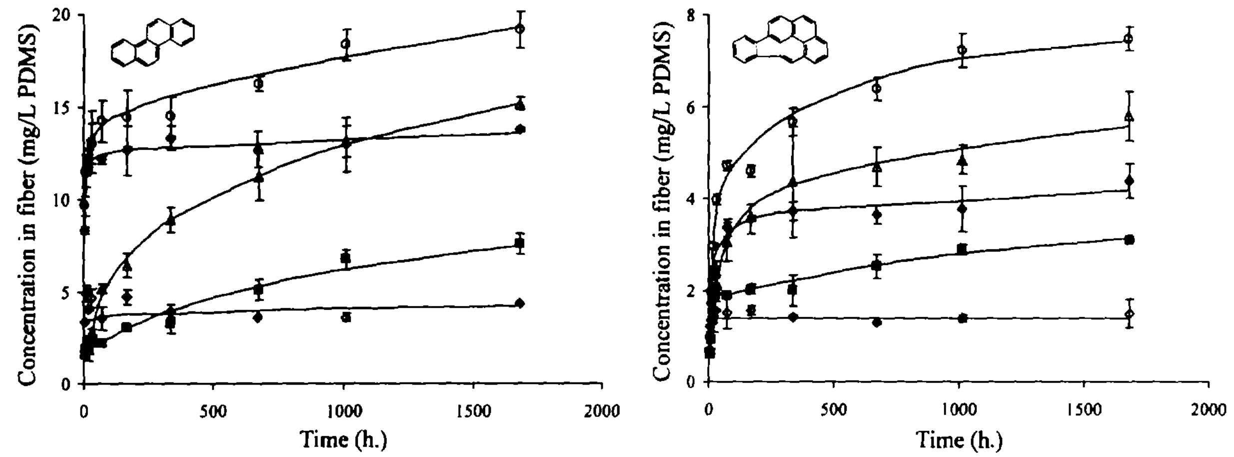

FIGURE 1. Concentration of chrysene and indeno[1,2,3-*cd*]pyrene in SPME fibers (mg/L PDMS) as a function of equilibration time (h) in slurries of OG2 (open diamonds), OG10 (closed diamonds), OG14 (closed squares), CG2 (open circles), and CG11 (open triangles) soils. To be able to optimally present all data in one graph, chrysene concentrations for OG10 and CG2 were divided by 3, and concentrations for OG2 and OG14 were multiplied by 5. Indeno[1,2,3-*cd*]pyrene concentrations for OG10 were divided by 5. Lines serve to guide the eye. Error bars represent standard deviations.

Samples. The 15 soil samples that were investigated in the present study had previously been collected from abandoned MGP sites. They were handled as described in ref *10* and stored air-dried at 4 °C. Soils were coded either as OG or CG, to indicate if they originated from oil or coal-fired gas plant sites, respectively. They were characterized in terms of elemental composition, organic carbon content, thermal gravimetric behavior, PAH concentrations, and SFE and water/XAD desorption, as reported in ref *10*. Furthermore, bioassays with earthworms (*E. fetida*) were performed as described in ref *12* to determine survival and bioaccumulation of PAHs upon a 14 day exposure to the soils (which resulted in equilibrium concentrations as evidenced by additional 28 day measurements (*12*)). To further characterize the soils, the so-called soot carbon fraction (i.e., the organic carbon fraction resisting thermal oxidation at 375 °C (*13*)) was determined in the present study.

PAH Concentration Measurements in Interstitial Water. Concentrations of PAHs in soil interstitial water were measured according to a slightly modified version of the methodology described by Ter Laak et al. (*14*). Amber-colored 7 mL glass vials (Supelco, Bellefonte, PA) were filled with either 1 g (soils OG2 and OG10) or 2 g (other samples) of soil, 5 mL of an aqueous solution containing 0.01 M calcium chloride and 25 mg/L sodium azide in Millipore water, and one or two 5 cm long SPME fibers (soil and fiber amount adjusted to remaining vial volume and expected concentrations in fibers, respectively). Before adding the fibers, vials were manually shaken to produce homogeneous suspensions. Vials were closed with aluminum-lined caps and put on a "Rock and Roller" apparatus (Snijders Scientific, Tilburg, The Netherlands) that caused gentle agitation of the soil–water–fiber system.

Two successive experiments were performed. The objective of the first experiment (PAH uptake kinetics in fibers) was to find a single, fixed equilibration time to be applied in the second experiment. To this end, systems were equilibrated for either 4, 8, 16, 30, 72, 168, 336, 672, 1008, or 1680 h. This experiment was conducted in triplicate for five soils only (OG2, OG10, OG14, CG2, and CG11). These soils were selected on the basis of their molar C/H ratio, total PAH concentration, and organic carbon content, to represent a sample set comprising the widest range in these characteristics. From this first experiment, 672 h was selected as the fixed equilibration time (see Results and Discussion), which was then applied in the second experiment, in which fibers were exposed in quadruplicate to all 15 soils.

Upon finishing all exposures, fibers were collected from the vials, wiped with wet tissue to remove any attached particles, cut into 1.25 cm length pieces, and placed in amber-colored HPLC vials filled with 0.2 (in a 250 *μ*L insert), 1.0, or 1.5 mL of acetonitrile (volume fit to expected concentrations). Finally, the internal standard (2-methylchrysene) was added, and the vials were vortexed for 1 min and stored at −20 °C. Prior to analysis, the vials were defrosted, vortexed again, and left at room temperature for 1 day. Extraction efficiencies were previously determined to be 99.6 ± 0.1% (*15*).

Instrumental Analysis. Concentrations of 13 PAHs (phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*e*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene, dibenz[*a,h*]anthracene, and indeno[1,2,3-*cd*]pyrene) in the fiber extracts were determined using a HPLC system consisting of a Varian Prostar 420 autosampler, a Gynkotec P580HPG pump, a Jasco FP-920 fluorescence detector, and a Vydac 201TP54 C_{18} column (kept at 25.0 °C). The mobile phase consisted of a mixture and flow gradient of methanol and water, which were degassed with a Shimadzu DGU-14A degasser.

Data Analysis. PAH concentrations in fibers (C_f) resulting from the second experiment were translated into freely dissolved PAH concentrations in interstitial water (C_w), using previously determined fiber–water partitioning coefficients (K_f values; see Table S3) and the equation $C_w = C_f/K_f$. The resulting C_w values were averaged for quadruplicate measurements (averaged relative standard error was 6.5%), and the mean concentrations served as a basis for the determination of soil–water distribution coefficients and the prediction of bioaccumulation and toxicity.

Results and Discussion

PAH Uptake Kinetics in Fibers. In Figure 1, results from the kinetics experiment are presented for a representative 4- and 6-ring PAH (chrysene and indeno[1,2,3-*cd*]pyrene, respectively). Data shown are concentrations in SPME fibers as a function of time (4 h up to 10 weeks). From this figure, it appears that the systems created from the five soils behaved kinetically differently. Systems containing the soils OG2 and OG10 reached equilibrium within 1–2 weeks, whereas for those with soils OG14, CG2, and in particular CG11, PAH concentrations in the fibers still increased after 6–10 weeks. Assuming that all fibers acted identically in all cases, the observed different uptake profiles must be ascribed to differences in soil behavior. The soils behaving differently is not surprising, as the five soils for which the uptake was studied were selected from the overall sample set to represent a broad range in soil characteristics (molar C/H ratio, total PAH concentration, and organic carbon content). The difference indicates that not the uptake into fibers but PAH desorption from the soils was the rate-limiting step in these systems. Obviously, PAH desorption from soils OG14 and CG2 was slow, and from CG11, it was very slow (systems with these soils are still not at equilibrium after a prolonged exposure time). The observed result for CG11 is consistent with SFE/XAD desorption kinetic measurements reported in ref *10*. It should be noted, though, that the present experiments only provide an indication of desorption and certainly cannot be considered as desorption kinetics measurements. After all, the extraction phase applied here does not behave like a fast infinite sink for PAH removal, such as Tenax or XAD. The slow/very slow desorption from the soils most probably is related to the PAH source (*16*). Still, no strong correlations were observed between the fast desorbing fraction (*F*) and soil characteristics, such as molar C/H ratio and organic matter and carbon content (*10*).

On the basis of Figure 1, it is difficult to define a universal equilibration time. The application of such a time is desirable, however, because it is experimentally unfeasible to perform uptake kinetics measurements for every single soil to be tested. Hence, we selected 4 weeks (672 h) as a standard exposure time for the current setup (i.e., gently rocking and rolling fibers in 7 mL vials filled with soil slurry). This selection is based on the following considerations: (i) systems created from at least two out of the five soils were at equilibrium within these 4 weeks; (ii) although PAH concentrations in fibers did increase after 4 weeks in soils OG14, CG2, and CG11, the increase was relatively small and proceeded very slowly. Note that slowly/very slowly desorbing chemical fractions in sediments and soils are generally considered nonbioavailable (*8*, *17*, *18*). Consequently, slow/very slow desorption should be excluded from SPME measurements that focus on bioavailability; (iii) for several field-contaminated sediments, 4 weeks also appeared to be sufficient for reaching equilibrium (Van der Heijden, S. A. and Jonker, M. T. O., unpublished data); and (iv) considering the extreme properties of some of the tested soils, four weeks will be sufficient for the majority of field-contaminated soils and sediments to reach near steady state.

Soil Organic Carbon–Water Distribution Coefficients (K_{oc}). Concentrations in interstitial water (C_w) resulting from the second experiment, in which all soils were equilibrated for the 4 week exposure time, were combined with concentrations in soils (C_s) and organic carbon fractions (f_{oc}), to obtain organic carbon-normalized soil–water distribution coefficients (K_{oc} values; $K_{oc} = C_s/(C_w f_{oc})$). The resulting averaged ($n = 4$) values for the 13 PAHs analyzed are presented in Table S1. This table shows that K_{oc} values vary between values similar to K_{ow} (i.e., values close or equal to those generally used in fate and effect modeling) and those ranging up to almost 3 orders of magnitude higher than K_{ow}. For dibenz[*a*,*h*]anthracene, log K_{oc} values of as high as 9.12 were observed in two soils. To our knowledge, such high values have not been reported before for soils, although similar values have been observed for sediments (*19*, *20*). In Figure 2, the variation in K_{oc} values for this set of soils is illustrated by plotting them against K_{ow} values. This figure clearly indicates that the generic K_{oc} values currently used for predicting environmental fate and effects of PAHs do not reflect real world partitioning behavior. This conclusion is not new (*3*, *4*); however, previous grounds to dispute the generic (low) K_{oc} concept generally were based on data for just a (very) limited number of chemicals and sediments or soils. In contrast, Table S1 presents a large data set (with all K_{oc} values measured by the same partitioning method), showing a very large variation among many soils for several chemicals. Only very recently, a similar variability in K_{oc} values was shown for sediments on the basis of an even larger set of samples and chemicals (*20*).

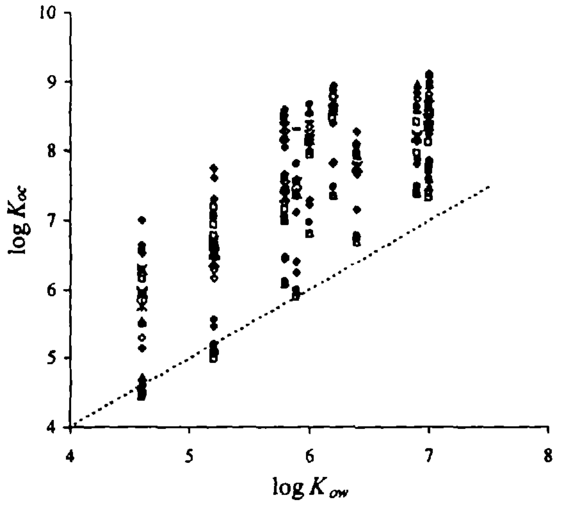

FIGURE 2. Log K_{oc} vs log K_{ow} values of 13 PAHs in 15 MGP soils (different soils are depicted by different markers). The dotted line represents the 1:1 relationship.

As the contact time between PAHs and soils in the field was comparable in all cases (i.e., several decades), the observed variation among K_{oc} values will most probably not be caused by slow sorption (*2*) but by a variation in the quality of the organic carbon instead (*3*, *21*). At former MGP sites, this variation in carbon quality will be due to the presence of natural organic carbon and/or multiple forms of anthropogenic carbon, such as soot, pitch, and coal tar. To investigate the cause of the variation in K_{oc} values in more detail, we performed linear regressions between soil–water distribution coefficients (K_d values) and different measures of organic carbon quality. These included the total organic carbon fraction (f_{oc}), the soot carbon fraction (f_{sc}), the soot carbon/total carbon ratio, and the molar C/H ratio (see Table S2). Additionally, regressions were performed with the soil C/N ratio, weathering index (i.e., the total concentration of the 2-ring PAHs divided by the total concentration of the 5- and 6-ring PAHs (*10*)), Σ16 EPA PAHs, and the amount of extractable hydrocarbons (see Table S2), as well as with the chemical properties log K_{ow}, total surface area (TSA), and molar volume (see Table S3).

As expected from the large variation in K_{oc} values, the *correlation between log K_d and total organic carbon fraction* proved to be very poor (r^2 values between 0.12 and 0.46 for individual chemicals; see Table S4). Similarly, for most of the other soil characteristics, correlation coefficients were low as well (averaged r^2 between 0.05 and 0.29). Only regressions with the molar C/H ratio resulted in a reasonable r^2 of 0.47 (averaged value for all chemicals; individual values between 0.29 for pyrene and 0.60 for benzo[*e*]pyrene; see Table S4). In contrast, correlations between log K_d values and chemical properties were highly significant (i.e., averaged r^2 values of 0.81, 0.85, and 0.89 were observed for correlations with log K_{ow}, TSA, and molar volume, respectively (see Table S5)). It should be noted, however, that regressions with chemical properties were performed for individual soils and thus do not explain any of the variation in log K_d values among soils. Still, 81% of this variation could be explained when combining soil and chemical properties and performing multiple

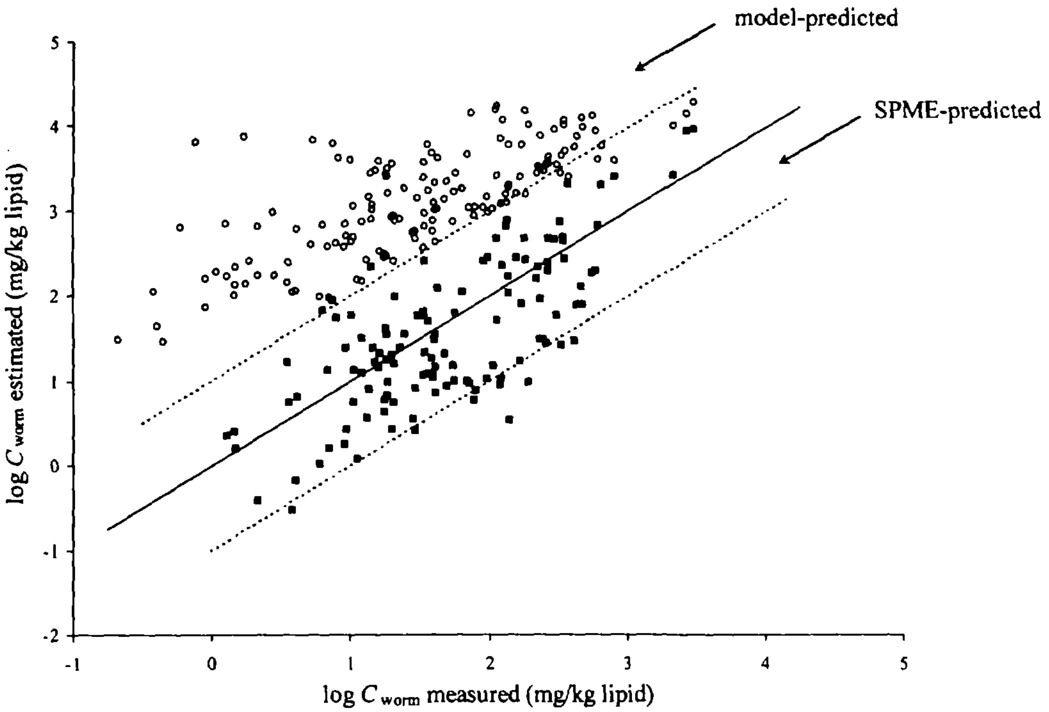

FIGURE 3. Predicted PAH concentrations in worms vs experimentally determined concentrations (both mg/kg lipid on a logarithmic scale). Solid squares represent concentrations predicted by SPME; open circles represent values predicted by current risk assessment methods (based on total soil concentrations and generic K_{oc} values, calculated from the one-parameter LFER for PAHs as reported by Nguyen et al. (35)). The solid line presents the 1:1 relationship; dotted lines give the one-order-of-magnitude deviation interval.

regression (using SAS for Windows; v 9.1.3). The resulting multiple linear regression model was as follows:

$$\log K_d = -1.98 + 0.03[\text{molar volume}] + 0.93[\text{C/H}] - 0.45[\text{weathering index}] - 10.3 f_{sc} \quad (1)$$

with an adjusted r^2 value of 0.81. The most important predictors are the first three independent variables (molar volume, molar C/H ratio, and weathering index), which together explained 78% of the variation in log K_d. Including a random coefficient accounting for a random soil effect on log K_d increased the r^2 to 0.95 (see Supporting Information Figures S1 and S2). In other words, there appears to be a random soil effect that explains an additional 14% of the variance in log K_d values. This effect is related to soil properties that were not measured, but it shows a strong covariance with PAH aqueous solubility (see Supporting Information). It should be noted that the previous linear regressions assume a linear sorption mechanism. For the hard carbon materials that constitute a significant fraction of the carbon pool in these soils (see Table S2), nonlinear adsorption is, however, believed to be the applicable mechanism (*3*). Nonlinear sorption can be accounted for by modeling K_d values by means of a dual domain model, combining linear absorption into natural organic carbon and nonlinear adsorption to hard (soot) carbon (*3*)

$$K_d = f_{aoc}K_{oc} + f_{sc}K_F C_w^{n-1} \quad (2)$$

with f_{aoc} being the amorphous, natural organic carbon fraction (i.e., $f_{oc} - f_{sc}$), K_F being the Freundlich sorption coefficient, and n being the Freundlich exponent accounting for nonlinearity. This model was solved by either assuming (*4*) or fitting K_F and n values (see Supporting Information), which resulted in r^2 values of 0.82 and 0.87, respectively. Inclusion of a random soil effect within the regression model yielded r^2 values of 0.93 and 0.94 for the models using assumed and fitted values for K_F and n, respectively (see Supporting Information). It is beyond the scope of the present paper to discuss these results in detail, but it is important to note is that all regression and modeling results indicate that f_{oc} alone has serious limitations for site-specific assessment of MGP soil–water distribution of PAHs: a conclusion previously also drawn for sediments and soot/soot-like materials (*20, 22, 23*). The present results demonstrate that additional or other soil properties are required to describe/predict sorption coefficients sufficiently accurately. These are properties somehow characterizing the fraction of hard carbon materials and might include the fraction obtained by thermal oxidation at 375 °C (f_{sc}), the C/H ratio, or the pitch fraction (*24*).

SPME-Predicted versus Measured PAH Bioaccumulation in Earthworms. Concentrations of individual PAHs in earthworm lipids (C_{worm}; mg/kg lipid) were estimated by combining SPME-derived freely dissolved aqueous concentrations with worm bioconcentration factors (BCF): $C_{worm} = C_w BCF$, with BCF values set to be equal to K_{ow} values (*25, 26*). The resulting C_{worm} data were then compared to experimentally determined lipid-normalized equilibrium concentrations in earthworms exposed to the soils (*12, 27*). In Figure 3, the predicted concentrations are plotted against measured ones (solid markers) for 11 PAHs (benzo[*b*]fluoranthene and benzo[*k*]fluoranthene were not included as these compounds were not chromatographically separated in refs *12* and *27*). The figure also includes a comparison between measured concentrations and concentrations predicted by using equilibrium partitioning theory and generic K_{oc} values (open markers) as a representation of current risk assessment model predictions. Although considerable scatter is evident, Figure 3 demonstrates that SPME is generally able to predict the bioaccumulation of individual PAHs in worms within a factor of 10. A factor of 10 may seem rather large, but it should be stressed that (i) current risk assessment methods would predict uptake from these soils as 10 to as much as 10 000 times too high (see Figure 3). Hence, the application of SPME provides a clear improvement of predicted risks over the current approach; (ii) predicted and measured PAH concentrations are based on chemical analyses performed in different labs by using different techniques (HPLC vs GC-MS); (iii) the results relate to a very complex sample set; and (iv) uptake of PAHs from water into worms was modeled by assuming that BCF values are equal to K_{ow} values, an assumption that may introduce a certain degree of uncertainty. Accurate determinations of BCF values for the species of interest could reduce such uncertainties. Considering the previous four issues, predictions of PAH body residues in worms within a factor of 10, as shown in Figure 3, are satisfactory.

Figure 3 additionally shows that all SPME-predicted data points (solid markers) are evenly distributed around the 1:1 line. Moreover, OG and CG samples appear to be distributed randomly, but PAH uptake is consistently underpredicted in some soils (i.e., OG2, CG11, CG15, and CG17) and overpredicted in soil CG2 (see Figure S5). However, there is no systematic, consistent over- or underprediction of specific (groups of) PAHs (see Figure S5). This suggests that SPME measurements and accompanying calculations rather closely mimic the mechanism that controls the uptake of PAHs in worms. In general, this mechanism is believed to be uptake via the aqueous phase only (i.e., desorption of PAHs from soil particles to water and subsequent uptake of PAHs from water into worms through the skin or the gut wall by hydrophobic partitioning). Alternatively, however, uptake by means of solid–liquid transfer might be the route of exposure (*10*). In this case, PAHs are transferred directly from soil solids to worm tissue or PDMS by immediate contact between the two phases. Experimental evidence for such a mechanism is lacking, however.

SPME-Predicted versus Observed Toxicity. In addition to predicting bioaccumulation, SPME-measured concentrations in interstitial water were also used to assess toxic effects (i.e., mortality) in worms upon exposure to the soils. This was performed by converting the predicted individual PAH concentrations in worm lipid to concentrations on a molar basis (mmol/kg lipid), summing the resulting concentrations, and comparing the totals with the literature threshold concentration known to cause acute toxicity (lethality) due to narcosis (i.e., the so-called lethal body burden (LBB) or critical body residue (CBR) (*28–31*)). This approach assumes that hydrophobic organic chemicals partition into biomembranes, where they act additively on a molar basis and cause a disturbance of the normal structure and/or functioning of the membrane (*29*). At a certain, constant sum membrane burden (LBB/CBR; between 50 and 200 mmol/kg lipid (*29–31*)), the disturbance becomes irreversible, and the organism will consequently die. Obviously, this approach is valid only when specifically acting toxicants are either absent or present below their threshold concentration for specific toxicity (which usually occurs in the environment). In the latter case, organic chemicals will simply contribute to the overall membrane burden and thus to the narcotic potency of the chemical mixture to which the organism is exposed. For more details on narcosis, LBB/CBR, and the above described approach for assessing toxicity, we refer to work by others (*29–32*). In particular, at former MGP sites, this way of assessing toxicity has potential, as the primary contaminants at these sites are PAHs, which are well-known narcotics.

In Figure 4, the estimated total internal molar concentrations (sum of 13 PAHs—benzo[*b*]fluoranthene and benzo[*k*]fluoranthene included again) are presented as bars for each of the 15 soils. Theoretically, molar concentrations that exceed the CBR (in Figure 4 set to 100 mmol/kg lipid) represent soils that should be lethal to organisms. Figure 4 therefore suggests that worms exposed to the soils OG10, OG18, and CG3 should die. When comparing these predictions to actual observations during earthworm bioassays (see ref *12*; results are summarized at the bottom of Figure 4), it appears that toxicity is predicted correctly in 13 out of 15 cases. Only one false negative (CG10) and one false positive (OG10) prediction were made. In other words, for these MGP soils, SPME properly predicted the actual toxicity in 87% of the cases. Moreover, the false positive prediction can be traced back to an artifact. The soil concerned (OG10) consisted of almost pure soot (87% of carbon; see Table S2). Fine soot particles might stick to the soft, hydrophobic fiber coating (PDMS) (*33*), and in case they cannot be wiped off, this would lead to an overprediction of C_f and thus of C_w. Consequently, K_d/K_{oc} values and toxicity would be under- and overestimated, respectively. Although the fibers exposed to this soil did not appear to retain any particles (no coloring of the fibers was observed), K_{oc} values in Table S1 support the occurrence of this incomplete phase separation artifact. After all, K_{oc} values for this soil are among the lowest values determined (in particular, values for the least hydrophobic PAHs are similar to K_{ow} values), whereas for a sample consisting of almost pure soot, one would expect K_{oc} values to be exceptionally high (*22*). The presumed incomplete phase separation would leave the current SPME technique less suitable for measurements in samples with high soot (or similar materials) contents.

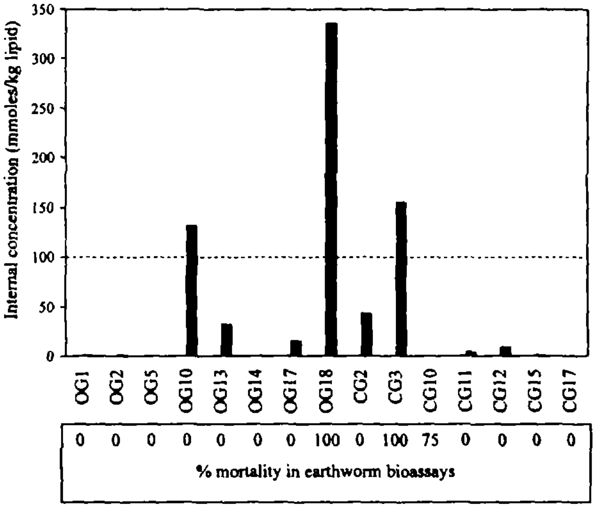

FIGURE 4. SPME-predicted total internal molar concentrations of the 13 PAHs studied (mmol/kg lipid) in earthworms virtually exposed to the 15 MGP soils. Bars that exceed the 100 mmol/kg lipid threshold should result in mortality. Actual mortality observed during bioassays with earthworms (*12*) is presented at the bottom of the graph.

The false negative value observed for sample CG10 indicates that, besides the PAHs analyzed, this soil contained other toxicants. Potential candidates might be nitrogen-containing chemicals, such as ammonia, because the C/N ratio determined for this soil was abnormally low as compared to that observed for the other soils (see Table S2). Ammonia is commonly found at former MGP sites since it was formed as a byproduct during gas production. However, soil CG10 was collected from the edge of a maintained lawn, so in this case, ammonia might have had its origin in fertilizing activities as well. On the other hand, the presence of other types of contaminants in this soil (e.g., metals or pesticides for lawn maintainance) cannot be excluded.

Finally, it is important to note that the CBR approach used in this study should notably be considered as a way to initially assess toxicity, as only 13 individual chemicals were analyzed. Although PAHs will probably be the most abundant chemicals at MGP sites, other organic chemicals will exist in the soils as well, contributing to the overall narcotic potency of the sample. Nevertheless, the current approach does provide a useful initial estimate, as clearly evidenced by the results presented. For samples for which total body residues in exposed organisms are predicted to be $\ll$1 mmol/kg, it is unlikely that other chemicals will contribute enough of a tissue residue to reach the CBR threshold. Conversely, in cases where the CBR is already exceeded, mortality is obvious (assuming the absence of artifacts, such as observed for soil OG10).

One way to deal with the above limitation is to use GC-FID or -MS for the analysis of SPME fibers and to quantify the total detector response. This way, all organic chemicals are included, but it is difficult to convert the analytical results to internal molar concentrations because the exact composition (and thus the molecular weights of the chemicals and their K_f and BCF values) of the mixture will be unknown. Nevertheless, in case the K_f and BCF values are proportional for the chemicals that are present, satisfactory estimates may be obtained *(34)*. Alternatively, by defining C_f thresholds above which effects occur (to be quantified in separate experiments for different biota), the results can directly be linked to toxicity. A major drawback of this approach is that it excludes the mechanistic (LBB/CBR) basis, which reduces the method to a strictly empirical one.

The SPME approach applied in the current study (using disposable fibers) is simple, inexpensive, and appears to provide a useful tool to screen PAH-contaminated MGP soils for toxic potency and bioaccumulation potential. Hence, this method might be considered a promising substitute for the more expensive and laborious bioassays, thereby contributing to a reduction in animal testing.

Acknowledgments

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Supporting Information Available

Tables of log K_{oc} values, soil characteristics, chemical properties, and r^2 values resulting from linear regressions; description of multiple linear regression analysis with a random coefficient mixed model; description of a dual domain model with a random coefficient; and figure of the ratio of measured over predicted C_{worm} vs log K_{ow}. This material is available free of charge via the Internet at http://pubs.acs.org.

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