Environ. Sci. Technol. **2008,** *42,* 7516–7521

Passive Dosing of Soil Invertebrates with Polycyclic Aromatic Hydrocarbons: Limited Chemical Activity Explains Toxicity Cutoff

PHILIPP MAYER*,† AND MARTIN HOLMSTRUP‡

Department of Environmental Chemistry and Microbiology, National Environmental Research Institute, University of Aarhus, PO Box 358, DK-4000 Roskilde, Denmark, Department of Terrestrial Ecology, National Environmental Research Institute, University of Aarhus, PO Box 314, DK-8600 Silkeborg, Denmark

Received March 26, 2008. Accepted July 17, 2008.

The partitioning of organic soil pollutants into soil organisms is driven by their chemical activity, which normally does not exceed that of the pure pollutant. Passive dosing with the silicone poly(dimethylsiloxane) (PDMS) was used to initiate and maintain the maximum chemical activity of 10 polycyclic aromatic hydrocarbons (PAHs) in toxicity tests with the springtail *Folsomia candida*. The test animals could move freely on the PDMS saturated with PAHs, resulting in direct contact and exposure to saturated air. After 7 days, springtail lethality correlated neither with the octanol−water partition coefficients of the PAHs nor with their molecular size, but with their melting point. All low-melting PAHs ($T_M \leq 110$ °C) caused 100% lethality, whereas all high-melting PAHs ($T_M \geq 180$ °C) caused no significant lethality. The lethality was successfully fitted to one chemical activity response curve for all PAHs tested, with effective chemical activity causing 50% lethality (Ea-50) of 0.058. It was also fitted to the PAH concentration in the PDMS, resulting in an EC_{PDMS}-50 of 8.7 mM. Finally, the combined exposure to anthracene and pyrene was described by the sum of chemical activities causing lethality, in good agreement with the chemical activity−response curve obtained.

Introduction

It is still difficult to characterize the exposure of organisms to hydrophobic organic chemicals (HOCs) in complex and heterogeneous media, such as soils and sediments (*1*, *2*). The best-established and most promising approach is the equilibrium partitioning theory, which is based on the assumption that bioconcentration in soil and sediment organisms can be explained by equilibrium partitioning between the matrix and organism (*3*−*6*). The equilibrium partitioning is controlled by the chemical activity of the contaminant and can be described by distribution coefficients such as K_D values and biota-to-soil accumulation factors (BSAF).

K_D and BSAF values are difficult to predict with high accuracy, vary between soils and sediments, can be concentration-dependent, may also be affected by cosolutes and are easily underestimated when soot particles are present (*7*−*10*). The application of generic or estimated distribution coefficients to the equilibrium partitioning theory can therefore introduce substantial error to the prediction of contaminant concentrations in organisms (*11*). It is therefore better to use measured freely dissolved pore-water concentrations (C_{free}) in the equilibrium partitioning calculations (*12*−*15*), an approach that works when the organism is in equilibrium with the interstitial water. This does not imply that water is the primary exposure medium from which contaminants enter the organism nor an important reservoir for the contaminants; however, it should be regarded as a reference medium for expressing effective concentrations for partitioning (*16*).

HOCs have very low aqueous solubilities, and some soils have a rather low water content. This makes the freely dissolved concentration a less obvious parameter for the expression of HOC exposure in soil. As an alternative, therefore, we would suggest the closely related chemical activity (*a*) (*16*), which is also applicable in dry soils, where the main routes of diffusive uptake might be through air and via direct contact between particles and organisms. Both processes are driven by differences in chemical activity. We chose the pure (subcooled) liquid form of the chemical as the reference state at which the chemical activity was set to be 1 (*16*, *17*).

A new passive dosing system is introduced in the present study to control chemical activity. It uses a silicone polymer loaded with the contaminant to control its chemical activity at the polymer surface and the overlying air in a closed test system. It is based on the principles of partitioning-driven administration (*18*), also known as partition-controlled delivery (*19*, *20*). Here, the contaminant is introduced by partitioning from a dominating polymeric phase, rather than by addition via a solvent extract. This approach has several advantages (*1*): the exposure can be kept constant (*2*), the test organisms are not coexposed to solvents, and (*3*) the exposure principle resembles partitioning from the soil matrix to some extent.

Polycyclic aromatic hydrocarbons were chosen as model contaminants in this study because they are among the most important soil pollutants and have a broad range of relevant physical chemical properties. The question of their relative toxicity to soil organisms is still debated. Many PAH mixtures, such as coal tar and creosote, are known to be highly toxic, whereas aged soils with high PAH levels as well as some individual PAHs that were freshly spiked at very high concentrations were found not to exert toxicity (*21*−*25*). It is hoped that an assessment of PAH exposure in soils, which is more precise and is based on the entity that controls diffusion and partitioning, will help to clarify these apparent contradictions.

The present study aims (i) to determine the toxicity of 10 PAHs at their maximum exposure levels to a soil invertebrate, (ii) to identify and explain cutoff phenomena in PAH toxicity, (iii) to demonstrate passive dosing for controlled direct-contact exposure, and (iv) to demonstrate that chemical activity can be used to quantify direct-contact exposure.

The first working hypothesis of this study is that the melting enthalpy of a substance determines its maximum potential for diffusion and partitioning into the test organism. This maximum potential for partitioning can be expressed as the chemical activity of the solid substance ($a_{crystal}$), is related to its melting point, and determines whether it can cause baseline toxicity at saturation (*26*). The baseline toxicity

* Corresponding author phone: +45 46 30 18 81; fax: +45 46 30 11 14; e-mail: phm@dmu.dk.
† Department of Environmental Chemistry and Microbiology.
‡ Department of Terrestrial Ecology.

10.1021/es801689y CCC: $40.75

Published on Web 08/22/2008

is defined here as the general type of toxicity that occurs when a critical concentration of contaminants is reached in lipid membranes; it is also known as narcosis (*27, 28*). The second working hypothesis is that baseline toxicity is exerted at a relatively constant level of chemical activity of the PAH when equilibrium partitioning governs its uptake into the lipid target membranes. This second hypothesis is based on theoretical considerations as well as the finding within human toxicology that many narcotics act at a level of chemical activity within the range 0.01−0.1 (*29*) and the observation within aquatic toxicology that many hydrocarbons (including chlorinated ones) exert their toxicity at a relatively constant fraction of their (subcooled) liquid solubility (*30*).

Material and Methods

Passive Dosing with PAHs. Naphthalene (99+%, Sigma), acenaphthene (99%, Aldrich), fluorene (99%, Aldrich), phenanthrene (99.5%, Aldrich), anthracene (99%, Acros), fluoranthene (99%, Aldrich), pyrene (>99%, Fluka), benzo[*a*]anthracene (99%, Aldrich), chrysene (99%, Cerilliant), and benzo[*a*]pyrene (98%, Cerilliant) were used as the model substances. Initial experiments were carried out with PAHs of lower purity, whereas the PAHs of higher purity were used in the definite test to minimize the risk of impurities' contributing to the toxicity. The octanol−water partition coefficients (log K_{OW}) and the molecular volumes of the PAHs were determined with the SPARC online calculator (*31*).

Medical grade silicone was made with the MDX4-4210 kit from Dow Corning supplied by the Institute of Anaplastology (Velten, Germany). It contained a prepolymer and a catalyst. The two components were mixed according to the instructions given by the supplier. The passive dosing vials were then prepared by adding 500 mg of the silicone mixture to each 10 mL vial, and the vials were left to cure for at least 72 h at room temperature. They were then placed in an oven at 100 °C at least overnight. Excess ethyl acetate was then added to remove impurities and oligomers and was retained for at least 72 h.

The passive dosing vials were loaded by partitioning from a methanol suspension of the respective PAH, which was placed above the silicone for at least 72 h. The PAH crystals maintained the maximum chemical activity of the PAH in the methanol, which was then transferred to the silicone by equilibrium partitioning. The loading by partitioning from methanol to silicone is a relatively fast and highly reproducible process (*32, 33*). The suspension was then discarded, and the silicone surface was wiped gently with lint-free tissue to remove traces of PAH suspension. A small volume of water was added to remove methanol and PAH crystals from the silicone. This cleaning step was repeated at least three times.

Crystal Activities. The chemical activity of a PAH in its solid crystal state ($a_{crystal}$, unitless) is numerically identical to its fugacity ratio, which also can be expressed as the ratio between regular solubility (S) and subcooled liquid solubility (S_L) (*16, 34*).

$$a = C_{free}/S_L \Rightarrow a_{crystal} = S/S_L \quad (1)$$

This was estimated for each PAH at 25 °C (T = 298 K) from its reported melting point (T_m, K), assuming the entropy of melting to be 56 J mol^{-1} K^{-1} (i.e., Walden's rule), as suggested by Yalkowsky et al. (*35*):

$$S/S_L = \exp[(6.8)(1 - T_m/T)] \quad (2)$$

Springtail Toxicity Test. *Folsomia candida* were cultured at 20 ± 1 °C with a 12:12 h light/dark photo period. The springtails were kept in Petri dishes containing a 5 mm layer of water-saturated charcoal/plaster-of-Paris mixture (1:8) and fed a diet of dried baker's yeast. They were further standardized as described by Krogh (*36*) to ensure that all individuals were of approximately the same age (41−44 days old) at the beginning of the experiment. They had an average wet weight of 91 μg and a dry weight of 34 μg at the beginning of the experiment.

The toxicity test was carried out with 5 vials per PAH and 10 springtails per vial. To avoid desiccation of the springtails, a 2 μL droplet of demineralized water was added to the center of the silicone floor before the springtails were introduced. The vials were closed with airtight screw caps containing Teflon-coated septa and were placed at 20 ± 0.1 °C for 7 days (12:12 h light:dark photoperiod). The springtails could move freely within the vials and were observed mainly on the dry silicone surface. The water droplet was used to maintain sufficient humidity within the vial. The water uptake from the droplet was very limited, so the uptake of hydrophobic test substances via drinking water should also be very limited. This leaves diffusive mass transfer from the headspace air and diffusive mass transfer by direct physical contact at the silicone-organism interface as the two main routes of exposure. An earlier study (*37*) had already demonstrated that headspace concentrations can be efficiently controlled by partitioning from loaded silicone.

At the end of the test, the springtails in each vial were transferred to a Petri dish with a moistened floor of plaster-of-Paris and inspected using a dissection microscope. Springtails able to walk in a coordinated manner when gently stimulated with a fine brush were considered as survivors. 100% of the springtails in the control treatment (silicone without PAH) survived during the 7-day toxicity test. The relationship between toxicity and chemical activity was modeled by a sigmoidal dose−response function with variable slope, also known as an uphill function or two-parameter logistic equation. Data were fitted by the least-squares method using GraphPad Prizm 4 (San Diego, CA).

An initial experiment conducted with PAHs of lower purity acted as a pilot experiment. The second and final experiment was conducted as described here: its results were in good agreement with those of the pilot experiment.

Confirmation of PAH Exposure. The springtails were removed from the passive dosing vials. The silicone surface was cleaned by wrist shaking with a small volume of water, which was then decanted. Additional water was added to the vials and kept under a lid for 2 h to obtain a thermodynamic equilibrium between PDMS and water. A subsample of the water was taken and mixed with an equal volume of methanol before being analyzed for the target PAH as described below. The measured concentrations in the water were divided by aqueous solubility values from the literature to derive a percentage saturation. The aqueous solubilities were mean literature values from ref *38* where available or, alternatively, from ref *17*. The PAHs in the PDMS were extracted by 10 mL of methanol at room temperature and kept at least overnight.

The water/methanol extract analysis of PAHs was carried out by HPLC with fluorescence detection (Agilent 1100 system with G1321A FLD operated at Ex: 260 nm; Em: 350, 420, 440 and 500 nm). The CP-Ecospher 4 PAH separation column was obtained from Varian Inc. (Palo Alto, CA) and was operated at 0.5 mL/min (28 °C, 30 μL injection). Methanol (HPLC grade from Merck, Darmstadt, Germany) and water (Super-Q treated, Millipore, MA) was used as the mobile phase: 50% methanol at t = 0−2 min, linear gradient from 50 to 75% methanol at t = 2−7 min, linear gradient from 75 to 100% at t = 7−35 min, and 100% methanol at t = 35−48 min. The PAH concentrations in the extracts were quantified by a five-point external standard curve. Analysis was carried out within 2 weeks after sampling. Signal integration was performed with HP Chemstation software (A.06.03, Agilent Technologies, Palo Alto, CA) and corrected manually where necessary.

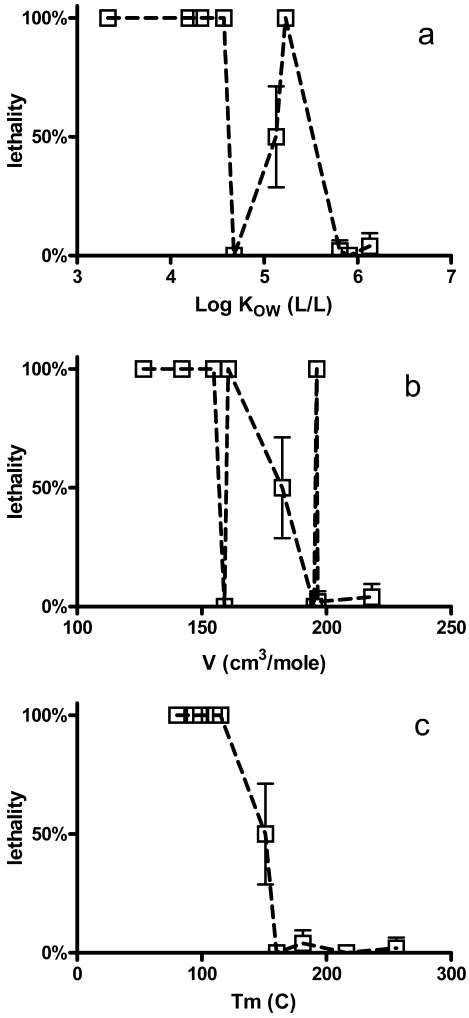

FIGURE 1. (a−c): Lethality of the springtail *F. candida* after 7 days of exposure to the 10 PAHs. Lethality is plotted against (a) their octanol−water partition coefficient (log K_{OW}), (b) their molecular volume (V), and (c) their melting point temperature (T_m). Error bars represent standard deviations of five replicates.

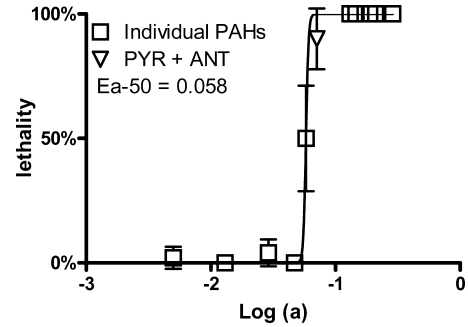

FIGURE 2. Lethality of *F. candida* after 7 days of exposure to the 10 PAHs and one PAH mixture plotted against the chemical activity (*a*, unitless) of the PAHs. Lethalities were fitted to the uphill function. Error bars represent standard deviations of five replicates.

Results and Discussion

PAH Toxicity at Saturation and Evidence of Melting Point Cutoff. The lethal effect of the 10 PAHs was plotted against their log K_{OW} values in Figure 1a, since log K_{OW} is often used in qualitative activity−structure relationships and to express or identify hydrophobicity cutoffs in toxicity. There was no clear trend between log K_{OW} values and lethality, indicating that neither hydrophobicity nor lipophilicity can explain why some of the PAHs were toxic at saturation, whereas others were not. The percentage lethality was then plotted against the molecular volume (V) of the PAHs in Figure 1b, since V often is used to identify and express size exclusion phenomena. There was again no clear trend. Pyrene (182.3 cm^3/mol) and fluoranthene (196.1 cm^3/mol) did exert toxicity, whereas the smaller PAH anthracene (159.1 cm^3/mole) did not. Size exclusion phenomena can consequently not explain the present observations of toxicity. Additional figures could be made with other relevant size parameters, such as molecular weight, the length or the effective cross section of the PAHs. However, for the tested PAHs, these parameter are highly correlated, and the resulting figures would thus not yield additional information. Finally, the percentage lethality was plotted against the melting point of the PAHs (Figure 1c), showing a clear relationship. All low-melting PAHs with melting points of 110 °C and below led to 100% lethality, whereas all high-melting PAHs with melting points of 180 °C and above did not lead to any significant lethality. This experimental observation of a melting point cutoff in single substance toxicity is in good agreement with earlier studies based on data from the literature (*26, 39*).

The melting points of the PAHs are directly related to their enthalpy of melting and, thus, to the thermodynamic stability of their crystals. The symmetry of the PAHs may play a role in this respect, since more symmetrical molecules tend to form more stable crystal structures. The highly symmetrical anthracene molecule, thus, has a much higher melting point (217.5 °C) than its isomer phenanthrene (99.5 °C), and its maximum chemical activity (0.013) is much lower than that of the latter (0.19) (eq 2).

The partitioning of organic substances into the cellular phospholipid membranes of the springtails is a spontaneous process driven by the partial molar free energy, which can be quantified by its chemical activity. The percentage lethality values of all 10 PAHs were thus plotted against their respective $a_{crystal}$ and fitted as one data set to the uphill function (Figure 2). The effective chemical activity causing 50% lethality (Ea-50) was determined to be 0.058 with a 95% confidence interval of 0.057 to 0.059 and an r^2 of 0.98. This result was in good agreement with that of the pilot experiment (Ea-50 = 0.060; 95% C.I.: 0.043−0.083). The exact Ea-50 value of 0.059 was largely determined by the toxicity of pyrene, and generalization of this value to the other PAHs awaits further confirmation. This Ea-50 value is well within the range of effective chemical activities (Ea-50) for a baseline toxicity of 0.01−0.1 which were recently calculated from a variety of published effective concentrations (EC-50) for algae, fish, tadpoles and mice (*16, 26, 40−45*).

The Ea-50 values of this passive dosing study can be extrapolated to the complex soil matrix if equilibrium partitioning governs the uptake into the springtails. A very recent study allows such an extrapolation to be evaluated. Styrishave et al. combined a 21-day *F. candida* soil toxicity test of pyrene (*46*) with matrix solid phase microextraction (matrix-SPME) (*47*). They determined EC-50 values for reproduction based on freely dissolved concentrations in soil interstitial water at various additions of organic matter and various degrees of aging. These values remained largely constant at approximately 23 μg/L, which is equivalent to an Ea-50 of 0.025 (eq 1 and S_L from ref *17*). The ratio between the acute Ea-50 (7 days, lethality) of this study and the chronic Ea-50 (21 days, reproduction) from Styrishave (*46*) is 2.3, which is rather low and well within the range of earlier reported acute-to-chronic ratios for three PAHs (*23*). The results of the two studies thus seem to be in good agreement with each other.

The lethality caused by the 10 PAHs was also plotted against their measured concentrations in the PDMS (C_{PDMS}), which was used for the passive dosing. The lethality of all 10

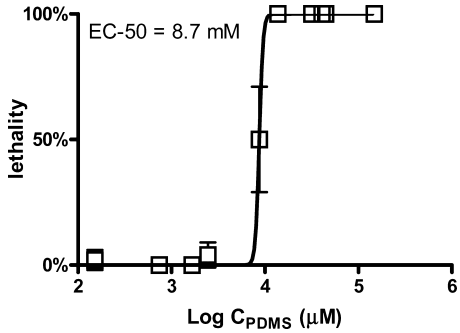

FIGURE 3. Lethality of *F. candida* after 7 days of exposure to the 10 PAHs plotted against the measured concentrations of the PAHs in the silicone PDMS. Lethalities were fitted to the uphill function. Error bars represent standard deviations of five replicates.

PAHs as a function of C_{PDMS} was successfully fitted to the uphill function with an r^2 of 1.00 (Figure 3), thus confirming the suitability of PDMS as a biomimetic material for the PAHs. The median effective concentration on a PDMS basis (EC_{PDMS}-50) was determined to be 8.7 mM, and this value could be used as a reference for biomimetic extractions with PDMS-coated SPME fibers (*48*).

Mixture Toxicity. The combined exposure to anthracene and pyrene was described by the sum of their chemical activities and leads to lethality, in good agreement with the chemical activity−response curve obtained (Figure 2). This is an early and very limited example illustrating how chemical activities might be used to characterize exposure to complex mixtures. The sum of chemical activities is suggested as a new exposure measure for mixture toxicity similar to the sum of toxic units (TUs). The main difference between these two approaches is that chemical activity is chemically defined, whereas toxic units are the result of toxicity testing.

The melting point cutoff has some important implications for our perception of the mixture toxicity of solid organic chemicals which did not exert toxicity when tested individually. The solubility of such solid chemicals is approximately additive in a mixture (*49*), so they can contribute to the exposure and, thus, the toxicity of a complex mixture. This is in good agreement with the observation that complex mixtures, such as coal tars, can be highly toxic, even though many of their constituents were observed to have limited or no acute toxicity. Relevant mixtures could include (*1*) the technical quality of a single chemical (*2*), the (isomeric) mixture of chemicals within a product, and (*3*) the complex mixture of chemicals present in the environment. The melting point cutoff suggests that the absence of toxicity of pure high-melting-point chemicals should not be extrapolated to a mixture.

Uptake Route. The PAHs were certainly not taken up via food, since no food was given to the animals during the test. Their uptake via drinking water was also considered to be negligible because of limited concentrations in the water and limited water intake. The main uptake routes of the PAHs in the present study are thus diffusive mass transfer through air and diffusive mass transfer by direct contact at the silicone-organism interface. Both are driven by chemical activity gradients and have been shown to be effective relative to their diffusive mass transfer through water (*50*). The results of the present study do not allow us to distinguish which of the two routes was dominant in the passive dosing vial or in contaminated soil. Nevertheless, there is no need to know the routes toward equilibrium in such situations. The observed relationship between chemical activity and toxicity as shown in Figure 2 is in good agreement with the PAH concentration (or chemical activity) at the target site being

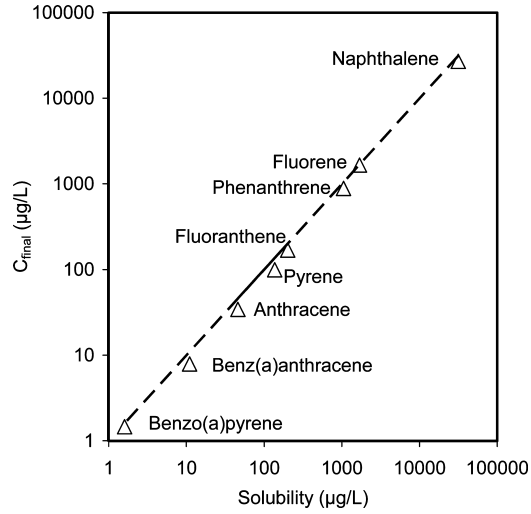

FIGURE 4. Equilibrium partitioning concentrations of PAHs in water at the end of the experiment plotted against their aqueous solubilities. Aqueous solubilities were mean literature values taken from ref *38* where available and, alternatively, from (*17*). Chrysene was not included because it could not be measured with sufficient precision, and acenaphtene was not included due to the lack of solubility data in both references. A perfect fit line (1:1) was included to serve as a visual reference.

controlled by equilibrium partitioning and baseline toxicity as the mode of toxic action.

Confirmation of maximum exposure ($a_{crystal}$). The validity of environmental toxicity tests relies on a sound analytical confirmation of the tested exposure levels. For this purpose, water was placed above the silicone after the experiment, and the equilibrium partitioning concentrations were measured in this water. These concentrations were then plotted against the respective aqueous solubilities in Figure 4. The measured concentrations were between 72 and 98% of the respective solubilities. The combination of saturating the silicone with the PAHs prior to the toxicity test and the analytical confirmation of PAH concentrations near their solubility limit after the test clearly demonstrates that the passive dosing system yielded stable chemical activities very close to those of the pure substance ($a_{crystal}$).

We suggest that future research be directed (*1*) at similar experiments with other groups of organic chemicals to explore the applicability domain of the findings obtained (*2*), at additional tests of PAHs to determine bioconcentration kinetics and effects on reproduction (*3*), at more extensive work on mixture toxicity to test whether toxicity can be linked to the sum of chemical activities, and (*4*) on additional experiments in soils to validate the relationship between chemical activity and toxicity in the complex soil matrix. The Ea-50 values obtained can then be directly compared to chemical activities in contaminated soil, which can be measured with recently developed equilibrium sampling techniques (*37, 51*).

In summary, the study shows how passive dosing from silicone can be used to control direct-contact exposure in toxicity tests and supports the hypothesis that baseline toxicity is exerted at relatively constant chemical activity. The chemical activity framework and a melting point cutoff in toxicity were able to explain why some PAHs were acutely toxic at saturation while others were not. It should finally be noted that the results obtained were successfully explained without considering aqueous concentrations.

Acknowledgments

We thank Margit Fernqvist for her skilled technical assistance and Fredrik Reichenberg for his valuable comments on the

manuscript. This research was funded externally by the European Commission within the scope of the FP 6 Integrated Projects NOMIRACLE (GOCE-003956) and OSIRIS (COGE-037017).

Supporting Information Available

Tables listing the physicochemical properties of the 10 PAHs and the toxicity observations are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Alexander, M. How Toxic are Toxic Chemicals in Soil? *Environ. Sci. Technol.* **1995**, *29*, 2713–2717.

(2) Alexander, M. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ. Sci. Technol.* **2000**, *34*, 4259–4265.

(3) Shea, D. Developing National Sediment Quality Criteria. *Environ. Sci. Technol.* **1988**, *22*, 1256–1261.

(4) Di Toro, D. M.; Zarba, C. S.; Hansen, D. J.; Berry, W. J.; Swartz, R. C.; Cowan, C. E.; Pavlou, S. P.; Allen, H. E.; Thomas, N. A.; Paquin, P. R. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ. Toxicol. Chem.* **1991**, *10*, 1541–1583.

(5) Jager, T.; Fleuren, R.; Hogendoorn, E. A.; De Korte, G. Elucidating the routes of exposure for organic chemicals in the earthworm, *Eisenia andrei* (Oligochaeta). *Environ. Sci. Technol.* **2003**, *37*, 3399–3404.

(6) Ma, W. C.; van Kleunen, A.; Immerzeel, J.; de Maagd, P. G. J. Bioaccumulation of polycyclic aromatic hydrocarbons by earthworms: Assessment of equilibrium partitioning theory in in situ studies and water experiments. *Environ. Toxicol. Chem.* **1998**, *17*, 1730–1737.

(7) Cornelissen, G.; Gustafsson, O.; Bucheli, T. D.; Jonker, M. T. O.; Koelmans, A. A.; Van Noort, P. C. M. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ. Sci. Technol.* **2005**, *39*, 6881–6895.

(8) Thorsen, W. A.; Cope, W. G.; Shea, D. Bioavailability of PAHs: Effects of soot carbon and PAH source. *Environ. Sci. Technol.* **2004**, *38*, 2029–2037.

(9) Bucheli, T. D.; Gustafsson, O. Quantification of the soot−water distribution coefficient of PAHs provides mechanistic basis for enhanced sorption observations. *Environ. Sci. Technol.* **2000**, *34*, 5144–5151.

(10) Xiao, B. H.; Yu, Z. Q.; Huang, W. L.; Song, J. Z.; Peng, P. A. Black carbon and kerogen in soils and sediments. 2. Their roles in equilibrium sorption of less-polar organic pollutants. *Environ. Sci. Technol.* **2004**, *38*, 5842–5852.

(11) ten Hulscher, T. E. M.; van Noort, P. C. M.; van der Velde, L. E. Equilibrium partitioning theory overestimates chlorobenzene concentrations in sediment porewater from lake Ketelmeer, the Netherlands. *Chemosphere* **1997**, *35*, 2331–2344.

(12) Kraaij, R.; Mayer, P.; Busser, F. J. M.; van Het Bolscher, M.; Seinen, W.; Tolls, J.; Belfroid, A. Measured pore-water concentrations make equilibrium partitioning work−A data analysis. *Environ. Sci. Technol.* **2003**, *37*, 268–274.

(13) You, J.; Landrum, P. E.; Trimble, T. A.; Lydy, M. J. Availability of polychlorinated biphenyls in field-contaminated sediment. *Environ. Toxicol. Chem.* **2007**, *26*, 1940–1948.

(14) Ter Laak, T. L.; Barendregt, A.; Hermens, J. L. M. Freely dissolved pore water concentrations and sorption coefficients of PAHs in spiked aged and field-contaminated soils. *Environ. Sci. Technol.* **2006**, *40*, 2184–2190.

(15) van der Wal, L.; van Gestel, C. A. M.; Hermens, J. L. M. Solid phase microextraction as a tool to predict internal concentrations of soil contaminants in terrestrial organisms after exposure to a laboratory standard soil. *Chemosphere* **2004**, *54*, 561–568.

(16) Reichenberg, F.; Mayer, P. Two complementary sides of bioavailability: accessibility and chemical activity of organic contaminants. *Environ. Toxicol. Chem.* **2006**, *25*, 1239–1245.

(17) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*; John Wiley & Sons Inc.: New York, 1993.

(18) Mayer, P.; Wernsing, J.; Tolls, J.; de Maagd, P. G. J.; Sijm, D. T. H. M. Establishing and controlling dissolved concentrations of hydrophobic organics by partitioning from a solid phase. *Environ. Sci. Technol.* **1999**, *33*, 2284–2290.

(19) Brown, R. S.; Akhtar, P.; Akerman, J.; Hampel, L.; Kozin, I. S.; Villerius, L. A.; Klamer, H. J. C. Partition controlled delivery of hydrophobic substances in toxicity tests using poly(dimethylsiloxane) (PDMS) films. *Environ. Sci. Technol.* **2001**, *35*, 4097–4102.

(20) Kiparissis, Y.; Akhtar, P.; Hodson, P. V.; Brown, R. S. Partition-controlled delivery of toxicants: A novel in vivo approach for embryo toxicity testing. *Environ. Sci. Technol.* **2003**, *37*, 2262–2266.

(21) Sverdrup, L. E.; Nielsen, T.; Krogh, P. H. Soil ecotoxicity of polycyclic aromatic hydrocarbons in relation to soil sorption, lipophilicity, and water solubility. *Environ. Sci. Technol.* **2002**, *36*, 2429–2435.

(22) van Brummelen, T. C.; van Gestel, C. A. M.; Verweij, R. A. Long-term toxicity of five polycyclic aromatic hydrocarbons for the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. *Environ. Toxicol. Chem.* **1996**, *15*, 1199–1210.

(23) Droge, S. T. J.; Paumen, M. L.; Bleeker, E. A. J.; Kraak, M. H. S.; van Gestel, C. A. M. Chronic toxicity of polycyclic aromatic compounds to the springtail *Folsomia candida* and the enchytraeid *Enchytraeus crypticus*. *Environ. Toxicol. Chem.* **2006**, *25*, 2423–2431.

(24) Jensen, J.; Sverdrup, L. E. Polycyclic aromatic hydrocarbon ecotoxicity data for developing soil quality criteria. *Rev. Environ. Contam. Toxicol.* **2003**, *179*, 73–97.

(25) Sverdrup, L. E.; Hagen, S. B.; Krogh, P. H.; van Gestel, C. A. M. Benzo(*a*)pyrene shows low toxicity to three species of terrestrial plants, two soil invertebrates, and soil-nitrifying bacteria. *Ecotoxicol. Environ. Saf.* **2007**, *66*, 362–368.

(26) Mayer, P.; Reichenberg, F. Can highly hydrophobic organic substances cause aquatic baseline toxicity and can they contribute to mixture toxicity? *Environ. Toxicol. Chem.* **2006**, *25*, 2639–2644.

(27) van Wezel, A. P.; Opperhuizen, A. Narcosis due to environmental pollutants in aquatic organisms: residue-based toxicity, mechanism, and membrane burdens. *Crit. Rev. Toxicol.* **1995**, *25*, 255–279.

(28) Di Toro, D. M.; McGrath, J. A.; Hansen, D. J. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. *Environ. Toxicol. Chem.* **2000**, *19*, 1951–1970.

(29) Brink, F.; Posternak, J. M. Thermodynamic analysis of the relative effectiveness of narcotics. *J. Cell. Compar. Physiol.* **1948**, *32*, 211–233.

(30) Bobra, A. M.; Shiu, W. Y.; Mackay, D. A predictive correlation for the acute toxicity of hydrocarbons and chlorinated hydrocarbons to the water flea (*Daphnia magna*). *Chemosphere* **1983**, *12*, 1121–1129.

(31) Karickhoff, S.; Carreira, L.; Hilal, S. SPARC online calculator, 2006; http://ibmlc2.chem.uga.edu/sparc/index.cfm.

(32) Mayer, P.; Karlson, U.; Christensen, P. S.; Johnsen, A. R.; Trapp, S. Quantifying the effect of medium composition on the diffusive mass transfer of hydrophobic organic chemicals through unstirred boundary layers. *Environ. Sci. Technol.* **2005**, *39*, 6123–6129.

(33) Booij, K.; Smedes, F.; van Weerlee, E. M. Spiking of performance reference compounds in low density polyethylene and silicone passive water samplers. *Chemosphere* **2002**, *46*, 1157–1161.

(34) Mackay, D.; Shiu, W. Y.; Ma, K. C. *Polynuclear Aromatic Hydrocarbons, Polychlorinated Dioxins and Dibenzofurans*; Lewis Publishers: Boca Raton, FL, 1992; Vol. II.

(35) Yalkowsky, S. H.; Orr, R. J.; Valvani, S. C. *Ind. Eng. Chem. Fundam.* **1979**, *18*, 351–353.

(36) Krogh, P. H. Does a heterogeneous distribution of food or pesticide affect the outcome of toxicity tests with Collembola. *Ecotoxicol. Environ. Saf.* **1995**, *30*, 158–163.

(37) Legind, C. H.; Karlson, U.; Burken, J.; Reichenberg, F.; Mayer, P. Determining chemical activity of (semi)volatile compounds by headspace solid-phase microextraction. *Anal. Chem.* **2007**, *77*, 2869–2876.

(38) De Maagd, P. G. J.; ten Hulscher, D. T. E. M.; van den Heuvel, H.; Opperhuizen, A.; Sijm, D. T. H. M. Physicochemical properties of polycyclic aromatic hydrocarbons: aqueous solubilities, *n*-octanol/water partition coefficients, and Henry's law constants. *Environ. Toxicol. Chem.* **1998**, *17*, 251–257.

(39) Lipnick, R. L. Narcosis: Fundamental and baseline toxicity mechanism for nonelectrolyte organic chemicals In *Practical Applications of Quantitative Structure−Activity Relationships (QSAR) in Environmental Chemistry and Toxicology*; Karcher, W., Devillers, J., Eds.; Kluwer: Dordrecht, 1990; Vol. 1.

(40) Ferguson, J. The use of chemical potentials as indices of toxicity. *Proc. R. Soc. London, B* **1939**, *127*, 387–404.

(41) Mayer, P.; Halling-Sorensen, B.; Sijm, D. T. H. M.; Nyholm, N. Toxic cell concentrations of three polychlorinated biphenyl congeners in the green alga *Selenastrum capricornutum*. *Environ. Toxicol. Chem.* **1998**, *17*, 1848–1851.
(42) Könemann, H. Quantitative structure−activity relationships in fish toxicity studies. Part 1: Relationship for 50 industrial pollutants. *Toxicology* **1981**, *19*, 209–221.
(43) Wong, P. T. S.; Chau, Y. K.; Rhamey, J. S.; Docker, M. Relationship between water solubility of chlorobenzenes and their effects on a freshwater green alga. *Chemosphere* **1984**, *9*, 991–996.
(44) Calamari, D.; Galassi, S.; Setti, F.; Vighi, M. Toxicity of selected chlorobenzenes to aquatic organisms. *Chemosphere* **1983**, *12*, 253–262.
(45) Petersen, G. Comparative studies of toxic effects and bioaccumulation of lipophilic substances in fish early life stages. Ph.D. thesis; University of Copenhagen, Denmark, 1997; p 133.
(46) Styrishave, B.; Mortensen, M.; Krogh, P. H.; Andersen, O.; Jensen, J. Solid phase microextraction (SPME) as a tool to predict the bioavailability and toxicity of pyrene to the springtail, *Folsomia candida*, under various soil conditions. *Environ. Sci. Technol.* **2008**, *42*, 1332–1336.
(47) Mayer, P.; Vaes, W. H. J.; Wijnker, F.; Legierse, K. C. H. M.; Kraaij, R. H.; Tolls, J.; Hermens, J. L. M. Sensing dissolved sediment porewater concentrations of persistent and bioaccumulative pollutants using disposable solid-phase microextraction fibers. *Environ. Sci. Technol.* **2000**, *34*, 5177–5183.
(48) Leslie, H. A.; Oosthoek, A. J. P.; Busser, F. J. M.; Kraak, M. H. S.; Hermens, J. L. M. Biomimetic solid-phase microextraction to predict body residues and toxicity of chemicals that act by narcosis. *Environ. Toxicol. Chem.* **2002**, *21*, 229–234.
(49) Banerjee, S. Solubility of organic mixtures in water. *Environ. Sci. Technol.* **1984**, *18*, 587–591.
(50) Mayer, P.; Fernqvist, M. M.; Christensen, P. S.; Karlson, U.; Trapp, S. Enhanced diffusion of PAHs in artificial and natural aqueous solutions. *Environ. Sci. Technol.* **2007**, *41*, 6148–6155.
(51) Reichenberg, F.; Smedes, F.; Jönsson, J. A.; Mayer, P. Vials with polymer coatings of multiple thicknesses for equilibrium sampling of hydrophobic organic compounds in soil. *Chem. Cent. J.* **2008**, 2–5.

ES801689Y