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BIOAVAILABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS FROM A HISTORICALLY CONTAMINATED SEDIMENT CORE

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Abstract—To determine changes in bioavailability of selected polycyclic aromatic hydrocarbons (PAHs) with sediment aging, *Lumbriculus variegatus* were exposed for 4 weeks to sediment core sections taken from a contaminated lake. Core depths included surficial (0 to 4 cm), 4- to 8-, 12- to 16-, 28- to 32-, and 44- to 48-cm sections deposited from approximately 1899 to 1993, and were known to be historically contaminated with PAHs. Bioaccumulation was maximal at the 12- to 16-cm depth (circa 1967) where sediment PAH concentrations were greatest. Accumulation was generally below detection limits in the 0- to 4-cm depths, even though sediment concentrations of some compounds were comparable to those at the 12- to 16-cm depth where accumulation was great enough to generate accurate kinetics curves. Accumulation peaked at about 96 h, then declined over the remainder of the study for the lower-molecular-weight PAHs. For most higher-molecular-weight PAHs, accumulation peaked at about 2 weeks, then declined only slightly after 4 weeks. The differential bioavailability observed between surficial and at-depth core sections raises questions concerning the adequacy of results generated from toxicity and bioaccumulation tests routinely conducted with surficial sediments.

Keywords—*Lumbriculus variegatus* Bioavailability Sediment core Polycyclic aromatic hydrocarbons Bioaccumulation

INTRODUCTION

Differential bioavailability of neutral organic contaminants from sediment has been shown to arise from relationships among the contaminant, sediment particles, and the duration that these substances are in contact with each other [1–3]. Enhanced uptake of some polycyclic aromatic hydrocarbons (PAHs) by bioassay organisms was observed when animals were exposed to recently dosed sediments versus historically contaminated or dosed and aged sediments in the laboratory [3,4]. Bioavailability of some PAH compounds was shown either to change or stay the same when organisms were exposed to dosed sediments left to "age" for certain lengths of time. For example, PAH compounds such as fluorene, phenanthrene, and pyrene were more available to test species, as determined by uptake clearances, in dosed sediment aged less than 1 week than in that dosed and aged 60 to 150 d [3,4]. However, in the same studies, no differential bioavailability was observed for compounds such as fluoranthene and benzo[*a*]pyrene (BAP) in aged versus nonaged sediments. Although differential bioavailability has only been well documented for PAH compounds, this phenomenon may exist for other classes of contaminants as well.

The bioavailability of some PAH compounds has been examined in cores taken from field-collected sediments. Contrary to the lack of change in bioavailability of BAP seen in the laboratory-dosed sediments, a significant increase in calculated accumulation factors (AFs) as a measure of bioavailability was observed in surficial sediments (0- to 2-cm depth; i.e., recently contaminated sediment) versus material taken at 4- to 8-cm depth (i.e., "aged" sediment) [5]. Little change in AF was seen for compounds such as pyrene and chrysene, indicating a minimal change in bioavailability of these compounds.

To investigate further the effect of sediment aging on bioavailability of PAHs, the oligochaete worm *Lumbriculus variegatus* was exposed to sediment core sections taken from Lake George (part of the St. Mary's River) in northern Michigan. Previous surveys have shown this area to be historically contaminated with PAHs, with peak contaminant concentrations occurring in the 1970s [6,7]. Although contaminant concentrations in surficial sediments of Lake George are estimated to be below effects levels, populations of benthic invertebrates continue to be depressed [8]. Our objectives were to determine uptake of selected PAHs in bioaccumulation assays over a period of 4 weeks and to compare uptake kinetics of selected PAH compounds in surficial sediments with those found at depth.

We expected PAH accumulation in animal tissue to decrease with core depth, assuming a loss of bioavailability with increased sediment–contaminant contact time. To determine bioavailability differences, kinetic modeling of contaminant uptake and accumulation factors were calculated for compounds in a variety of core depths reflecting contaminant contact time and sediment diagenesis. Differences in bioavailability of PAHs to bioassay organisms that may occur with sediment depth would be important in estimating hazard associated with sediment contamination in areas where mixing occurs, as due to dredging or storm events.

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METHODS

Animals

Lumbriculus variegatus were used for the study because of their continuous feeding behavior and inability to biotransform PAH compounds [4]. Animals were reared in the laboratory at room temperature in 37-L glass aquaria, according to Phipps et al. [9]. Shredded and presoaked unbleached paper towels were used as a substrate, and the animals were fed a diet of trout chow three times per week. A flow of 8 to 10 L of fresh well water per day was passed through the aquaria.

Sediment

Bottom sediments were collected at a 16-m depth in October 1993 from Lake George (46°29.437′ N, 84°07.479′ W). A 25- × 25-cm box corer was used that allowed collection of an undisturbed core that included the uppermost flocculent layer. Cores were inspected for their integrity immediately upon recovery. Four replicate subcores from each of two box corers (designated cores C and E) were collected and extruded from the core sampler into 10-cm-diameter polybutyrate tubes. Vacuum was applied to the top of the tubes as they were inserted into the sediment to minimize compaction. Cores were cut into 4-cm sections. All four replicate subcore 4-cm sections from the same depth were composited into 1-quart solvent-rinsed glass jars with foil-lined caps. In addition, another subcore from a separate box core was sectioned into 1-cm sections for ^{210}Pb radiometric dating studies. All sample jars were kept cool (approx. 4°C) in the dark until used in laboratory exposures. To obtain homogeneous aliquots in replicate exposure beakers, composited sections were manually mixed prior to use in bioaccumulation assays. As few indigenous organisms were observed in the cores, sediment was not sieved prior to use.

Reference sediment used in the bioaccumulation and depuration assays was collected from Lake Michigan. The sediment was collected at a 45-m depth by Ponar grab approximately 8 km off the coast of Grand Haven, Michigan. The sediment was passed through a 1-mm screen sieve to remove debris and indigenous organisms before use in the assays. This sediment has previously been used as a negative control [10], and minimal uptake of PAHs by *L. variegatus* was expected.

Bioaccumulation assay

Sediment core sections (0- to 4-cm, 4- to 8-cm, 12- to 16-cm, and 28- to 32-cm depths) approximating 100 g dry weight were placed into 300-ml glass beakers. Peak concentrations of PAHs were estimated to be present in the 12- to 16-cm core sections, according to sedimentation rates and ^{210}Pb radiometric dating studies [6]. Single or duplicate exposure beakers were prepared for each of the sampling intervals, 6 h, 24 h, 48 h, 96 h, 1 week, 2 weeks, and 4 weeks. Single exposure beakers were used for two of the sampling intervals (96 h and 2 weeks) because of the limited amount of sediment available. Beakers that contained sediment from the 44- to 48-cm depth of core C (preindustrial-era depth) and beakers containing the Lake Michigan sediment were prepared as control and reference sediment exposures, respectively. The Lake Michigan reference sediment was used as a control for mortality, as a previous study used this sediment [10]. Therefore, results of organism lipid and weight analysis from this work could be compared to the previous study. Five groups of 40 *L. variegatus* were taken from the culture aquaria, blotted dry, and weighed to determine mean wet weight at the beginning of the study.

Beakers were filled with fresh well water, then allowed to settle overnight before animals were added. Forty *L. variegatus* (approximating a 1:30 dry worm weight to sediment organic carbon ratio) were placed into each beaker the next day. Each beaker was placed into an aquarium with aerated well water extending 5 cm over the top of the beakers. Half of the aquarium water was discarded and replaced with fresh water every other day during the 28-d exposure period. Oxygen concentration and pH were monitored weekly. Oxygen concentration remained over 90% of saturation, and pH ranged from 8.3 to 8.5 over the course of the experiment. At the end of each exposure interval, the number of animals on the sediment surface and any dead animals were noted. Animals were removed from the sediment and allowed to purge gut contents by standing 24 h in fresh, clean well water before wet-weight analysis and subsequent contaminant concentration or lipid determination. Wet weights were determined by blotting excess water from groups of worms recovered from individual exposure beakers and then weighing on tared foil. After weighing and counting, animals for chemical analysis were placed onto acetone-rinsed aluminum foil and frozen until extraction. In addition, a sample of sediment from exposure beakers was taken after each sampling interval, placed into a solvent-rinsed vial, and refrigerated for later dry:wet weight, contaminant, and organic carbon analyses. The accumulation assay and all analytical work were performed under gold fluorescent light ($\lambda > 500$ nm) to minimize photodegradation of the PAHs. All exposures were conducted at ambient temperature (17 ± 2°C).

Depuration study

Readily available radiolabeled PAHs were used to determine depuration rates in *L. variegatus*: [12-^{14}C]benz[*a*]anthracene (BAA, 49 mCi/mmol, Amersham Ltd., Amersham, UK); [9-^{14}C]phenanthrene (PHE, 13.1 mCi/mmol, Sigma Chemical Co., St. Louis, MO); [3-^{14}C]fluoranthene (FLA, 55 mCi/mmol, Sigma Chemical); [7-^{14}C]benzo[*a*]pyrene (BAP, 16.2 mCi/mmol, Sigma Chemical); and [^{3}H]chrysene (CHR, 340 mCi/mmol, Sigma Chemical). All compounds were dissolved in an acetone carrier. Compound radiopurity was greater than 95% for all compounds prior to use, as determined by thin-layer chromatography, using hexane:benzene (8:2, v/v) and liquid scintillation counting (LSC).

Individual depuration experiments were conducted for each radiolabeled PAH. Well water was dosed with nanomolar concentrations of the contaminant, mixed, and allowed to stand for 2 h prior to adding *L. variegatus*. Animals were exposed for 6 to 20 h (exposure intervals were terminated when sufficient activities, e.g., >5,000 dpm per organism, of the radioisotopes in animal tissues were reached). After exposure, groups of 12 animals were placed into individual beakers containing 50 g wet-weight Lake Michigan sediment with overlying well water for depuration. Animals were sam-

pled from depuration media at 6, 24, 48, 72, 96, and 120 h. Additional samples were taken at 140 h and 256 h for BAA and BAP depuration studies. Animals were gently sieved from the sediment, weighed, and counted for radioisotope activity via LSC.

Analyses

Sediment and animal tissue were analyzed for 13 individual PAH compounds and 2 compound groups: fluorene (FLE), phenanthrene, anthracene (ANT), fluoranthene, pyrene (PYR), benz[*a*]anthracene, benzo[*b*]fluoranthene (BBF), benzo[*e*]pyrene (BEP), benzo[*a*]pyrene, perylene (PER), indeno[*1,2,3-cd*]pyrene (IP), dibenz[*a,h*]anthracene (DBA), benzo[*ghi*]perylene (BGP), chrysene/triphenylene, and benzo[*k*]fluoranthene/benzo[*j*]fluoranthene (BKF/BJF). Individual compounds could not be differentiated for the chrysene/triphenylene (CHR) and benzo[*j*]fluoranthene (BJF) groups according to chromatogram retention times, and data for these groups are reported as mixtures of the two compounds. Sediment samples of 3 to 8 g wet weight were weighed into 250-ml amber glass bottles fit with Teflon-lined screw caps and spiked with four deuterated surrogate standards (d_8-naphthalene, d_{10}-anthracene, d_{12}-benz[*a*]anthracene, and d_{12}-benzo[*e*]pyrene) added directly to the sediment surface. Precombusted sodium sulfate was added to desiccate the sediment. Samples were mixed with 100-ml extraction solvent (1:1 methylene chloride:acetone, v/v), then pulse-sonicated for 4 min using a Tekmar high-intensity ultrasonic processor (375 W at 20% power). After an 18- to 24-h settling period, extraction solvents were decanted onto glass wool-plugged champagne columns. Three additional 10-ml volumes of extraction solvent were added, one at a time, and decanted, for a total extraction solvent volume of 130 ml. Samples were evaporated under nitrogen to 5 ml using a Turbo-Vap® processor. Cyclohexane (20 ml) was added and the samples evaporated to 1 ml prior to cleanup.

Animal tissue samples (50 to 120 mg wet weight) were placed in a Ten Brock tissue homogenizer and spiked with surrogate standards. Samples were extracted with 2 × 6 ml ethyl acetate:acetone (5:1, v/v), followed by extraction with 2 × 5 ml cyclohexane. The extracts were combined and evaporated to 1 ml, exchanged into hexane, and evaporated to a final volume of 1.0 ml.

Sediment cleanup was performed by constructing silica gel columns. Slurries of precombusted silica gel and hexane were poured into 9-mm-i.d. glass champagne columns topped with 2- to 3-mm sodium sulfate. The columns were pre-eluted with 15 ml hexane at a flow rate of 2 ml/min. Samples were added to the columns and eluted with 12 ml hexane. Next, 25 ml 15% methylene chloride in hexane was eluted and saved for PAH analysis. The eluates were transferred into evaporator tubes and reduced to 1 ml, with exchange into hexane. The eluates were spiked with an internal standard (d_{10}-acenaphthene, d_{10}-fluoranthene, d_{12}-chrysene, and d_{12}-perylene) prior to gas chromatography (GC)/mass spectrometry-selected ion monitoring (MS-SIM).

Tissue sample cleanup was performed by passing the extracts through a pre-rinsed 0.2-mm nylon filter unit connected to a 1-ml syringe. Samples were spiked with internal standards and analyzed in the same way as sediment samples. Analysis was conducted on a Hewlett-Packard model 5890 series II gas chromatograph equipped with a Hewlett-Packard model 5971 mass spectrometer detector. The GC analysis employed a 30 m × 0.25 i.d. DB5 column, helium carrier gas at 60 ml/min, with an oven temperature profile of: 50°C for 1 min, 50°C to 150°C at 25°C min^{-1}, 150°C to 230°C at 6°C min^{-1}; 230°C to 300°C at 3°C min^{-1}; hold for 3.33 min. Splitless injection was used. The MS-SIM analysis in electron impact mode included an ionization voltage of 70 eV, 200 mA filament emission, and 1,588 to 1,772 V for the electron multiplier.

Recovery of surrogate standards averaged 90.75% ± 0.08 SD. In addition to isotopically labeled surrogate standards added to the samples, quality-control measures included method blanks and analysis of National Institute of Standards and Technology Organics in Marine Sediment Standard Reference Material, where recoveries of 70 to 120% were obtained. Confirmation of the identity of individual PAHs required the presence of appropriate qualifier ions in the mass spectrum and retention times within 2% of calibration standards.

Lipid content of replicate groups of three to five individual *L. variegatus* were analyzed after exposures of 48 h, 1 week, and 4 weeks to each of the depth levels (except the surficial sediment, 0- to 4-cm depth) and reference sediments used in the bioaccumulation assay. To determine accurate changes in lipid content during exposures, lipid analysis was also performed on animals directly obtained from culture aquaria. A microgravimetric procedure with a chloroform/methanol extraction was used for all lipid determinations [11].

Total organic carbon (TOC) content of sediment samples was determined by drying the sediment to constant weight, treating with 1 N HCl to remove carbonates, redrying, and assaying organic carbon on a Perkin-Elmer 2400 CHN elemental analyzer. Samples were measured against an acetanilide standard. Triplicate analyses yielded differences of 0 to 3%.

Kinetics model

Because uptake curves reflected a decrease in bioavailability of the contaminant over time despite constant or nearly constant sediment concentrations, the following model was used [2]:

$$C_a = [k_s C_s^0 (e^{-\lambda t} - e^{-k_d t})] / [k_d - \lambda] \quad (1)$$

where

C_a = the concentration of contaminant in the animal (ng·g wet weight $^{-1}$)
k_s = the uptake rate coefficient (g dry sediment·g tissue^{-1}·h^{-1})
C_s^0 = the concentration of contaminant in the sediment at time = 0 h (ng·g dry weight $^{-1}$)
k_d = the depuration rate (h^{-1})
λ = the rate constant for the apparent reduction in the bioavailable concentration with time (h^{-1}), and
t = time (h).

To be valid, the assumption was made that biotransformation of PAHs by *L. variegatus* was sufficiently slow to avoid

significant loss over the course of the assay. Previous studies have confirmed that *L. variegatus* does not measurably biotransform with pyrene or BAP [12]. Other assumptions of this model included that all residues in the organisms were in a common pool equally available for depuration and that the rate of depuration was directly proportional to the concentration within the organisms. Depuration rates used in Equation 1 were estimated by regressing log-transformed k_d derived from experimental data against first-order molecular connectivity indices [13] for BAP, PYR [10], FLA, PHE, BAA, and CHR.

The data from the depuration experiments were fit to a first-order decay:

$$C_a(t) = C_a^0 \cdot e^{-k_d t} \quad (2)$$

where

C_a^0 = the PAH concentration in the animals at the beginning of the depuration experiment ($ng \cdot g^{-1}$ wet weight).

Statistics

Analysis of variance (ANOVA) was used to test for overall significant differences in animal lipid content after 48 h, 1 week, and 4 weeks of exposure. Student's *t* tests were used to test for significant differences in organic carbon concentrations, weight gain, and lipid percentages at the beginning and end of the study for each of the sediment depths. Differences were considered significant between the test categories at the 0.05 probability level.

RESULTS

Sediment

The sediment depths chosen for the exposures represented material deposited from approximately 1899 to the present (J. Robbins, personal communication). Concentrations of the 15 PAH compounds fluctnated among replicate sediment samples for each of the depths over the course of the study, but no obvious trends in PAH concentrations were apparent from 6-h through 4-week exposure periods (Table 1). The 12- to 16-cm depth contained the highest concentrations of ANT, BBF, BEP, IP, DBA, BGP, PHE, FLA, PYR, BAA, CHR, BKF/BJF, and BAP. The surficial depth (0 to 4 cm) contained the highest mean concentration of FLE (100 ± 38 SD $ng \cdot g^{-1}$ dry weight, cores C and E). The lowest PAH concentrations were obtained in the control depth, 44 to 48 cm, except for PER ($455\ ng \cdot g^{-1}$ dry weight; Table 1). The PER concentration at the 44- to 48-cm depth was comparable to that at the 0- to 4-, 12- to 16-, and 28- to 32-cm depths. The presence of PER in preindustrial-era depths was likely due to indirect biosynthesis through chemical reduction of PAH precursors and is not attributed to anthropogenic sources [14]. Reference Lake Michigan sediment contained fairly low PAH concentrations, where all individual PAH compounds were below $70\ ng \cdot g^{-1}$ dry weight.

Sediment TOC concentrations ranged from 1.56 to 3.33% and differed significantly among sediment depths, with the highest concentration at the 12- to 16-cm depth (Table 1). The lowest TOC concentration (1.56 to 1.67%) occurred at the 28- to 32-cm depth. Individual PAH compound concentrations and TOC concentrations did not significantly differ in any of the depth levels between 6-h and 4-week exposure intervals (*t* test: $p > 0.05$).

Bioassay results

No obvious toxicity of test sediments to *L. variegatus* was apparent during the course of the exposures. Less than 1% of the animals were on the sediment surface at the end of the exposure intervals, suggesting no sediment avoidance by *L. variegatus*. However, no significant reproduction was observed during the 4-week bioaccumulation study. The percentage of animals recovered after each of the exposure intervals ranged from 89.0% (after 24-h exposures) to 102.5% (after 2-week exposures). The number of animals recovered from reference sediment after 4 weeks increased from 40 to 43 and 45 in the duplicate exposures. Significant losses in animal wet weights were observed in all sediment depths, including the reference Lake Michigan sediment during the exposures. Mean wet weight/individual animal was 2.44 ± 0.25 mg at the start of the study and dropped to 1.56, 1.75, 1.19, 1.36, 1.39, and 1.37 mg/individual at the 0 to 4 cm, 4 to 8 cm, 12 to 16 cm, 28 to 32 cm, 44 to 48 cm, and Lake Michigan sediment levels, respectively. Previous studies with *L. variegatus* exposed to reference Lake Michigan sediment also showed a 14% decrease in animal wet weight over a 1-week exposure period [10]. Despite the weight decrease, lipid content increased significantly in animals exposed to the 4- to 8-cm depth (ANOVA: $F = 16.789$; $d.f. = 2, 10$; $p = 0.001$; Table 2). Animal lipid content did not significantly change over the 4-week exposure intervals for the other depths or Lake Michigan reference sediment exposures (Table 2).

Bioaccumulation

Concentration of PAH compounds in animals was low relative to sediment concentrations throughout the 4-week bioaccumulation study (Table 3). Concentrations of compounds in animal tissue were not different from those obtained from *L. variegatus* exposed to reference Lake Michigan sediment for many of the sampling times. Therefore, uptake curves could not be obtained for those depth levels (e.g., uptake clearance values could not be determined for any 0- to 4-cm depths, only two compounds at the 28- to 32-cm depths, and only one compound at the 44- to 48-cm depth, Table 4). The greatest accumulation of compounds occurred at the 12- to 16-cm depth were uptake clearances could be determined for 11 of the 15 compounds analyzed. However, even at the 12- to 16-cm depth, compound concentrations were relatively low; the highest compound concentration obtained at this depth was FLA after 52 h of exposure ($365\ ng \cdot g^{-1}$ wet animal weight).

The shape of individual uptake curves varied according to compound molecular weights. For example, the lower-molecular-weight compounds PYR and FLA (mol. wt. 202.3; Fig. 1) were taken up rather quickly, and accumulation of these compounds in animal tissue peaked between 48 h and 96 h and then decreased to the end of the study. Both BAA (Fig. 1) and CHR (mol. wt. 228.3) exhibited similar uptake

Table 1. PAH and TOC concentrations in sediment cores taken from Lake George, Michigan

	Concentrations at increasing core depths								
	0–4 cm		4–8 cm		12–16 cm		28–32 cm		44–48 cm
Congener	Core C	Core E	Core C	Core E	Core C	Core E	Core C	Core E	Core C
Fluorene	80.1 (12.0)[a]	120.5 (42.3)	42.5 (4.0)	46.3 (1.5)	68.7 (6.9)	71.1 (2.2)	20.6 (4.2)	16.8 (8.6)	2.8 (1.3)
Phenanthrene	743.6 (10.7)	1,005.7 (369.2)	351.2 (38.9)	388.7 (44.1)	911.5 (105.8)	894.2 (143.9)	281.8 (26.7)	214.5 (86.7)	20.6 (12.3)
Anthracene	219.4 (28.2)	278.5 (121.3)	150.9 (15.8)	137.0 (25.4)	322.1 (16.0)	404.9 (80.8)	101.8 (9.8)	58.0 (18.7)	4.5 (2.6)
Fluoranthene	1,069.5 (129.7)	1,550.7 (492.7)	481.8 (11.6)	535.8 (13.7)	1,876.3 (197.7)	1,818.7 (218.9)	533.0 (116.4)	311.9 (146.1)	11.6 (1.2)
Pyrene	803.0 (98.3)	1,154.9 (369.2)	374.0 (6.9)	420.0 (22.3)	1,522.3 (159.9)	1,445.2 (139.0)	370.0 (89.5)	202.1 (76.0)	4.8 (0.2)
Benz[*a*]anthracene	520.0 (15.1)	711.0 (232.9)	239.9 (25.1)	278.9 (19.7)	1,113.1 (41.2)	1,095.2 (52.0)	303.6 (48.5)	167.6 (70.0)	4.7 (0.7)
Chrysene + triphenylene	611.1 (16.6)	928.1 (361.1)	293.0 (8.0)	327.3 (26.6)	1,214.5 (52.5)	1,215.2 (91.5)	376.4 (46.8)	205.5 (83.3)	8.0 (1.3)
Benzo[*b*]fluoranthene	623.9 (71.2)	780.8 (100.1)	286.0 (60.6)	315.4 (76.9)	1,507.3 (127.7)	1,376.2 (116.4)	385.0 (45.3)	231.4 (120.7)	7.6 (0.5)
Benzo[*k*]fluoranthene + Benzo[*j*]fluoranthene	566.9 (73.0)	845.2 (148.8)	277.7 (41.0)	303.7 (25.2)	1,103.3 (182.0)	1,152.2 (176.7)	359.2 (72.4)	190.6 (114.9)	6.6 (1.3)
Benzo[*e*]pyrene	512.8 (12.0)	697.9 (155.2)	260.8 (19.6)	282.0 (22.5)	1,095.6 (24.8)	1,032.1 (104.3)	277.7 (28.1)	158.6 (71.0)	4.8 (0.6)
Benzo[*a*]pyrene	598.3 (19.6)	1,007.0 (324.0)	314.1 (2.1)	349.3 (1.6)	1,355.1 (123.4)	1,256.2 (146.7)	335.5 (38.9)	183.4 (80.7)	7.9 (3.1)
Perylene	338.4 (12.6)	535.2 (171.2)	196.0 (4.2)	217.0 (2.3)	482.6 (21.3)	482.9 (&72.3)	612.0 (150.8)	620.7 (255.1)	454.8 (3.6)
Indeno[*1,2,3-cd*]pyrene	549.0 (4.8)	857.5 (196.0)	321.4 (9.4)	355.5 (22.0)	1,404.4 (215.5)	1,193.7 (163.6)	393.3 (54.6)	227.5 (103.0)	7.2 (0.4)
Dibenz[*a,h*]anthracene	122.2 (8.4)	200.0 (62.4)	67.5 (2.2)	75.2 (2.1)	308.7 (53.7)	265.7 (6.6)	92.7 (13.1)	45.4 (20.9)	—[b]
Benzo[*ghi*]perylene	559.3 (58.5)	932.2 (329.8)	307.3 (3.0)	347.9 (9.1)	1,198.0 (168.5)	1,088.6 (69.7)	332.1 (51.4)	188.2 (84.0)	3.9 (1.0)
% Organic carbon	2.85 (0.04)	2.82 (0.06)	2.76 (0.07)	2.76 (0.04)	3.22 (0.13)	2.98 (0.09)	1.90 (0.03)	1.62 (0.05)	1.89 (0.04)

Concentrations are in $ng \cdot g^{-1}$ dry weight and represent the mean of duplicate samples from day 1 and day 28 of bioaccumulation assays.
[a] ± 1 SD.
[b] Concentration below detection limit.

Table 2. Lipid content of *Lumbriculus variegatus* (mean % ± 1 SD, $n = 5$) after exposure to sediments from different depths for various exposure durations

	Lipid content with increasing exposure			
Core-depths tested	0 Hours	48 Hours	1 Week	4 Weeks
Animals from culture aquaria	7.5 ± 3.6	—	—	—
4–8 cm	—	4.4 ± 0.8	10.1 ± 2.0	12.9 ± 3.1
12–16 cm	—	8.5 ± 0.7	—	14.9 ± 11.7
28–32 cm	—	5.9 ± 3.1	8.2 ± 4.4	7.8 ± 6.0
44–48 cm	—	10.0 ± 2.3	8.6 ± 3.3	9.4 ± 3.5
Lake Michigan reference sediment	—	—	—	9.2 ± 3.4

Table 3. PAH accumulation ($ng \cdot g^{-1}$ wet weight) and accumulation ratios (ARs) in *Lumbriculus variegatus* after 28-d exposure in sediment core sections taken from Lake George, Michigan

	Accumulation and ARs at increasing core depths								
	0–4 cm		4–8 cm		12–16 cm		28–32 cm		44–48 cm
Compound	Core C	Core E	Core C	Core E	Core C	Core E	Core C	Core E	Core C
Fluorene									
Accumulation	bd[a]	3	bd	bd	38	bd	bd	bd	bd
AR	nc[b]	nc	nc	nc	nc	nc	nc	nc	nc
Phenanthrene									
Accumulation	bd	31	5	8	286	12, 13[c]	bd	7	6
AR	nc	nc	nc	nc	nc	nc	nc	nc	nc
Anthracene									
Accumulation	6	6	4	4	18	19, 21	bd	bd	bd
AR	nc	nc	nc	nc	nc	nc	nc	nc	nc
Fluoranthene									
Accumulation	bd	15	12	6	126	51, 89	bd	8	bd
AR	nc	nc	nc	nc	0.018	0.007	nc	nc	nc
Pyrene									
Accumulation	bd	bd	bd	bd	105	60, 91	bd	bd	bd
AR	nc	nc	nc	nc	0.019	0.011	nc	nc	nc
Benz[*a*]anthracene									
Accumulation	9	8	12	11	70	58, 68	13	6	bd
AR	nc	nc	nc	.011	.017	.014	nc	nc	nc
Chrysene + triphenylene									
Accumulation	11	14	14	17	122	96, 109	4	10	bd
AR	nc	nc	nc	.014	.028	.020	nc	nc	bc
Benzo[*b*]fluoranthene									
Accumulation	11	19	16	19	199	209, 214	19	21	bd
AR	nc	nc	.015	nc	.036	.039	nc	nc	nc
Benzo[*k*]fluoranthene + benzo[*j*]fluroanthene									
Accumulation	85	29	49	35	162	208, 217	66	29	9
AR	nc	nc	.049	.032	.040	nc	.080	nc	nc
Benzo[*e*]pyrene									
Accumulation	22	22	31	34	249	246, 227	41	20	bd
AR	nc	nc	.032	.033	.063	.061	nc	nc	nc
Benzo[*a*]pyrene									
Accumulation	8	11	11	11	150	159, 142	23	12	bd
AR	nc	nc	.010	.009	.031	.032	nc	nc	nc
Perylene									
Accumulation	7	10	11	12	60	54, 59	212	227	119
AR	nc	nc	.016	.015	nc	0.028	0.114	0.082	0.064
Indeno[*1,2,3-cd*]pyrene									
Accumulation	bd	bd	bd	bd	91	91, 93	bd	bd	bd
AR	nc	nc	nc	nc	0.018	0.020	nc	nc	nc
Dibenz[*a, h*]anthracene									
Accumulation	3	bd	bd	bd	bd	28	4	7	bd
AR	nc	nc	nc	nc	nc	nc	nc	nc	nc
Benzo[*ghi*]perylene									
Accumulation	bd	bd	bd	bd	80	74, 63	bd	bd	bd
AR	nc	nc	nc	nc	0.018	nc	nc	nc	nc

[a]Concentration below detection limit.
[b]Not calculated.
[c]Results from duplicate analyses.

curves, but peak accumulation occurred after 96 to 170 h of exposure. Uptake curves for higher-molecular-weight compounds (mol. wt. 252.3 to 276.3) did not show the dramatic increase then decrease in accumulation over time, but rather reached an accumulation plateau (apparent steady state) after 100 to 336 h of exposure (Fig. 2), although accumulation decreased slightly at the last sampling time (672 h) in most data sets.

For most compounds, k_s did not consistently increase or decrease with sediment depth (Table 4), although accumulation was too low to calculate k_s values from many of the core depths. The k_s values were greatest for the lower-molecular-weight compounds (PYR and FLA) and lowest for the higher-molecular-weight compounds (BGP and IP) and ranged from 0.0005 to 0.0067 g dry sediment $\cdot$ g tissue$^{-1} \cdot h^{-1}$ (Table 3). The k_s values for PER could be compared across

Table 4. Uptake rate coefficients (k_s) and depuration rate coefficients (k_d) for PAH congeners from bioassay sediment core sections

PAH congener	k_s (g dry sediment · g tissue^{-1} · h^{-1}) at increasing core depths							k_d^{exp} [a] (h^{-1})	k_d^{est} [b] (h^{-1})
	4–8 cm		12–16 cm		28–32 cm		44–48 cm		
	Core C	Core E	Core C	Core E	Core C	Core E	Core C		
FLA	nd[c]	nd	0.0062	0.0067	nd	nd	nd	0.0208	0.0197
PYR	nd	nd	0.0039	0.0064	nd	nd	nd	0.0256	0.0198
BAA	nd	0.0013	0.0019	0.0034	nd	nd	nd	0.0090	0.0089
CHR + TRI	nd	0.0016	0.0024	0.0038	nd	nd	nd	0.0090	0.0089
BBF	0.0014	nd	0.0014	0.0024	nd	nd	nd	nd	0.0036
BKF + BJF	0.0021	0.0021	0.0019	0.0025	0.0015	0.0015	nd	nd	0.0036
BEP	0.0017	0.0017	0.0018	0.0026	nd	nd	nd	nd	0.0036
BAP	0.0009	0.0008	0.0012	0.0019	nd	nd	nd	0.0032	0.0036
PER	0.0014	0.0016	0.0012	0.0050	0.0031	0.0033	0.0027	nd	0.0036
IP	nd	nd	0.0005	0.0007	nd	nd	nd	nd	0.0015
BGP	nd	nd	0.0005	nd	nd	nd	nd	nd	0.0015

Standard errors of the model values ranged from 0.00009 to 0.00171.
[a] k_d (h^{-1}) was calculated from experimental data.
[b] k_d (h^{-1}) was estimated from the regression model (Equation 3).
[c] Not determined.

four core depths and fluctuated with sediment depth as well as core replicate samples (Table 4).

Accumulation ratios (ARs) for PAH compounds calculated as ($C_{a(dry\ wt)}$/fraction lipid) (C_s/fraction organic carbon)$^{-1}$ ranged from 0.018 to 0.114 at peak accumulation and from 0.007 to 0.114 after 4-week exposure intervals. Mean ARs from all data sets in the study were 0.042 ± 0.02 SD at peak accumulation and 0.032 ± 0.02 SD after 4-week exposures. The ARs did not follow a consistently increasing or decreasing pattern with individual compound molecular weights or sediment depth.

Depuration results

Elimination of radiolabeled PAHs was most rapid for the lowest-molecular-weight compound, PHE (mol. wt. 178.2, k_d = 0.0399 h^{-1}). The natural-log transformations of the first-order elimination model (Equation 2) yielded linear regressions with r^2 = 0.726 (BAP), 0.909 (CHR), 0.906 (BAA), 0.901 (FLA), and 0.917 (PHE). Elimination was slowest for BAP and was not as consistent at early sampling times of the depuration study as with other compounds that showed more rapid elimination (Table 4), thus the lower coefficient of determination for BAP. The regression of experimental k_d with first-order molecular connectivity indices yielded the following equation (Fig. 3):

$$\ln(k_d) = -1.2807\ (\pm 0.105)\ {}^1\chi^v + 2.807\ (\pm 0.622)$$
$$r^2 = 0.988 \qquad (3)$$

where ${}^1\chi^v$ is the first-order valence index of the PAH. Estimated k_d values from this regression ranged from 0.0015 to

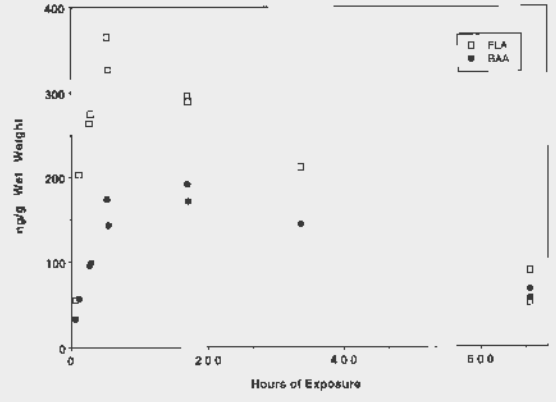

Fig. 1. Typical uptake curves for fluoranthene (FLA) and benz[*a*]anthracene (BAA). Data points were generated from the E 12- to 16-cm core depth bioaccumulation assay. Curves represent model predicted values.

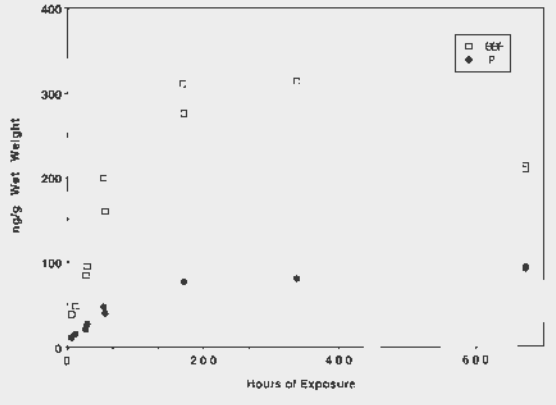

Fig. 2. Typical uptake curves for benzo[*b*]fluoranthene (BBF) and indeno(*1,2,3,-cd*)pyrene (IP). Data points were generated from the E 12- to 16-cm core depth bioaccumulation assay. Curves represent model predicted values.

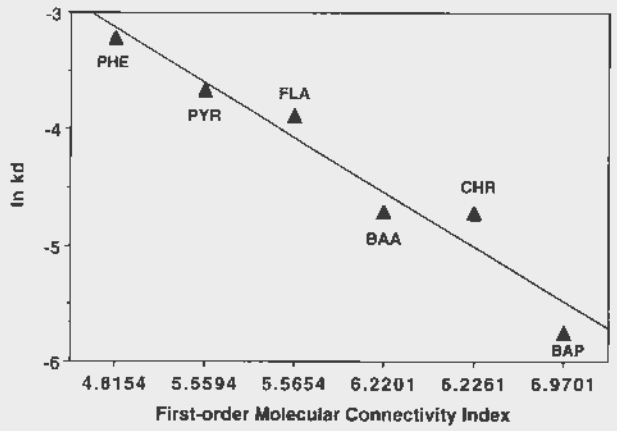

Fig. 3. Relationship of log-transformed k_d (h^{-1}) to first-order molecular connectivity indices of PAH congeners used in depuration study.

0.0198 h^{-1} and were used in Equation 1 to determine k_s (Table 4).

DISCUSSION

We expected the bioavailability of PAH compounds to decrease with increasing sediment/contaminant contact time (i.e., deeper sediments). Bioavailability of lower-molecular-weight PAHs (fluorene, phenanthrene) to *Diporeia* spp., described by uptake clearances from sediment, has been found to decrease significantly with sediment/contaminant aging [3]. Similarly, a preliminary study that used *L. variegatus* exposed to a mixture of PAHs showed reduced k_s values for lower-molecular-weight compounds after aging the sediment for 1.5 months (P. Van Hoof, personal communication). In contrast, data from the present study show that accurate uptake curves for the 15 compounds studied could not be generated from surficial sediment (0- to 4-cm depth) because of the lack of tissue accumulation, even though sediment concentrations of some compounds at 0- to 4-cm depths were comparable to those at 12- to 16-cm depths.

For example, FLA concentration at the 0- to 4-cm core C depth was 1,070 $ng \cdot g^{-1}$ compared to 1,876 $ng \cdot g^{-1}$ at the 12- to 16-cm core C depth. Calculation of C_a using an uptake clearance of 0.006 g dry sediment $\cdot g^{-1} \cdot h^{-1}$ (mean k_s from cores C and E at 12- to 16-cm depth) would yield a tissue concentration of 299 $ng \cdot g^{-1}$ dry weight, 30 times greater than what was actually obtained after 168 h of exposure. Likewise, the BAP concentration in animal tissue should have been 111 $ng \cdot g^{-1}$ after 2 weeks of exposure in 0- to 4-cm sediment if bioavailability were comparable across sediment depths. This calculated value is approximately 13 times greater than what was actually obtained in tissue at the 0- to 4-cm depth.

A portion of the lower-molecular-weight compounds may have been eliminated from animal tissues during the 24-h gut-purging period (e.g., elimination via passive diffusion through animal integument). Previous studies reported an average 2% decrease in total pyrene tissue concentration when *L. variegatus* were placed in water for 24 h after exposure in whole sediment [10]. However, any small decrease in tissue concentration should have occurred uniformly among all exposure depths.

To estimate depuration rates for modeling bioavailability more accurately, experimental data was obtained for PAH compounds representing a range of solubilities (log K_{ow} values ranged from 4.5 to 6.1). To best estimate depuration rate coefficients from the experimental data for all PAH compounds studied, we chose to regress experimental k_d against first-order molecular connectivity indices of the corresponding compounds. Similar studies have used other physical chemical relationships (e.g., log K_{ow}) to estimate rate coefficients for classes of compounds from experimentally derived data [15–17]. However, parameters such as log K_{ow} often cover a large range of values for a specific compound. For example, log K_{ow} values reported for BAP have ranged from 5.98 to 6.50 [18,19] and will contribute a rather wide range of error to the estimated k_d, depending on which value of K_{ow} is chosen. Molecular connectivity indices, although identical for some PAH compounds, are molecular descriptors that minimize estimation error.

Where uptake curves could be accurately obtained to compare k_s values across depths, little difference was observed. Uptake coefficients were similar from 4- to 8-cm to 28- to 32-cm depths for BKF/BJF, and varied by less than a factor of five with no apparent trends for PER from 4- to 8-cm to 44- to 48-cm depths. Previous studies have similarly shown the bioavailability of higher-molecular-weight PAH compounds to change minimally with increased sediment/contaminant contact time. For example, *Diporeia* spp. and *Chironomus riparius* exposed to CHR, BEP, and BAP exhibited no significant changes in k_s from sediments aged from 3 to 180 d [3,4]. This evidence, however, contrasts the lack of contaminant uptake from the 0- to 4-cm surficial sediment when concentrations of compounds were comparable to sediment concentrations of depths where measurable uptake occurred.

The uptake rate coefficients calculated by the model used in Equation 1 may be slightly inflated, as *L. variegatus* significantly lost weight during the 28-d study, violating one of the model assumptions. However, uptake rate is primarily driven by the first few sampling points during a bioaccumulation assay. Worm weight loss during the first 96 h of the assays was less than 20% and would minimally affect the estimated k_s values. Uptake rate coefficients of selected PAHs obtained in the present study are comparable to k_s values previously published for *Diporeia* spp.; uptake coefficients in laboratory-dosed sediments generated for BAP, BAA, and PYR were within the range of values we obtained in Lake George sediment using *L. variegatus* [2,15]. Uptake rate coefficients also compared favorably with those obtained for BAP and PYR in a pilot study that used both spiked sediment and historically contaminated sediment with *L. variegatus*. However, our uptake coefficients are approximately an order of magnitude lower than the range of 0.039 to 0.132 $g \cdot g^{-1} \cdot h^{-1}$ calculated for PYR in another study that used *L. variegatus* [10]. The PYR uptake rate coefficients in that study were found to decrease with increasing PYR concentration ranging from 0.4 $ng \cdot g^{-1}$ to 269 $\mu g \cdot g^{-1}$ dry sediment, an observation not substantiated by our data.

One reason for the variability in uptake coefficients obtained with PYR when using *L. variegatus* as an indicator species may be the selection of the model used to estimate the rate of contaminant uptake. The model used in this study included a rate constant, λ, to describe the decline in bioavailability of the contaminant to the animals. The model used in a previous study did not include this rate constant, for the shape of the uptake curve for PYR did not indicate a significant reduction in uptake during the exposure period [10]. However, if the accumulation data from the previous study is fitted to Equation 1, k_s values for PYR decrease from 0.130 to 0.067 (using a 1:10 animal dry-weight to sediment organic-carbon ratio) and 0.156 to 0.058 g sediment·g^{-1} organism·h^{-1} (using a 1:50 animal dry-weight to sediment organic-carbon ratio) [10].

Use of a particular model will depend on the general shape of uptake curves produced over the exposure intervals. Rate constants that account for losses in bioavailability are generally not included in models when kinetics curves show an asymptotic approach to a plateau. If the exposure period is not carried out long enough, the gradual decrease in bioavailability may uot be seen, as may be the case with the higher-molecular-weight PAHs (Fig. 2). Substantially different uptake rate coefficients can be obtained when various models are used to evaluate kinetics for the same contaminant and species.

The initial rise and then decline in accumulation that we observed with the lower-molecular-weight compounds has been reported [2]. The reason for the peak in accumulation was originally postulated to result from changes in bioavailability over the course of the bioassay as the contaminant comes to equilibrium with the sediment particles and interstitial water. Our latest speculation is that the initial rise in accumulation may be due to the dissolved concentration of contaminant in interstitial water. As this concentration is depleted, the initial uptake rate is slowed. If the rate of desorption from sediment particles is not able to maintain a concentration comparable to that at the beginning of the experiment, the decline in accumulation after this initial rise would occur because the accumulation rate from feeding is not as fast as from that obtained from the dissolved phase in interstitial water. Because concentrations of higher-molecular-weight compounds are not as soluble in interstitial water as are the lower-molecular-weight compounds, this initial peak in the uptake curve is not as apparent, or is not observed at all in a 28-d bioaccumulation assay (Fig. 2).

Accumulation factors (AFs) are used to describe maximum bioaccumulation on a lipid/organic carbon-normalized basis when conditions are at steady state. Because the ARs that we obtained were an average 10 to 150 times lower than AFs obtained from two other 4-week bioaccumulation studies using *Macoma nasuta* and *Diporeia* spp. exposed to various PAH compounds [5,15], steady state was apparently not attained in this study. The initial pulse of PAH uptake and then decline in accumulation, as shown in Figures 1 and 2, indicates that the major source of contaminant, sediment, may not be representative of overall accumulation in a 28-d study such as this. Guidelines for bioaccumulation tests suggest that assays be run for 28 d; in general, this is the time taken to achieve tissue residues within 80% of steady state [20]. Aside from the fact that accumulation was below detection limits for many compounds, we only achieved about 30% of the steady-state tissue residue in depths where accumulation could be accurately determined. Peak accumulation was usually observed before the end of the 28-d exposure periods, and accumulation would likely have continued to drop had the assays been extended beyond 4 weeks.

Reasons for not achieving steady-state conditions are open to speculation. One explanation may be that physiological stress was placed on the test species during the assays. Organisms did not reproduce during the 28-d tests and generally lost weight, an indication of less than optimal growth conditions. However, lipid levels either stayed constant or significantly rose during the assays, and they were comparable to lipid levels reported for *L. variegatus* elsewhere [12]. Furthermore, a general decline in organism health with a subsequent decline of contaminant uptake should have been indicated equally in all depth levels tested. Instead, a large decrease in contaminant bioavailability was seen in 0- to 4-cm depths, compared to 12- to 16-cm depths.

This substantial decrease in contaminant bioavailability iu the surficial layer is more likely due to compositional differences among the sediment depths. Although particle-size aualysis was uot performed on the sediment studied, microscopic observation of sediment particles did not appear to differ among depths. Furthermore, sediment TOC did not vary by more than 0.4% between 0- to 4-cm and 12- to 16-cm depths. However, relative amounts of oxygen-containing functional groups that comprise the organic carbon molecules change with burial and weathering. Such changes in the oxygen:carbon and hydrogen:carbon ratios of organic carbon molecular structure can affect nonpolar contaminant binding [21]. Differential binding of PAH compounds to colloids, micro-particles, or different forms of humic or fulvic acids might explain the differences in bioavailability observed. Certainly, more experimental data that involve analysis of these factors must be considered before an accurate explanation can be made concerning the differential bioavailability among sediment depths.

The differential bioavailability observed in this study raises questions concerning the suitability of conventional 10-d toxicity studies, as well as the accuracy of accumulation results obtained from surficial sediments. Do conventional 10-d toxicity tests adequately determine the steady-state tissue concentrations in indicator species such as *L. variegatus*? Although the use of this species has been recommended for use in bioaccumulation tests of 28-d duration [9], exposure to sediments low in nutrients may limit its use, short of providing additional food over the duration of the exposure, which would alter bioavailability. In addition, toxicity or accumulation assays that use surficial sediments to assess contamination may not be accurate in estimating the bioavailability of contaminants found at depth.

Finally, our data raise questions about the general quality of Lake George sediments. Although contaminant concentrations in surficial depths of the sediment core may contribute minimally to the bioaccumulation of PAHs by benthic organisms, the reason why benthic populations have

not returned to preindustrial levels remains unknown. Further research on conditions resulting in atypical accumulation from Lake George sediments is likely to yield insights into other factors influencing the benthos of this system.

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REFERENCES

1. **Varanasi, U., W.L. Reichert, J.E. Stein, D.W. Brown** and **H.R. Sanborn.** 1985. Bioavailability and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment from an urban estuary. *Environ. Sci. Technol.* **19**:836–841.
2. **Landrum, P.F.** 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi*. *Environ. Sci. Technol.* **23**:588–595.
3. **Landrum, P.F., B.J. Eadie** and **W.R. Faust.** 1992. Variation in the bioavailability of polycyclic aromatic hydrocarbons to the amphipod *Diporeia* (spp.) with sediment aging. *Environ. Toxicol. Chem.* **11**:1197–1208.
4. **Harkey, G.A., P.F. Landrum** and **S.J. Klaine.** 1994. Comparison of whole sediment, elutriate and pore-water exposures for use in assessing sediment-associated organic contaminants in bioassays. *Environ. Toxicol. Chem.* **13**:1315–1329.
5. **Ferraro, S.P., H. Lee II, R.J. Ozretich** and **D.T. Specht.** 1990. Predicting bioaccumulation potential: A test of a fugacity-based model. *Arch. Environ. Contam. Toxicol.* **19**:386–394.
6. **Upper Great Lakes Connecting Channels Study Management Committee.** 1980. Upper Great Lakes connecting channels study, Vol. 2—Final Report. Environment Canada, Ottawa, Ontario.
7. **Kauss, P.B.** and **Y.S. Hamdy.** 1991. Polycyclic aromatic hydrocarbons in surficial sediments and caged mussels of the St. Marys River, 1985. *Hydrobiologia* **219**:37–62.
8. **Burt, A.J., P.M. McKee, D.R. Hart** and **P.B. Kauss.** 1991. Effects of pollution on benthic invertebrate communities of the St. Marys River, 1985. *Hydrobiologia* **219**:63–81.
9. **Phipps, G.L., G.T. Ankley, D.A. Benoit** and **V.R. Mattison.** 1993. Use of the aquatic oligochaete *Lumbriculus variegatus* for assessing the toxicity and bioaccumulation of sediment-associated contaminants. *Environ. Toxicol. Chem.* **12**:269–279.
10. **Kukkonen, J.** and **P.F. Landrum.** 1994. Toxicokinetics and toxicity of sediment-bound pyrene in *Lumbriculus variegatus* (Oligochaeta). *Environ. Toxicol. Chem.* **13**:1457–1468.
11. **Gardner, W.S., W.A. Frez** and **E.A. Cichocki.** 1985. Micromethod for lipids in aquatic invertebrates. *Limnol. Oceanogr.* **30**:1099–1105.
12. **Harkey, G.A.** 1993. Investigation of the bioavailability of sediment-associated hydrophobic organic contaminants via laboratory bioassays. Ph.D. thesis. Clemson University, Clemson, SC.
13. **Hall Associates Consulting.** 1993. *Molconn-X PC™ Package, Version 2.0.* Quincy, MA.
14. **Neff, J.M.** 1979. *Polycyclic Aromatic Hydrocarbons in the Aquatic Environment.* Applied Science, London, UK.
15. **Landrum, P.F.** and **W.R. Faust.** 1991. Effect of variation in sediment composition on the uptake rate coefficient for selected PCB and PAH congeners by the amphipod *Diporeia* sp. In M.A. Mayes and M.G. Barron, eds., *Aquatic Toxicology and Risk Assessment: Fourteenth Volume.* STP 1124. American Society for Testing and Materials, Philadelphia, PA, pp. 263–279.
16. **Oliver, B.G.** 1987. Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. *Environ. Sci. Technol.* **21**:785–790.
17. **Landrum, P.F.** 1988. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: Role of physiological and environmental variables. *Aquat. Toxicol.* **12**:245–271.
18. **Mackay, D., A. Bobra** and **W.Y. Shin.** 1980. Relationships between aqueous solubility and octanol–water partition coefficients. *Chemosphere* **9**:701–711.
19. **Miller, M.M., S.P. Wasik, G. Huang, W. Shiu** and **D. Mackay.** 1985. Relationships between octanol water partition coefficients and aqueous solubility. *Environ. Sci. Technol.* **19**:522–529.
20. **U.S. Environmental Protection Agency.** 1989. Guidance manual: Bedded sediment bioaccumulation tests. EPA/600/x-89/302, ERLN-N111. Environmental Research Laboratory—Narragansett, Newport, OR, pp. 25–27.
21. **Grathwohl, P.** 1990. Influence of organic matter from soils and sediments from various origins on the sorption of some chlorinated aliphatic hydrocarbons: Implications on K_{oc} correlations. *Environ. Sci. Technol.* **24**:1687–1693.