SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 20, No. 8, pp. 1752–1761, 2001
© 2001 SETAC
Printed in the USA
0730-7268/01 $9.00 + .00

IMPACT OF SEDIMENT MANIPULATION ON THE BIOACCUMULATION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM FIELD-CONTAMINATED AND LABORATORY-DOSED SEDIMENTS BY AN OLIGOCHAETE

PATRICIA L. VAN HOOF,† JUSSI V.K. KUKKONEN,‡ and PETER F. LANDRUM*†
†Great Lakes Environmental Research Laboratory, 2205 Commonwealth Boulevard, Ann Arbor, Michigan 48105, USA
‡University of Joensuu, Department of Biology, P.O. Box 111, FIN-80101 Joensuu, Finland

(*Received* 11 *July* 2000; *Accepted* 12 *December* 2000)

Abstract—The accumulation kinetics of polycyclic aromatic hydrocarbons (PAHs) by the freshwater oligochaete *Lumbriculus variegatus* were measured for field-contaminated and laboratory-dosed sediment. In addition, sediment manipulations typically used for homogenization and dosing in bioaccumulation assays were compared. Rather than an asymptotic approach to steady state, both resident and dosed PAH accumulation exhibited a peak during the 14-d assays, with steeper declines being noted for the lower-molecular-weight compounds. Lack of evidence of a peak for higher-molecular-weight PAHs may be due to slower kinetics and the short length of the assay. Relative to minimally mixed sediment, slurried sediment enhanced the accumulation of less-soluble resident PAHs, did not affect moderately soluble PAHs, and reduced the uptake of the more-soluble PAHs, fluorene and phenanthrene. Aging sediment after mixing reduced the availability of highly to moderately soluble resident PAHs but had no effect on less-soluble PAHs. A similar effect was noted for dosed PAHs, though a larger reduction in bioavailability was observed. Dosed PAH uptake clearance coefficients (k_s) exceeded those of minimally mixed resident PAHs by factors of 3 to 4 for pyrene and 26 for benzo[*a*]pyrene. These results demonstrate that sediment manipulations and contamination history need to be considered when measuring PAH bioaccumulation.

Keywords—Bioavailability Polycyclic aromatic hydrocarbons Sediment aging Toxicokinetics

INTRODUCTION

Bioaccumulation studies are used to assess the rate and extent of contaminant accumulation by an organism from various media, including air, water, food, soil, and sediment. For aquatic organisms, routes of exposure to hydrophobic organic contaminants are from pore water or overlying water via absorption of the freely dissolved component or ingestion of the sediment-associated component. The relative importance of each exposure route is a complex function of compound hydrophobicity, contact time with sediment, desorption kinetics, sediment characteristics, organism absorption efficiency, feeding behavior, burrowing, and reproductive behavior. The availability of polycyclic aromatic hydrocarbons (PAHs) to epibenthic and infaunal deposit feeders is far less than that of other classes of compounds with similar physicochemical properties [1–3]. The reduced availability of PAHs has been ascribed to sequestration of a fraction of the total compound, rendering it unavailable to partition into pore water even over large time scales [4–7]. The decline in bioavailable or labile fractions with increasing contact time or aging is hypothesized to result from slow solute diffusion into interior regions of organic matrices [8,9] and/or entrapment within intraparticle micropores or polymeric microvoids [10,11]. Additionally, for PAHs, reduced labile fractions can result from preferred partitioning to the aromatic components of particulate organic matter [12] or incorporation into ash and soot matrices. Thus, the solute–sorbent contact time and the source of PAHs (i.e., pyrogenic, petrogenic, and diagenetic) will have a significant influence on partitioning, sorption kinetics, and bioavailability.

Using laboratory-dosed sediments with relatively short equilibration periods (<60 d) results in bioaccumulation that depends on solute–sorbent contact time and may not reflect the bioavailability of resident contaminants. The PAH uptake by bioassay organisms is frequently greater when using freshly dosed versus field-contaminated sediment or, alternatively, dosed and subsequently aged sediment [6,13,14]. Cases are reported, however, in which uptake kinetics have increased with increasing equilibration time [15,16].

Another consideration when dosing sediments is the impact of slurrying the sediment (i.e., adding water and stirring vigorously) on sediment characteristics (e.g., particle size distribution and pore-water composition) and bioavailability. The slurry technique is used to assure homogenization of dosed compound or resident contamination in sediment. Slurrying sediment was noted, however, to increase the rate and extent of microbial degradation of PAHs [17,18]. The authors suggested that bioavailability was enhanced by improved equilibration between dissolved and sorbed phases and the disruption of aggregates.

As part of a 1993 study by the U.S. Environmental Protection Agency to develop a standardized protocol for a bioaccumulation test with *Lumbriculus variegatus,* our interests were specifically focused on the impact of sediment manipulation on the accumulation PAHs from field-contaminated and laboratory-dosed sediments. The freshwater oligochaete *L. variegatus* is a useful organism for bioaccumulation studies, because it is exposed to all possible accumulation routes, is not known to metabolize PAHs or chlorinated hydrocarbons, egests sediment at the surface for easy collection, is environmentally tolerant, and can be easily cultured. We examined the bioaccumulation behavior of a complex mixture of PAHs

* To whom correspondence may be addressed (landrum@glerl.noaa.gov).

using historically contaminated sediment collected from the Little Scioto River (LSR) in north-central Ohio, USA. Sediment manipulations included gently hand-mixed without being slurried; slurried and dosed with trace levels of radiolabeled pyrene and benzo[*a*]pyrene, followed by a 10-d equilibration period; and slurried, dosed, and equilibrated for 42 d. These manipulations are typically used for homogenization and dosing in bioaccumulation assays. In addition to estimating the kinetics of PAH accumulation, sediment avoidance and mortality impacts were noted.

MATERIALS AND METHODS

Lumbriculus variegatus were reared in 37-L glass aquaria containing well water under low intensity (20 lux), gold fluorescent light ($\lambda > 500$ nm), and a light:dark photoperiod of 10:14 h at 21 ± 2°C. Shredded and presoaked, unbleached paper towels were used as a substrate in the aquaria. A flow of 8 to 10 L of fresh water per day was passed through the aquaria, and animals were fed with Trout Chow (~3 g; Purina Brand®, St. Louis, MO, USA) three times per week. Well water was used for the culture and assays with a nominal pH of 7.2, hardness of 470 mg/L as $CaCl_2$, and alkalinity of less than 1 mg/L as $CaCO_3$.

The site on the LSR was selected for this study because of its location downstream from an abandoned railroad yard suspected to be a source of creosote contamination (40°34.3′ N, 83°12.23′ W). Eckman grab samples of surficial sediment (0–5 cm) were homogenized and press-sieved gently by hand through a 1-mm screen to remove indigenous animals and large debris. Sediment organic carbon (OC) content measured 9.07% ± 0.61%. Lake Michigan, USA, sediment (43°3.27′ N, 86°38.71′ W) was used as a minimally polluted reference sediment to monitor the condition of the animals between assays (OC = 1.97% ± 0.06%).

Dosing compounds included [^{3}H]pyrene (specific activity = 25.2 Ci/mmol; Chemsyn Science Laboratories, Lenexa, KS, USA) and [^{14}C]benzo[*a*]pyrene (B*a*P; specific activity = 16.2 mCi/mmol; Sigma Chemical, St. Louis, MO, USA). Radiopurity was determined to be greater than 97% for both compounds as determined by thin-layer chromatography using hexane:benzene (8:2, v/v) and liquid scintillation counting. Nonlabeled PAH compounds were quantified using the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) SRM 1491. This solution contained 5 μg/ml of fluorene (FLE), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo[*a*]anthracene (B*a*A), chrysene (CHR), benzo[*b*]flouranthene (B*b*F), benzo[*e*]pyrene (B*e*P), B*a*P, perylene (PER), indeno[*1,2,3-cd*]pyrene (IP), and benzo[*g,h,i*]perylene (B*g*P). Seven perdeuterated PAHs (≥98% purity) were used either as surrogates to monitor analytical recoveries or as internal standards. All preparative and analytical procedures as well as bioassays were performed under gold fluorescent lights ($\lambda > 500$ nm) to avoid PAH photodegradation.

To compare the bioaccumulation of laboratory-dosed and environmentally resident PAH compounds (assays 2 and 3), LSR sediment was treated with [^{3}H]PYR and [^{14}C]B*a*P. The sediment, suspended in an aqueous slurry (3900 g of wet sediment and 1,000 ml of well water), was dosed with dropwise additions of 90 μCi of PYR in 160 μl of acetone and 17.7 μCi of B*a*P in 1,800 μl of acetone. These trace levels are less than 0.5% of the resident PYR and B*a*P concentrations in LSR sediment. The slurry was stirred vigorously for 4 h at room temperature, then kept at 4°C for 3 d. The overlying water was decanted and replaced with fresh well water.

Four static renewal assays with *L. variegatus* were conducted to compare dosed versus resident PAH bioaccumulation and to examine the effects of sediment manipulation. Assay 1 was conducted with undosed, gently hand-mixed LSR sediment. This treatment was judged to be the least-disturbance sediment manipulation and was used as a reference for assessing the impacts of slurry homogenization in assays 2 and 3. Each time-point beaker (400 ml) contained 100 g of wet sediment and was replicated three times. The beakers were placed in an aquarium filled with well water allowing for free exchange of water between beakers. On the following day, a group of 40 *L. variegatus* was added to each beaker. This yielded an animal dry weight:sediment OC ratio of 1:100 for LSR sediment and of 1:10 for Lake Michigan sediment. Time-point samples were taken randomly at 8, 24, 48, 96, 168, and 336 h. The Lake Michigan reference sediment was sampled at 168 and 336 h. Half of the well water in the aquarium was changed every day, and oxygen levels remained at greater than 60% saturation.

Assay 2 was conducted with LSR sediment 10 d after dosing with radiolabeled PAHs and slurry homogenization. This bioassay was used to assess the impacts of slurry mixing on resident PAH bioavailability. Additionally, the use of dosed PAHs as surrogates for resident PAHs was evaluated. The bioassays for radiolabeled PAHs required 40 g of wet sediment to be distributed to fifteen 50-ml glass beakers, whereas the lower sensitivity of gas chromatography/mass spectrometry analysis of nonlabeled PAHs required 100 g of sediment to be distributed to nine 400-ml beakers. The beakers were placed in the aquarium filled with well water. On the following day, 12 test organisms were carefully added to each 50-ml beaker, and 40 were added to each of the 400-ml beakers, always maintaining a 1:100 ratio of animal dry weight to sediment OC. The exposures were conducted as in assay 1, including reference sediment assays, except that the large beakers were sampled only after 96, 168, and 336 h.

Assay 3 was similar to assay 2. However, in assay 3, the exposure was run 42 d after dosing the sediment to assess the impact of additional solute–sorbent contact time on bioavailability.

At each sampling time in assays 1, 2, and 3, the following observations and samples were taken from each beaker: the number of *L. variegatus* not burrowed into the sediment was noted for possible sediment-avoidance behavior; the number of surviving *L. variegatus* was recorded for toxicity determinations; surviving animals were measured for wet weight, and PAH body burdens were analyzed by liquid scintillation counting or gas chromatography/mass spectrometry; and the sediment in each beaker was sampled for dry:wet weight ratios and PAH concentrations.

Assay 4 was conducted with undosed but slurried, homogenized LSR sediment aged for 10 d. This assay was run because of the observed mortality in assays 2 and 3. Sediment loading and exposure conditions were the same as in the previous assays, but with assay 4, we wanted to evaluate the effect of adding water and vigorous mixing on the potential toxicity of this sediment. In this assay, only survival of the organisms was monitored at 168 and 336 h.

At the end of each time interval, animals were gently sieved from the sediment, rinsed in well water, counted, blotted dry, and weighed. Gut purging was not intentionally done, but sam-

pling logistics caused a minimum 3-h delay between placing animals in petri dishes filled with well water and weighing the sample. Animal samples for radionuclide analysis (2–4 individuals in quadruplicate) were placed in scintillation cocktail and sonicated with a high-intensity ultrasonic disrupter (Tekmar, Cinncinnati, OH, USA) for 2 min to maximize the extraction of PAHs. Tritium and ^{14}C activities were quantified via liquid scintillation counting after 2 d. The data were corrected for quench using the external standards ratio method after correcting for background. Dosed-sediment samples were taken in duplicate from each beaker for contaminant concentration, dry:wet weight ratios, and OC. The dry:wet weight ratios were determined by weighing a wet sediment sample and then drying at 90°C to constant weight. Radionuclide concentrations in 100 mg of wet sediment were measured using the same method as for animal samples. The OC content was determined by drying sediment samples, treating 100 mg of dry sediment with 1 N HCl to remove carbonates, redrying, and then analyzing OC on a 2400 CHN Elemental Analyzer (Perkin-Elmer, Norwalk, CT, USA).

For nonlabeled PAHs, triplicate animal samples (100–300 mg wet wt) and duplicate sediment samples (~250 mg dry wt from the beginning and end of each assay) were extracted and analyzed as described previously [16]. Limits of detection ranged from 0.6 to 4.5 for organisms or 2 to 18 ng/g for sediments for 250-mg samples. Surrogate recoveries ranged between 60 and 70% for FLE, PHE, and ANT but exceeded 90% for high-molecular-weight PAHs. Recoveries of PAHs from a standard reference marine sediment (NIST SRM 1941) generally exceeded 90%. Percentage relative deviation between duplicates was less than 30% for concentrations greater than 1 ng/g.

The accumulation data were fit to a two-compartment model that provides for a decline in uptake with time [6]:

$$dC_a/dt = k_s C_s^o e^{-\lambda t} - k_d C_a \quad (1)$$

Or, in the integrated form,

$$C_a = [k_s C_s^o (e^{-\lambda t} - e^{-k_d t})]/[k_d - \lambda] \quad (2)$$

where C_a is the concentration of the compound in the organism (ng/g wet wt), C_s^o is the initial contaminant concentration in bulk sediment (ng/g dry wt), k_s is the uptake clearance coefficient of the compound from both pore water and sediment (g dry sediment/g wet organism/h), λ is the rate constant for the change in uptake (1/h), k_d is the depuration rate constant (1/h), and t is time (h).

The mechanism for a decline in uptake is as yet undetermined. It may result from physicochemical processes that cause a loss in bioavailability as described by the term $C_s^o e^{-\lambda t}$. On the other hand, a reduction in uptake may result from a decreasing uptake clearance coefficient (i.e., $k_s e^{-\lambda t}$), a parameter that includes assimilation efficiency and feeding rate. Thus, the λ parameter could incorporate changes occurring in both k_s and C_s^o. The nonlinear fit of accumulation data to Equation 2 yields estimates of k_s, λ, and k_d. Because the number of data points were limited in assays 2 and 3, reliable estimates could not be obtained for all three variables simultaneously. Therefore, the k_d values determined in assay 1 were used to fix k_d in assays 2 and 3, so that k_s and λ could be estimated. It was assumed that k_d was a function of the organism and would have the least amount of change between the various assay conditions. Directly measured depuration rate constants are available from a study using Lake Michigan sediment [16], but the relatively lower OC content of the sediment and differences in the condition of the animals warranted using those values for comparison purposes only.

With compounds for which no evidence of a reduction in uptake during the time of the bioassay was found, a simplified equation was applied:

$$C_a = [k_s C_s (1 - e^{-k_d t})]/k_d \quad (3)$$

The nonlinear fit of data to Equation 3 yields estimates for k_s and k_d. Testing the significance of differences between model estimates or slopes from regressions lines was determined using Student's t test. Differences were considered to be significant when $p < 0.05$.

RESULTS

Most of the animals avoided burrowing into the LSR sediment in all four assays during the first 9 h of exposure. Only in assay 2 was there evidence of worms still on the sediment surface at 24 h, though they were completely burrowed by 48 h. Animals in reference beakers burrowed into Lake Michigan sediment within the first hour.

Exposure of *L. variegatus* to dosed and slurried LSR sediment (assays 2 and 3) resulted in a 35 ± 8% decline in worm number after 14 d relative to initial stocking. For reference sediment assays, an increase in number of 24 ± 11% was observed after 14 d. Average wet worm weights, however, declined similarly ($p < 0.05$) for both sediments, by an average of 61 ± 4% for all LSR assays compared to 58 ± 4% for reference sediment assays after the 14-d exposure. A large portion of the weight loss occurred during the first 48 h (20–30%), especially when the animals avoided the sediment in assay 2. When LSR sediment was minimally mixed (assay 1), worm numbers increased (23 ± 12%), though not to the degree observed in the corresponding reference sediment assays (66 ± 8%, $p < 0.05$). Average worm weights for this assay followed the same decline as noted for the slurried assays. Due to the high mortality observed in the slurried exposures (assays 2 and 3), a fourth assay was run to determine whether vigorous mixing of sediment before exposure induced mortality. Assay 4 was not dosed with radiolabeled compounds. Worm numbers declined by 12 ± 9%, but not to the extent observed in assays 2 and 3 ($p < 0.01$). An increase in worm number of 22 ± 10% in the reference sediment for assay 4 demonstrated that the condition of the animals had not changed since assays 2 and 3 were run ($p < 0.05$).

The accumulation of selected PAHs by *L. variegatus* from LSR sediment during a 14-d period is shown in Figure 1. For minimally mixed LSR sediment, PAH uptake curves fell into three general types of toxicokinetics. One form of behavior, exhibited by low-molecular-weight PAH compounds (group I = FLE, PHE, ANT, PYR, and FLA), involved a rapid initial uptake followed by a significant decline in tissue residue. Peak residues occurred between 2 and 4 d. Only FLE, PHE, and ANT appeared to approach steady state during the 14-d bioassay. A second form of behavior, exhibited by B*a*A and CHR (group II), had slower initial uptake rates followed by a gradual decline in tissue residue. Peak residues occurred at 7 d, and steady state was not achieved within the 14-d assay. A third type of accumulation appeared to be sigmoidal in shape, first exhibiting an asymptotic approach to a low residue level and then a large, linear increase in uptake (group III = B*b*F, B*e*P, B*a*P, PER, IP, and B*g*P). The duration of the assay was too

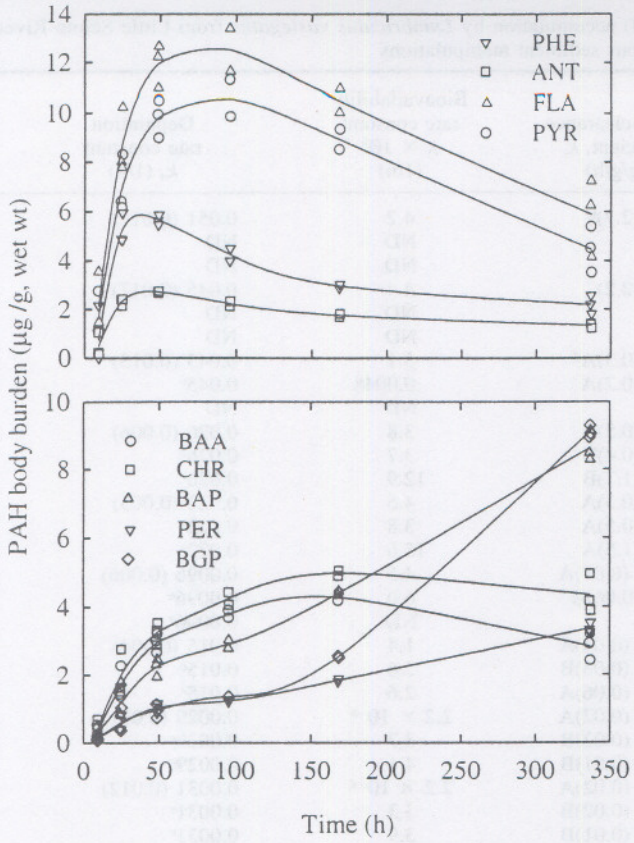

Fig. 1. Resident polycyclic aromatic hydrocarbon (PAH) body burdens of *Lumbriculus variegatus* exposed to minimally mixed Little Scioto River, Ohio, USA, sediment (assay 1). Lines define data trends for phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo[*a*]anthracene (B*a*A), chrysene (CHR), benzo[*a*]pyrene (B*a*P), perylene (PER), and benzo[*g,h,i*]perylene (B*g*P).

short to observe either a possible peak in accumulation or steady state for these high-molecular-weight compounds.

Estimates of toxicokinetic parameters for the first-order depuration/first-order declining availability model (Eqn. 1) of assay 1 are listed in Table 1. Due to the absence of early time points for assays 2 and 3, reliable estimates of three fitting parameters could not be obtained simultaneously. Therefore, the k_d values from assay 1 were used in Equation 1 to allow for an estimation of the other two toxicokinetic parameters. Concentrations of individual PAHs in LSR sediment ranged from 11 to 408 µg/g (dry wt) and remained unchanged for the duration of the bioassays ($p < 0.05$). Uptake clearance coefficients (k_s, mg/g/h) decreased exponentially with increasing log K_{ow} values for assay 1:

$$k_s = (1.35 \times 10^5)\, e^{-2.09 \cdot \log K_{ow}} \quad (4)$$

with $r^2 = 0.961$. Anthracene was omitted from the correlation because of its exceptionally low k_s value. The k_s values ranged from 1.8 to 12.3 mg/g/h for group I, from 0.35 to 0.72 mg/g/h for group II, and from 0.07 to 0.13 mg/g/h for group III.

The initial uptake of some low-molecular-weight PAHs was reduced, whereas others were unaffected, by slurry mixing and by sediment aging. Uptake curves demonstrating the influence of sediment mixing and aging are shown in Figure 2. With group I compounds for which k_s values could be determined, slurrying sediment resulted in no significant change in k_s for ANT and PYR but a significant increase in k_s for FLA (assay 2 vs assay 1). Estimates of k_s for assay 3 (aged 42 d) were not significantly different from assay 2 (aged 10 d) but were less reliable without early time points. However, aging of sediment for 42 d did result in a dramatic drop in low-molecular-weight PAH tissue residues by 7 d and a faster approach to steady state relative to that in assays 1 and 2. With group I compounds for which k_s values could not be determined in assays 2 and 3 due to faster kinetics (e.g., FLE and PHE), initial uptake appeared to be reduced and reached an apparent steady state more quickly.

Initial uptake of high-molecular-weight PAHs was enhanced by sediment slurrying for both time intervals of sediment aging. With group II compounds (i.e., B*a*A and CHR), vigorous mixing of sediments followed by 10 d of equilibration enhanced k_s by a factor of two relative to assay 1 (Fig. 2 and Table 1). Aging the sediment for 42 d resulted in less enhancement but an earlier approach to steady state compared to aging for 10 d. With group III compounds (i.e., B*b*F–PER), vigorous mixing of sediments enhanced k_s by a factor of three relative to assay 1. Aging the sediment for 42 d resulted in the same degree of enhanced uptake relative to the shorter equilibration interval. With the group III compounds IP and BGP, Equation 1 did a poor job of simulating their biphasic uptake behavior (Fig. 1). Consequently, only a rough comparison could be made between assays. Vigorous mixing of sediments (aged 10 d) enhanced k_s values by a factor of two compared to assay 1. Aging the sediment for 42 d resulted in a threefold increase in k_s relative to assay 1.

Similar to the relationship observed in the minimally manipulated assay 1, the slurried assays also exhibited strongly negative exponential relationships of k_s versus K_{ow}. The slopes for the log-normalized regressions of assay 2 ($\log k_s = -0.78$ [0.06] $\cdot \log K_{ow} + 4.67$ [0.39]; $r^2 = 0.952$) and assay 3 ($\log k_s = -0.72$ [0.11] $\cdot \log K_{ow} + 4.31$ [0.69]; $r^2 = 0.863$) were shallower relative to those of assay 1 ($\log k_s = -0.91$ [0.06] $\cdot \log K_{ow} + 5.13$ [0.36]; $r^2 = 0.958$) but were not significantly different ($p < 0.05$).

Generally, the shapes of the uptake curves for dosed B*a*P and PYR by *L. variegatus* (Fig. 3) appeared to be similar to those of resident B*a*P and PYR (Fig. 2). Again, a more rapid loss of available PYR and an earlier approach to steady state were observed in sediment aged 42 d relative to that aged 10 d. However, estimated k_s values were much greater for dosed than for resident PAHs. Uptake clearance coefficients for laboratory-dosed PYR (Table 2) were two- to threefold higher than those of resident PYR in assays 2 and 3 (Table 1). Unlike resident PYR, laboratory-dosed PYR exhibited a significant reduction in k_s as the sediment aged for a longer time period. For laboratory-dosed B*a*P, uptake clearance coefficients (Table 2) were eightfold higher than those of resident B*a*P in assays 2 and 3 (Table 1). Similar to resident B*a*P, no significant difference in availability was noted between assays in which the sediment was aged 10 versus 42 d.

Depuration rate constants for resident PAHs in assay 1, determined as a fitting parameter in Equation 1, are listed in Table 1. A separate measurement of depuration rate constants would have been preferable, but the lack of uncontaminated LSR sediment prohibited such assays. Because the k_d values from assay 1 were used in Equation 1 to estimate the other two toxicokinetic parameters for these assays, k_d values for assays 2 and 3 are not available. Estimated k_d values were compared to those measured for *L. variegatus* in Lake Michigan sediment [16]. Despite having a much lower OC content

Table 1. Model results of resident polycyclic aromatic hydrocarbon (PAH) accumulation by *Lumbriculus variegatus* from Little Scioto River, Ohio, USA, sediment under various sediment manipulations

PAH	Assay[a]	Sediment concentration, C_s^0 (μg/g, dry wt)	Uptake-clearance coefficient, k_s (mg/g/h)	Bioavailability rate constant $\lambda \times 10^3$ (1/h)	Depuration rate constant k_d (1/h)
Flourene	1	11.0	12.3 (2.7)[b]	4.2	0.051 (0.017)
	2	11.9	ND[c]	ND	ND
	3	11.0	ND	ND	ND
Phenanthrene	1	35.0	8.8 (2.2)	4.4	0.045 (0.017)
	2	37.3	ND	ND	ND
	3	36.0	ND	ND	ND
Anthracene	1	81.6	1.8 (0.3)A[d]	3.1	0.045 (0.013)
	2	59.3	2.3 (0.2)A	0.0048	0.045[e]
	3	54.2	ND	ND	ND
Flouranthene	1	115	4.1 (0.5)A	3.8	0.026 (0.006)
	2	101	5.6 (0.4)B	3.7	0.026[e]
	3	95.0	6.1 (1.7)B	12.9	0.026[e]
Pyrene	1	108	3.4 (0.3)A	4.6	0.022 (0.005)
	2	93.8	4.3 (0.5)A	3.8	0.022[e]
	3	93.1	5.3 (1.5)A	15.6	0.022[e]
Benzo[*a*]anthracene	1	112	0.72 (0.07)A	4.5	0.0096 (0.006)
	2	109	1.4 (0.06)B	6.0	0.0096[e]
	3	108	ND	ND	0.0096[e]
Chrysene	1	265	0.35 (0.04)A	1.4	0.015 (0.004)
	2	252	0.71 (0.03)B	2.6	0.015[e]
	3	255	0.46 (0.06)A	2.6	0.015[e]
Benzo[*b*]flouranthene	1	408	0.11 (0.02)A	2.2×10^{-8}	0.0029 (0.011)
	2	339	0.38 (0.02)B	3.7	0.0029[e]
	3	351	0.38 (0.01)B	4.1	0.0029[e]
Benzo[*e*]pyrene	1	254	0.13 (0.02)A	2.2×10^{-8}	0.0031 (0.012)
	2	228	0.35 (0.02)B	3.3	0.0031[e]
	3	232	0.36 (0.01)B	3.9	0.0031[e]
Benzo[*a*]pyrene	1	383	0.11 (0.02)A	5.1×10^{-8}	0.0032 (0.011)
	2	341	0.34 (0.02)B	3.4	0.0032[e]
	3	342	0.36 (0.01)B	3.1	0.0032[e]
Perylene	1	143	0.12 (0.03)A	4.6×10^{-8}	0.0034 (0.013)
	2	122	0.38 (0.02)B	3.9	0.0034[e]
	3	125	0.36 (0.01)B	3.2	0.0034[e]
Indeno[1,2,3-*cd*]pyrene	1	321	0.067 (0.01)A		4.7×10^{-11} (0.0013)
	2	246	0.14 (0.03)B		0.0010 (0.0013)
	3	251	0.20 (0.03)B		0.0014 (0.0011)
Benzo[*g/u*]perylene	1	325	0.076 (0.01)A		4.3×10^{-11} (0.0013)
	2	257	0.14 (0.02)B		0.0012 (0.0014)
	3	262	0.21 (0.03)B		0.0014 (0.0011)

[a] Assay 1 = minimally mixed; assay 2 = slurry mixed, aged 10 d; assay 3 = slurry mixed, aged 42 d.
[b] Values in parentheses are standard errors of the estimates.
[c] ND = not determined.
[d] Dissimilar uppercase letters denote significant difference ($p < 0.05$) between assays for a specific compound.
[e] Used k_d value from assay 1.

(0.5%), Lake Michigan k_d values are not significantly different ($p < 0.05$) from those estimated in this study. The LSR log-normalized depuration rate constants decreased linearly with increasing PAH log K_{ow} values according to the equation

$$\log k_d = -0.59(0.03) \cdot \log K_{ow} + 1.40(0.19) \quad (r^2 = 0.971;\ p < 0.001). \qquad (5)$$

Depuration rate constants for laboratory-dosed PYR and B*a*P (Table 2, assays 2 and 3) were not significantly different ($p < 0.05$) from resident PYR and B*a*P values (Table 1, assay 1).

The k_d values for resident and laboratory-dosed B*a*P have relatively large errors associated with them due to the lack of observations during the downward portion of the curve. Consequently, a longer length of time is required for observing accumulation of B*a*P and other high-molecular-weight PAHs to more accurately determine these toxicokinetic parameters.

Most resident PAHs have λ values that range between 0.0026 and 0.0046 h^{-1} for both minimally mixed and slurry-mixed assays (Table 1). Corresponding half-lives range from 270 to 150 h, respectively. By the end of these 14-d assays, availability (i.e., decline in C_s or k_s) was reduced by 60 to 80%, respectively. Deviations from this range included assay 3, in which λ values were three- to fourfold larger for FLA and PYR. This enhanced loss of availability in aged sediment was apparent for the other group II compounds (B*a*A and CHR), though λ could not be quantified very well (see CHR in Fig. 2). Negligible λ values for high-molecular-weight PAHs (i.e., B*b*F–B*g*P) in assay 1 may have resulted from the short time scale during which the observations were made. Only the linear portion of the curve was seen during the 14-d assay; longer periods may be required to observe any decline in availability. For the faster kinetics exhibited by high-molecular-weight PAHs in assays 2 and 3, uptake curves appeared to level off (see B*a*P in Fig. 2). Associated rate constants were similar to those for low-molecular-weight PAHs in nonaged

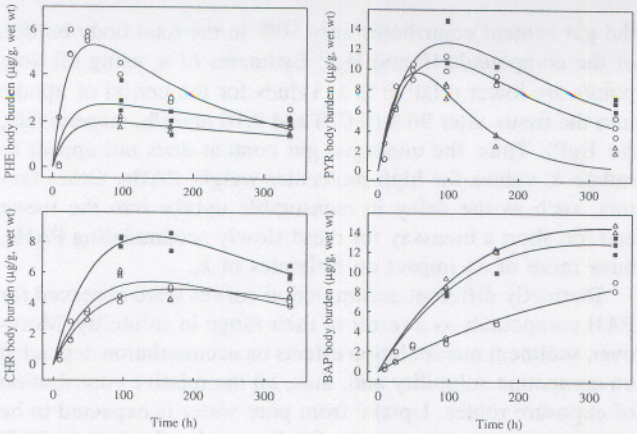

Fig. 2. Resident polycyclic aromatic hydrocarbon (PAH) body burdens of *Lumbriculus variegatus* resulting from exposure to Little Scioto River, Ohio, USA, sediment under various mixing and aging regimes: minimally mixed (○; assay 1), slurried and aged 10 d (■; assay 2), and slurried and aged 42 d (△; assay 3). Lines represent best-fit model results for phenanthrene (PHE), pyrene (PYR), chrysene (CHR), and benzo[*a*]pyrene (B*a*P).

sediment assays. Unlike low-molecular-weight PAHs, sediment aging appears to have no influence on λ values for high-molecular-weight PAHs.

Laboratory-dosed PYR λ values (Table 2) fell within the range seen for most resident PAHs (0.0026–0.0046 h^{-1}). Unlike with resident PYR, no significant increase in λ was observed with dosed PYR aged for 42 versus 10 d. Although laboratory-dosed B*a*P λ values were lower than the corresponding resident B*a*P for assays 2 and 3, large errors are associated with these curves.

Because of the unexpectedly slow kinetics of these bioaccumulation studies, 14 d of exposure was not long enough to reach steady state for most of the PAHs in assays 1 and 2. In assay 3, however, uptake curves for resident and dosed PAH compounds in groups I and II appear to have leveled off (Figs. 2 and 3). The biota–sediment accumulation factors (BSAFs) are calculated by dividing worm tissue concentrations (14 d) normalized to lipid content by sediment concentrations normalized to OC content. The BSAF values for resident PAHs in assay 3 were 0.38 for FLE, 0.28 for PHE, 0.33 for ANT, 0.21 for FLA, 0.16 for PYR, 0.13 for B*a*A, and 0.12 for CHR. For dosed PYR, the BSAF estimate, 0.68, was fourfold higher than the resident PYR value. The apparent BSAFs for resident PAHs decreased exponentially with increasing compound log K_{ow} according to the equation

$$\mathrm{BSAF} = 8.162 \cdot e^{-0.719 \cdot \log K_{ow}} \quad (r^2 = 0.933;\ p < 0.001) \quad (6)$$

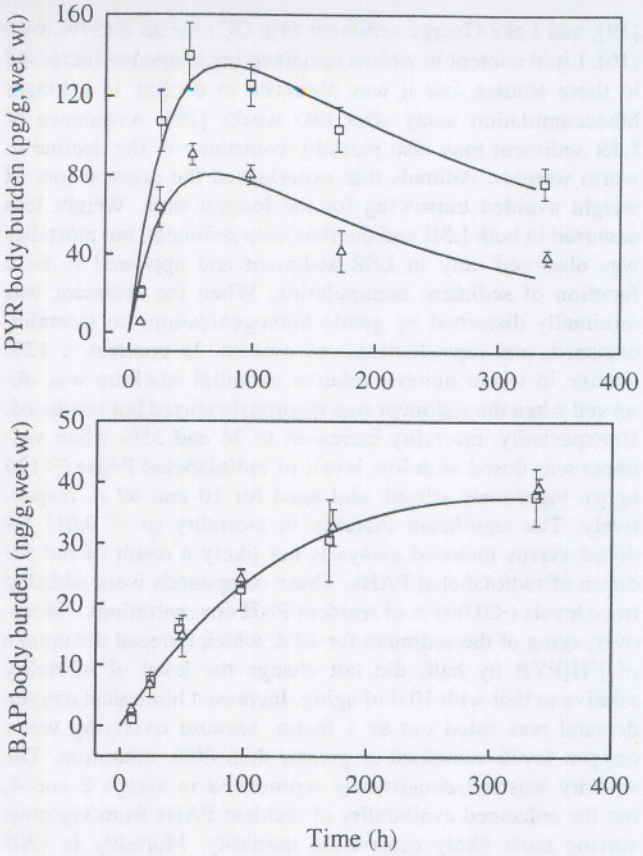

Fig. 3. *Lumbriculus variegatus* body burdens of [^{3}H]pyrene (PYR) and [^{14}C]benzo[*a*]pyrene (B*a*P) dosed into slurries of Little Scioto River, Ohio, USA, sediment and aged for 10 d (□; assay 2) or 42 d (△; assay 3). Error bars represent one standard deviation. Lines represent best-fit model results.

DISCUSSION

Characterizing the bioaccumulation of sediment-associated contaminants by a test species is simplified by holding certain parameters constant: animal weight, lipid content, and animal density. In our study, weight loss, mortality, and reproduction occurred, thus complicating bioaccumulation assessments. The test species *L. variegatus,* incubated in an aquarium with paper toweling as substrate and fed a diet of trout chow, lost weight when transferred to reference sediment with low OC content (2% OC) as well as when exposed to LSR sediment with 9% OC. Similar weight losses were observed previously for these worms when exposed to low OC sediment: Lake Michigan sediment (<1% OC) for 7 d (14% loss) and 28 d (56% loss)

Table 2. Model results of laboratory-dosed polycyclic aromatic hydrocarbon (PAH) accumulation by *Lumbriculus variegatus* from Little Scioto River, Ohio, USA, sediment under various sediment manipulations

PAH	Assay[a]	Sediment concentration C_s^o (ng/g dry wt)	Uptake-clearance coefficient, k_s (mg/g/h)	Bioavailability rate constant λ × 10^3 (1/h)	Depuration rate constant, k_d (1/h)
[^{3}H]pyrene	2	0.415	14.4 (2.1)A[b]	2.7 (0.6)A	0.036 (0.008)A
	3	0.419	9.2 (1.3)B	4.3 (0.9)A	0.036 (0.008)A
[^{14}C]benzo[*a*]pyrene	2	123	2.9 (0.6)A	5.0 × 10^{-9} (1.8)	0.009 (0.006)A
	3	119	2.9 (0.2)A	1.0 (1.4)	0.006 (0.003)A

[a] Assay 2 = slurry mixed, aged 10 d; assay 3 = slurry mixed, aged 42 d.
[b] Values in parentheses are the standard errors of the estimate. Dissimilar uppercase letters denote significant difference ($p < 0.05$) between assays for a specific compound.

[19], and Lake George sediment (3% OC) for 28 d (57% loss) [16]. Lipid content in worms remained unchanged or increased in these studies, but it was observed to decline in a longer bioaccumulation assay after two weeks [19]. Avoidance of LSR sediment may also partially contribute to the decline in worm weights. Animals that experienced the greatest loss of weight avoided burrowing for the longest time. Weight loss occurred in both LSR and the reference sediment, but mortality was observed only in LSR sediment and appeared to be a function of sediment manipulation. When the sediment was minimally disturbed by gentle homogenization, no mortality occurred, and reproduction was evident. In contrast, a 12% decline in worm number relative to initial stocking was observed when the sediment was vigorously stirred but not dosed. Unexpectedly, mortality increased to 36 and 33% when sediment was dosed with low levels of radiolabeled PAHs (<125 ng/g), vigorously stirred, and aged for 10 and 42 d, respectively. The significant increase in mortality ($p < 0.01$) for dosed versus undosed assays is not likely a result of the addition of radiolabeled PAHs. These compounds were added at trace levels (<0.001% of resident PAH concentrations). Moreover, aging of the sediment for 42 d, which reduced the uptake of [^{3}H]PYR by half, did not change the level of mortality relative to that with 10 d of aging. Increased biological oxygen demand was ruled out as a factor, because overlying water oxygen levels remained at greater than 60% saturation. The severity was not consistently reproduced in assays 2 and 4, but the enhanced availability of resident PAHs from vigorous stirring most likely caused the mortality. Mortality in LSR sediment (vigorously mixed, total PAH = 2.2 mg/g) was comparable to that of Lake Michigan sediment dosed with high levels of pyrene (200–300 μg/g) [19].

When bioaccumulation in tissue is low relative to sediment concentrations, the gut contents become an important contribution to an organism's total contaminant concentration [20]. Because our procedure most likely did not allow for complete purging of the gut, an estimate was made to determine the contribution of the residual gut content to the animal's total PAH body burden. According to Mount et al. [20], the sediment in the gut of *L. variegatus* varies from 7 to 37% of its total dry weight (average = 20%). Kukkonen and Landrum [21] measured a value of 14.7 ± 4.8%. The amount of sediment in the gut of a worm for our study, then, would average 0.15 and 0.11 mg (dry wt), estimate based on Mount et al. [20] and Kukkonen and Landrum [21], respectively. The animals were purged for a minimum of 3 h. Therefore, residual sediment in the gut would range from 0.013 to 0.018 mg after 3 h of purging ($t_{1/2}$ = 0.98 h [20]). Another means of estimating residual sediment in the gut assumes that most of the body burden at 48 h for a slowly absorbed compound such as B*g*P is from the gut contents. The mass of sediment needed to account for a total worm concentration of 952 ng/g (wet wt) of B*g*P is 0.013 mg (average wet worm weight = 4.5 mg), which is in agreement with the aforementioned range. Consequently, the contribution of PAHs from gut contents after a 3-h purge ranges from 5 to 24% for group I and II compounds that reached an apparent steady-state concentration. The impact on the BSAF values, therefore, is minimal. Estimates of k_s values are also minimally impacted for these low-molecular-weight PAHs. For the high-molecular-weight PAHs in group III, the majority of uptake appears to result from residual gut contents at times longer than 96 h. A significant increase in uptake rate occurs after 96 h, with most occurring in the tissue. By 14 d, the gut content contributes only 10% to the total body burden of the compounds IP and B*g*P. Estimates of k_s using all time points are lower relative to k_s values for the period of uptake into the tissue after 96 h (0.076 and 0.10 mg/g/h, respectively, for B*g*P). Thus, the unpurged gut content does not appear to inflate k_s values for high-molecular-weight PAHs. Other factors, such as the delay in measurable uptake into the tissue and too short a bioassay for these slowly accumulating PAHs, have more of an impact on estimates of k_s.

Distinctly different accumulation curves were observed for PAH compounds as a result of their range in solubility. Moreover, sediment manipulation effects on accumulation depended on compound solubility and, thus, on the relative contribution of exposure routes. Uptake from pore water is expected to be an important exposure route for low-molecular-weight PAH compounds due to their higher solubilities, whereas ingestion of sediment particles is the major route of exposure for high-molecular-weight PAHs [6,22–24]. Accumulation by *L. variegatus* of group I low-molecular-weight PAHs was characterized by a peak in uptake at 48 h and both reduced and enhanced availability as a result of sediment slurrying. An explanation for this low-molecular-weight PAH accumulation behavior may involve a reduction in the amount of labile fraction (i.e., freely dissolved, rapidly desorbed) of PAH compound as the assay progresses. Possible mechanisms for this loss include bioaccumulation into tissue, packaging within fecal material [6], and microbial degradation. The nonlabile fraction of sediment-associated PAH is kinetically limited from reaching equilibrium with the dissolved phase due to impeded desorption. Slurrying the sediment is expected to increase the bioavailability of the nonlabile fraction by disrupting aggregates and enhancing mass transfer between the sorbed and dissolved phases. Aging is expected to reduce bioavailability of nonequilibrated compounds by allowing diffusion of PAHs further within the aggregate particle and increasing the nonlabile fraction of compound, which is a process known as sequestration. For group I PAHs, slurrying sediment reduced availability of the more soluble compounds (i.e., FLE and PHE) but enhanced bioavailability for PAHs with lower solubilities (i.e., ANT in particular, PYR and FLA only a suggestion). The ANT solubility is intermediate between those of group I and group II PAHs. Aging the sediment for 42 d resulted in reduced availability for all compounds, but not as significantly for FLE and PHE. Enhanced accumulation of group II and III PAHs (i.e., B*a*A–B*g*P) by *L. variegatus* resulted from the slurrying of sediments. For these strongly sorbed compounds, desorption is probably enhanced, finer particles are more available for ingestion, or both. Aging reduced the availability of B*a*A and CHR but had no influence on the high-molecular-weight PAHs B*b*F–PER. For IP and B*g*P, aging enhanced uptake, but not significantly.

A comparison of PAH isomer ratios in *L. variegatus* tissue relative to those of bulk sediment further suggests that compound solubility is influential in controlling bioaccumulation. The ratio of the tricyclic isomers PHE and ANT points toward a preferential accumulation of PHE over ANT. In sediment, the PHE:ANT ratio was 0.49 ± 0.03, whereas in tissue, the ratio was 2.1 ± 0.2 for 96 h or less in assay 1, despite having similar log K_{ow} values. The tissue ratio remained at 1.7 thereafter. This implies that PHE was more available to the worm than ANT; that is, PHE had a larger labile fraction. It is possible that a relatively larger labile fraction for PHE arises from an aqueous solubility (6.62 mmol/m^3) that is approximately 16-

fold greater than that of ANT (0.42 mmol/m^3) [25]. In contrast, accumulation of PHE for the slurried assays was half that of the minimally mixed sediment, whereas accumulation of ANT was similar. The ratio in tissue was only 0.9 to 1.1 at 96 h and was further reduced, to a level not significantly different from that in sediment (0.6–0.7), after 14 d. Thus, it would appear that PHE was depleted from the labile pool much more rapidly in slurried than in minimally mixed assays. Ratios of other isomers, such as FLA:PYR and B*e*P:B*a*P, were not significantly enriched in tissue relative to sediment, and they did not change with time. The difference in solubilities between these pairs is, at most, a factor of 1.8.

One possible influence on the worms' uptake of PAHs is extensive mineralization of the dissolved-phase PAH by bacteria. Because of the high level of PAH contamination at this site, it is expected that microbes capable of utilizing PAHs for growth are present [26,27]. Low-molecular-weight PAHs, such as naphthalene and PHE, are known to degrade rapidly in sediments from the dissolved phase, whereas strongly sorbed PAHs, such as B*a*A and B*a*P, are more resistant to microbial attack [26–29]. The sharp decline of FLE and PHE in worm tissue between 2 to 4 d in assay 1 requires a large removal process, more than that for all the other compounds. Even with an exponential loss coefficient, the model overpredicts body burdens for PHE and FLE after the peak for assay 1. A diminished supply of FLE and PHE in the dissolved phase (before the exposure assay) would also explain the reduced peak at 48 h and the faster approach to steady state for the mixed and aged assays relative to the minimally mixed assay. Previous studies showed that slurrying sediment enhanced both the rate and extent of biodegradation of PAHs, probably as a result of improved equilibration between dissolved and sorbed phases, and the disruption of aggregates [17,18]. Other bioaccumulation studies have reported a rapid decline in accumulation of PHE in both water-only [30] and bulk-sediment exposures [6,15,16,31,32], along with an unexplained loss of PHE in exposure media. Recently, Jager et al. [33] hypothesized that the cause of the peak in accumulation of PAHs by earthworms was due to microbial degradation of the more available pore-water fraction. Clearly, further studies are needed to identify under what field conditions microbial degradation becomes an important competing process with bioaccumulation.

Reduction of the uptake clearance rate with increased exposure length may also result from a narcotic effect of nonpolar compounds on animal physiology [32,34]. Mortality and weight loss were evident for *L. variegatus* exposed to vigorously stirred LSR sediment; consequently, feeding and respiration may have been impacted as well. Body burdens averaged 0.37 mmol/kg for total PAHs, approaching the range of the critical body burden (2–8 mmol/kg) required to produce 50% mortality in aquatic organisms [35]. The reduction in the clearance uptake rate would have to be substantial to achieve the low low-molecular-weight PAH levels observed after 7 d. A similar decline in k_s would be expected for high-molecular-weight PAHs if the feeding rate is controlling accumulation. Looking at λ (assay 2 in Table 1), which can reflect loss of bioavailability due to a physiological response, values range from 0.0026 to 0.006 for FLA–PER and are not significantly different ($p < 0.05$). For the minimally mixed assay 1, λ values for FLE–B*a*A are also very similar, ranging from 0.0031 to 0.0046. Reductions in egestion rates due to contaminant exposure have been observed for oligochaetes [36,37] and polychaetes [38]. Thus, a reduction in feeding rate could possibly account for the downturn in all PAH accumulation curves, regardless of when the peak occurs. The large elimination rates for the low-molecular-weight PAHs result in an early and sharp decline in accumulation, whereas the small elimination rates of high-molecular-weight PAHs result in a slower decline.

Laboratory-dosed PAHs were expected to show more evidence of increased sequestration when sediment was aged than resident PAHs, which had more time to approach equilibrium. As expected, k_s values for dosed PYR declined with sediment aging. Whereas the accumulation of resident PYR appears to be lower the longer that it is aged, comparison of k_s values is difficult due to the lack of early time points for assays 2 and 3. The rate for loss of available resident PYR (λ) was greater with increased aging, unlike dosed PYR λ values, which were unchanged. The unexpected reduction in availability of resident PYR aged for 42 d (relative to both assays 1 and 2) implies vigorous mixing altered the pseudoequilibrium partitioning by enhancing sequestration. Even after 42 d of equilibration, the larger k_s values for dosed versus resident PYR (factors of two to three) illustrate how significantly contact time impacts bioavailability. For dosed B*a*P, k_s values are eightfold greater than resident B*a*P values. Additional aging for 32 d was too short a time to impact B*a*P k_s values or to estimate λ. The slow sorption rates of B*a*P into this high-percentage OC sediment requires long equilibration periods for dosed B*a*P to mimic resident B*a*P kinetics. Assuming that the minimally mixed k_s values for resident PAHs are fairly representative of LSR field bioavailability, dosed assays overestimated the availability of PYR by factors of 3 to 4 and of B*a*P by a factor of 26.

Comparison of *L. variegatus* uptake-clearance coefficients in LSR sediment relative to sediments with lower-percentage OC (<1%) reveals comparable availability of dosed B*a*P but greater availability of dosed PYR for this higher-percentage OC sediment. When k_s values are normalized for OC (k_{uoc}, mg OC/g/h), B*a*P LSR values for assays 2 and 3 (0.243 and 0.240 mg OC/g/h, respectively) are similar to those for Lake Michigan (0.246 mg OC/g/h [21]). Values of k_{uoc} for dosed PYR are higher in LSR sediment (1.20 and 0.76 mg OC/g/h for assays 2 and 3, respectively) than in Lake Michigan sediment (0.276 ± 0.019 mg OC/g/h [19] and 0.237 ± 0.004 [39]) and Lake Höytiäinen's 3.6% OC sediment (0.170 mg OC/g/h [22]). The high level of PAH contamination in LSR may have possibly enhanced the uptake of dosed PYR. A similar toxicokinetic effect was observed for *Diporeia* sp. accumulation of PYR when exposed to a mixture of PAHs [14,32].

The *L. variegatus* uptake-clearance coefficients of resident PAHs in creosote-contaminated LSR sediment are greater than those measured for a surficial sediment contaminated with combustion-derived particulate PAHs, but they are equal to or less than those measured at depth in Lake George (part of the St. Mary's river) in northern Michigan, USA. Harkey et al. [16] observed almost no measurable accumulation of any PAHs by *L. variegatus* from the 0- to 4-cm interval of cores taken from Lake George, despite the sediment having similar PAH concentrations to those of sediment at deeper intervals. At the 12- to 16-cm interval, however, k_{uoc} values for PHE, PYR, and FLA were not significantly different from those of LSR ($p < 0.05$), whereas high-molecular-weight PAH coefficients were 2- to 10-fold higher than those of LSR. Both exposures describe a strong, inverse relationship of log k_{uoc} with log K_{ow} (log $k_{uoc} = -0.9091 \cdot \log K_{ow} + 1.081$, with $r^2 = 0.958$ for LSR; log $k_{uoc} = -0.4242 \cdot \log K_{ow} - 1.237$, with

$r^2 = 0.902$ for Lake George). For Lake George toxicokinetics, evidence of peaks in accumulation even for the high-molecular-weight PAHs could be seen due to faster kinetics and longer length of the bioassay (28 d). The disparate accumulation kinetics of Lake George and LSR may be due to differences in sediment composition (e.g., 3% versus 9% OC), contaminant concentrations (ppb versus ppm), or the source of PAH (creosote, a liquid, versus particle-bound soot). Diagenetic processes may be responsible for the increased availability of PAHs downcore in Lake George.

Apparently, BSAF estimates for *L. variegatus* exposed to LSR sediment are not reliable due to the slow accumulation kinetics observed for these compounds. Selection of a 14-d assay was based on previous experience with faster kinetics occurring in lower-percentage OC sediments where the sediment was stirred vigorously. The organisms in assay 3 appeared to reach a steady state for low-molecular-weight PAHs, but whether the sediment manipulation altered the available fraction of the compounds is not clear. Comparison of these types of assays with in situ exposures or native species collected along with the sediment would help to elucidate associated artifacts.

As a result of this study, we have the following recommendations regarding a standardized protocol for bioaccumulation tests using *L. variegatus*. First, minimize weight loss of the animal during the assay by preconditioning the organism in clean sediment with a percentage OC similar to that of the test sediment. Second, minimize mortality by testing the toxicity of the sediment before any bioaccumulation assay. Third, minimize reproduction impacts by using smaller individuals (5–9 mg wet wt) that divide to a lesser degree, as recommended by Leppänen and Kukkonen [22]. Fourth, assess whether gut contents will unduly influence body-burden measurements. Fifth, perform a preliminary assay to determine the length of time required to achieve steady state with all compounds of interest. Finally, minimize sediment manipulations to avoid altering contaminant bioavailability.

Acknowledgement—We wish to thank Allen Burton and his students for collection of the LSR sediment samples. We also wish to thank Warren Faust for his technical assistance in performing sample extractions. This paper represents Great Lakes Environmental Research Laboratory contribution 1182.

REFERENCES

1. Landrum PF, Faust WR. 1991. Effect of variation in sediment composition on the uptake rate coefficient for selected PCB and PAH congeners by the amphipod *Diporeia* sp. In Mayes MA, Barron MG, eds, *Aquatic Toxicology and Risk Assessment,* Vol 14. STP 1124. American Society for Testing and Materials, Philadelphia, PA, pp 263–279.
2. Tracey GA, Hansen DJ. 1996. Use of biota-sediment accumulation factors to assess similarity of nonionic chemical exposure to benthically-coupled organisms of differing trophic mode. *Arch Environ Contam Toxicol* 30:467–475.
3. Ma WC, van Kleunen A, Immerzeel J, De Maagd PGJ. 1998. Bioaccumulation of polycyclic aromatic hydrocarbons by earthworms: Assessment of equilibrium partitioning theory in in situ studies and water experiments. *Environ Toxicol Chem* 17:1730–1737.
4. Farrington JW, Goldberg ED, Risebrough RW, Martin JH, Bowen VT. 1983. U.S. "Mussel Watch" 1976–1978: An overview of the trace metal, DDE, PCB, hydrocarbon, and artificial radionuclide data. *Environ Sci Technol* 17:490–496.
5. Prahl FG, Carpenter R. 1983. Polycyclic aromatic hydrocarbon (PAH)-phase associations in Washington coastal sediment. *Geochim Cosmochim Acta* 47:1013–1023.
6. Landrum PF. 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi. Environ Sci Technol* 23:588–595.
7. McGroddy SE, Farrington JW. 1995. Sediment porewater partitioning of polycylic aromatic hydrocarbons in three cores from Boston Harbor, Massachusetts. *Environ Sci Technol* 29:1542–1550.
8. Brusseau ML, Rao PSC. 1989. Sorption nonideality during organic contaminant transport in porous media. *Crit Rev Environ Control* 19:33–99.
9. Chiou CT. 1989. Theoretical considerations of the partition uptake of nonionic organic compounds by soil organic matter. In Sawhney BL, Brown K, eds, *Reactions and Movement of Organic Chemicals in Soils.* Soil Science Society of America, Madison, WI, pp 1–29.
10. Steinberg SM, Pignatello JJ, Sawhney BL. 1987. Persistence of 1,2-dibromoethane in soils: Entrapment in intraparticle micropores. *Environ Sci Technol* 21:1201–1208.
11. Pignatello JJ. 1990. Slowly reversible sorption of aliphatic halocarbons in soils. *Environ Toxicol Chem* 9:1107–1115.
12. Chiou CT, McGroddy SE, Kile DE. 1998. Partition characteristics of polycyclic aromatic hydrocarbons on soils and sediments. *Environ Sci Technol* 32:264–269.
13. Varanasi U, Reichert WL, Stein JE, Brown DW, Sanborn HR. 1985. Bioavailability and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment from an urban estuary. *Environ Sci Technol* 19:836–841.
14. Landrum PF, Eadie BJ, Faust WR. 1991. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia* sp. *Environ Toxicol Chem* 10:35–46.
15. Landrum PF, Eadie BJ, Faust WR. 1992. Variation in the bioavailability of polycyclic aromatic hydrocarbons to the amphipod *Diporeia* (spp.) with sediment aging. *Environ Toxicol Chem* 11: 1197–1208.
16. Harkey GA, Van Hoof PL, Landrum PF. 1995. Bioavailability of polycyclic aromatic hydrocarbons from a historically contaminated sediment core. *Environ Toxicol Chem* 14:1551–1560.
17. Mueller JG, Lanz SE, Blattman BO, Chapman PJ. 1991. Benchmark scale evaluation of alternative biological treatment processes for the remediation of PCP- and creosote-contaminated materials: Slurry phase bioremediation. *Environ Sci Technol* 25: 1055–1061.
18. White JC, Alexander M, Pignattello JJ. 1999. Enhancing the bioavailability of organic compounds sequestered in soil and aquifer solids. *Environ Toxicol Chem* 18:182–187.
19. Kukkonen J, Landrum PF. 1994. Toxicokinetics and toxicity of sediment-associated pyrene to *Lumbriculus variegatus* (Oligochaeta). *Environ Toxicol Chem* 13:1457–1468.
20. Mount DR, Dawson TD, Burkhard LP. 1999. Implications of gut purging for tissue residues determined in bioaccumulation testing of sediment with *Lumbriculus variegatus. Environ Toxicol Chem* 18:1244–1249.
21. Kukkonen J, Landrum PF. 1995. Effects of sediment-bound polydimethylsiloxane on the bioavailability and distribution of benzo[*a*]pyrene in lake sediment to *Lumbriculus variegatus. Environ Toxicol Chem* 14:523–531.
22. Leppänen MT, Kukkonen JVK. 1998. Relative importance of ingested sediment and pore water as bioaccumulation routes for pyrene to oligochaete (*Lumbriculus variegatus,* Müller). *Environ Sci Technol* 32:1503–1508.
23. Landrum PF, Robbins JA. 1990. Bioavailability of sediment-associated contaminants to benthic invertebrates. In Baudo R, Geisy JP, Muntau H, eds, *Sediments: Chemistry and Toxicity of In-Place Pollutants.* Lewis, Boca Raton, FL, USA, pp 237–263.
24. Kukkonen J, Landrum PF. 1995. Measuring assimilation efficiencies for sediment-bound PAH and PCB congeners by benthic organisms. *Aquat Toxicol* 32:75–92.
25. Miller MM, Wasik SP, Huang GL, Shiu WY, Mackay D. 1985. Relationships between octanol-water partition coefficients and aqueous solubility. *Environ Sci Technol* 19:522–529.
26. Herbes SE, Schwall LR. 1978. Microbial transformation of polycyclic aromatic hydrocarbons in pristine and petroleum-contaminated sediments. *Appl Environ Microbiol* 35:306–316.
27. Heitkamp MA, Cerniglia CE. 1987. Effects of chemical structure and exposure on the microbial degradation of polycyclic aromatic hydrocarbons in freshwater and estuarine ecosystems. *Environ Toxicol Chem* 6:535–546.

28. Wodzinski RS, Coyle JE. 1974. Physical state of phenanthrene for utilization by bacteria. *Appl Microbiol* 27:1081–1084.
29. Bauer JE, Capone DG. 1985. Degradation and mineralization of the polycyclic aromatic hydrocarbons anthracene and naphthalene in intertidal marine sediments. *Appl Environ Microbiol* 50:81–90.
30. Van Hattum B, Cid Montanes JF. 1999. Toxicokinetics and bioconcentration of polycyclic aromatic hydrocarbons in freshwater isopods. *Environ Sci Technol* 33:2409–2417.
31. Roesijadi G, Anderson JW, Blaylock JW. 1978. Uptake of hydrocarbons from marine sediments contaminated with Prudhoe Bay crude oil: Influence of feeding type of test species and availability of polycyclic aromatic hydrocarbons. *J Fish Res Board Can* 35:608–614.
32. Landrum PF, Dupuis WS, Kukkonen J. 1994. Toxicokinetics and toxicity of sediment-associated pyrene and phenanthrene in *Diporeia* spp.: Examination of equilibrium-partitioning theory and residue-based effects for assessing hazard. *Environ Toxicol Chem* 13:1769–1780.
33. Jager T, Anton Sanchez FA, Muijs B, van der Velde EG, Posthuma L. 2000. Toxicokinetics of polycyclic aromatic hydrocarbons in *Eisenia andrei* (Oligochaeta) using spiked soil. *Environ Toxicol Chem* 19:953–961.
34. Kane Driscoll S, Landrum PF, Tigue E. 1997. Accumulation and toxicokinetics of fluoranthene in water-only exposures with freshwater amphipods. *Environ Toxicol Chem* 16:754–761.
35. Landrum PF, Lee H II, Lydy MJ. 1992. Toxicokinetics in aquatic systems: Model comparisons and use in hazard assessment. *Environ Toxicol Chem* 11:1709–1725.
36. Keilty TJ, White DS, Landrum PF. 1988. Sublethal responses to endrin in sediment by *Limnodrilus hoffmeisteri* (Tubificidae), and in mixed culture with *Stylodrilus heringianus* (Lumbriculidae). *Aquat Toxicol* 13:227–250.
37. Lotufo GR, Fleeger JW. 1996. Toxicity of sediment-associated pyrene and phenanthrene to *Limnodrilus hoffmeisteri* (Oligochaeta: Tubificidae). *Environ Toxicol Chem* 15:1508–1516.
38. Weston DP. 1990. Hydrocarbon bioaccumulation from contaminated sediment by the deposit-feeding polychaete *Abarenicola pacifica*. *Mar Biol* 107:159–169.
39. Harkey GA. 1993. Investigation of the bioavailability of sediment-associated hydrophobic organic contaminants via laboratory bioassays. PhD thesis. Clemson University, Clemson, SC, USA.