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UPTAKE AND DEPURATION OF NONIONIC ORGANIC CONTAMINANTS FROM SEDIMENT BY THE OLIGOCHAETE, *LUMBRICULUS VARIEGATUS*

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Abstract—Uptake of sediment-associated contaminants by the oligochaete *Lumbriculus variegatus* was evaluated after 1, 3, 7, 14, 28, and 56 d of exposure to a field-collected sediment contaminated with DDT and its metabolites, dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE), or to a field-collected sediment contaminated with polycyclic aromatic hydrocarbons (PAHs). Depuration of contaminants by oligochaetes in a control sediment or in water was also evaluated over a 7-d period after 28 d of exposure to the field-collected sediments. Accumulation of PAHs with a log octanol-water partitioning coefficient (log K_{ow}) <5.6 typically reached a peak at day 3, followed by a lower plateau between days 7 and 56 of the sediment exposure. Similarly, 4,4′-DDT exhibited a peak in accumulation at day 14 followed by a decline at days 28 and 56. In contrast, accumulation of PAHs with a log K_{ow} >5.6 or DDD and DDE typically exhibited a steady increase from day 1 to about day 14 or 28, followed by a plateau. Therefore, exposures conducted for a minimum of 14 to 28 d better reflected steady-state concentrations for DDT and its metabolites and for PAHs. Depuration rates for DDT and its metabolites and high-K_{ow} PAHs were much higher in organisms held in clean sediment relative to both water-only depuration and model predictions. This suggests that depuration in clean sediment may artificially accelerate depuration of hydrophobic compounds. Comparisons between laboratory-exposed *L. variegatus* and oligochaetes collected in the field from these sediments indicate that results of laboratory tests can be extrapolated to the field with a reasonable degree of certainty.

Keywords—Sediment Bioaccumulation Oligochaetes *Lumbriculus variegatus*

INTRODUCTION

Standard guides have been published for conducting 28-d sediment bioaccumulation tests in the laboratory with the oligochaete *Lumbriculus variegatus* [1,2]. These standards also describe procedures for measuring uptake and depuration of contaminants accumulated from sediment. *Lumbriculus variegatus* is recommended in these standards for use in bioaccumulation testing with sediments based on ease of culture and handling, known chemical exposure history, adequate tissue mass for chemical analyses, tolerance of a wide range of sediment physicochemical characteristics, low sensitivity (in terms of survival) to contaminants associated with sediment, and amenability to long-term exposures without feeding.

Both the American Society for Testing Materials (ASTM) [1] and the U.S. Environmental Protection Agency (U.S. EPA) [2] recommend conducting sediment exposures with *L. variegatus* for 28 d to achieve a steady state between sediment and the organisms. This exposure duration is based on a summary of studies with a variety of organisms other than oligochaetes. Using these various organisms, >80% of steady-state concentration was approached in 83% of tests conducted for 28 d [1]. Numerous investigators have conducted sediment bioaccumulation studies with *L. variegatus* (e.g., [3–8]). However, only a limited number of studies have evaluated bioaccumulation kinetics of sediment-associated contaminants with *L. variegatus* [9–17].

The first objective of the present study was to evaluate bioaccumulation of sediment-associated contaminants by *L. variegatus* over a 56-d exposure period. Specifically, oligochaetes were exposed to a field-collected sediment contaminated with DDT and its metabolites or to a field-collected sediment contaminated with polycyclic aromatic hydrocarbons (PAHs). Uptake of contaminants was evaluated after 1, 3, 7, 14, 28, and 56 d of exposure to sediment. Depuration of contaminants by oligochaetes in a control sediment or in water was also evaluated over a 7-d period after 28 d of exposure to the contaminated sediments using additional exposure beakers.

Only two previous investigations have compared the bioaccumulation of sediment-associated contaminants by oligochaetes exposed in the field and in the laboratory [4,6]; however, neither of these studies estimated steady-state concentrations of compounds accumulated by oligochaetes in laboratory exposures. Therefore, the second objective of the present study was to compare the concentrations of sediment-associated contaminants in native oligochaetes collected from the field to concentrations of these compounds in *L. variegatus* at steady state in laboratory exposures.

MATERIALS AND METHODS

Sediment collection

Sediment contaminated with DDT and its metabolites was collected using a ponar grab sampler in February 1993 from

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the Huntsville Spring Branch near Huntsville (AL, USA) [18] starting at 2.5 km upstream from the confluence with Indian creek. Sediment contaminated with PAHs was also collected in February 1993 from the Little Scioto River near Dayton (OH, USA) [16] using an Eckman grab sampler (Geneq, Montreal, QC, Canada). A total of about 70 grabs were collected along about 150 meters of each stream (totaling about 70 L of sediment from each stream). Each grab sample of sediment (from about 0–5 cm depth) was placed in a metal tray from which about 1 L of sediment was transferred to a 114-L polyethylene drum for chemical analysis of sediment and for laboratory bioaccumulation testing. The remainder of the sediment in each tray was rinsed with river water through a U.S. standard stainless-steel sieve bucket (30; 600-μm opening) to isolate native oligochaetes from the collection area. Five groups of native oligochaetes (ranging from 0.31 to 0.59 g wet wt) from the Huntsville sediment were collected, blotted, weighed, and frozen for chemical analysis. One composited sample of native oligochaetes (5.1-g wet wt) was collected from the Little Scioto sediment for chemical analysis. Samples of native oligochaetes were transported on dry ice and stored in a walk-in freezer at −20°C before analysis. A control sediment was collected from Pequaywan Lake (MN, USA) [19]. Sediment was held in the dark in a walk-in cooler at 4°C for less than three weeks before the start of the exposures.

Culturing and testing of oligochaetes

Mixed-age *L. variegatus* were mass cultured in well water (hardness 290 mg/L as $CaCO_3$, alkalinity 255 mg/L as $CaCO_3$, pH 7.8, from a deep well at the Columbia Environmental Research Center, Columbia, MO, USA) using procedures outlined in [6,20]. Exposures were conducted in 4-L glass beakers containing 1 L of whole sediment and 3 L of overlying well water. The water volume of each beaker was calibrated to contain 4 L using a glass standpipe that exited through the side of the beaker and was held in place with a silicone stopper [6]. Exposure beakers were held in a temperature-controlled water bath at 23°C on a 16:8-h light:dark photoperiod at about 200 lux [1,2]. Overlying water in the beakers was continuously aerated with a glass pipette (~3 bubbles/s) and received one volume addition of overlying water per day [6]. Oligochaetes were not fed during the sediment exposures. The exposures to the Little Scioto sediment were started one week after the start of the exposures to the Huntsville sediment.

Four days before the start of the exposures, sediment was homogenized with a hand-held electric drill and stainless steel auger in the drums used for shipment and storage of sediment. The sediment was then scooped into the beakers and overlying water was added to the beakers through a piece of nylon screen to minimize resuspension of the sediment. Water delivery and aeration were started after the beakers were placed in the waterbath. On the day before the start of the exposures, oligochaetes were removed from the culture with an aquarium net, placed in beakers containing well water, and rinsed to remove most of the toweling and debris. Without a substrate, oligochaetes tend to form tight masses in the beakers, which facilitates the transfer of organisms to the sediment exposure beakers. On day 0, the oligochaetes were combined into a glass pan and rinsed with well water to break up the masses of oligochaetes and remove any remaining debris. A stainless-steel dental pick was used to separate oligochaetes into masses of about 1 g. The clumps of oligochaetes were removed from the pan with the dental pick, touched against the rim of the pan to remove excess water and placed into a pretarred weigh boat. A 1.0- to 1.2-g sample (wet wt; about 0.20–0.25 g dry wt) of oligochaetes was then transferred immediately to the exposure beakers containing sediment. In the Huntsville sediment (sediment with the lowest total organic carbon [TOC] concentration), there was greater than a 50:1 ratio of TOC in sediment to organism dry weight at the start of the sediment exposures (ASTM [1] and U.S. EPA [2] recommend testing a minimum of 1 g of oligochaetes/replicate at a ratio of TOC in sediment to organism dry wt of 50:1). Three samples of oligochaetes were also collected at the start of the sediment exposures for chemical analyses.

Operation of the exposure systems and behavior of oligochaetes were observed daily. Dissolved oxygen and conductivity of the overlying water were measured weekly in all beakers. Total hardness, pH, alkalinity, and total ammonia were measured on the day before the exposures started and on days 27 and 55 of the exposures in the beakers with control sediment and in five randomly selected beakers with Huntsville and Little Scioto sediments. Ranges of water quality for each parameter measured in the overlying water were dissolved oxygen 7.1 to 8.5 mg/L, pH 8.2 to 8.6, alkalinity 190 to 262 mg/L (as $CaCO_3$), total hardness 222 to 288 mg/L (as $CaCO_3$), conductivity 504 to 646 μS at 25°C, and total ammonia typically <0.2 mg/L.

Triplicate samples of the control sediment and duplicate samples of the Huntsville and Little Scioto sediments were collected for chemical analyses on the day when homogenized sediments were placed into the exposure beakers (4 d before the start of the exposures). One sample was also collected from each sediment for particle size, moisture, and TOC analysis. In addition, on days 28 and 56 of the exposures, duplicate samples of sediments were collected from the Huntsville and Little Scioto exposure beakers for chemical analyses. Sediment from these beakers (still containing oligochaetes) was sampled by pouring off the overlying water and then homogenizing the sediment with a glass rod. All sediment samples for chemical analysis were placed in 120-ml precleaned glass jars and refrigerated at 4°C until analysis.

Three replicate exposure beakers were sampled to collect oligochaetes for chemical analyses on days 1, 3, 7, 14, 28, and 56 of exposure to the Huntsville and Little Scioto sediments and on days 28 and 56 of the exposure to the control sediment (two additional replicate beakers also containing oligochaetes were sampled from the Huntsville and Little Scioto sediments on day 28 of the exposures). Toxicity to oligochaetes (based on reduced biomass) was observed in the exposures to Little Scioto sediment (see *Results* section). Therefore, the replicate samples of oligochaetes collected from Little Scioto sediment were composited at days 7 to 56 in the uptake study and during the depuration study.

Oligochaetes were isolated from the sediments by passing the samples through a series of U.S. standard stainless-steel sieves 6 (3.35mm opening) followed by an 18 (1.0mm opening) and 35 (300μm opening), and then collecting the contents of each sieve in a glass pan. The oligochaetes were separated from detritus using a stainless-steel dental pick or glass pipette. *Lumbriculus variegatus* were separated from any native oligochaetes based on behavior (native oligochaetes typically form a tight, spring-like coil whereas *L. variegatus* do not; [1]). Once isolated, all *L. variegatus* from a beaker were combined into a composite sample. The sample was blotted, weighed, placed in a precleaned container, and frozen at

−20°C. In addition, two composited samples of native oligochaetes were isolated on day 28 from the Huntsville sediment and one composited sample of native oligochaetes was isolated on day 28 from the Little Scioto sediment.

On day 28 of the exposure, *L. variegatus* were isolated from 20 beakers containing Huntsville sediment and 20 beakers containing Little Scioto sediment and transferred to 1-L beakers for a 7-d depuration study. Half of the depuration beakers contained 200 ml of the control sediment with overlying well water and the other half of the depuration beakers contained only well water. Test conditions in the depuration study were similar to the conditions in the uptake study. Oligochaete samples were collected for chemical analysis from the duplicate beakers after a 12-h and 1-, 2-, 3-, and 7-d depuration period.

Analyses of sediment and oligochaete samples

Physical analyses of the sediments included particle size, moisture, and TOC (Table 1; see [21] for a description of the methods). Chemical analyses of sediment included organochlorine (OCs) pesticides, polychlorinated biphenyls (PCBs), and selected PAHs (Table 1). Preliminary analyses of sediment samples from the Huntsville and Little Scioto indicated that there was an excess of acid-volatile sulfides relative to simultaneously extracted metals [22] that would limit the bioavailability of metals in the sediment samples. Hence, metal analyses were not conducted on the oligochaete samples. Chemical analyses of oligochaete samples from exposure to Huntsville sediment included OCs and PCBs and from exposure to Little Scioto sediment included OCs, PCBs, and PAHs. Oligochaete samples were also analyzed for percent lipids.

Chemical analysis of sediment and oligochaete samples was performed by the Geochemical and Environmental Research Group at Texas A&M University (College Station, TX, USA). Kemble et al. [23] describe the methods used to extract and analyze PAHs, OCs, and PCBs in the sediment samples. Average percent spike recovery for 22 OCs in sediment was 94%, average percent spike recovery for 22 PCB congeners in sediment was 104%, and average percent spike recovery for 25 PAHs in sediment was 97%. Differences between analyses of compounds in duplicate samples of the Huntsville and Little Scioto sediments was <30% for 90% of the comparisons.

Oligochaete samples were homogenized and extracted using a Teckmar® Tissumizer (Varian, Walnut Creek, CA, USA), sodium sulfate, and methylene chloride [24–26]. Extracts of the oligochaete samples were split into two portions. One was used to measure percent lipid and the second was used for measuring PAHs, OCs, or PCBs. A 20-ml aliquot of the total extract volume of about 300 ml was filtered and concentrated to 1 ml. A 100-μl subsample was then removed, evaporated to dryness, and weighed. Percent lipid was calculated using the mass of the dried subsample and the concentrated sample. Extracts for chemical analyses of PAHs, OCs, and PCBs were fractionated using adsorption chromatography to isolate the aliphatic fraction and the PAH/OC/PCB fraction. Lipid interference in the PAH/OC/PCB fraction was eliminated with further processing using high-pressure liquid chromatography [25]. The quantitative analyses were performed by capillary gas chromatography with electron capture for OCs and PCBs and a mass spectrometer detector in the selective ion monitoring mode for PAHs [25]. Average percent spike recovery from the oligochaete samples was 85% for dibromooctafluorobiphenyl (range 55–100%), 89% for PCB congener 103 (range 55–101%), and 81% for PCB congener 198 (range 48–92%).

Statistical analyses

Biota-sediment accumulation factors (BSAFs) were calculated during uptake and depuration as the ratio of the chemical concentration in oligochaetes (normalized to lipid) to the concentration in sediment (normalized to organic carbon content at the start of the exposure) [1,27]. Variability was observed in the individual measures of lipids during the exposures; therefore, an average lipid concentration of 2.3% (dry wt) for all of the uptake exposures was used to calculate BSAFs (Table 2). Log-K_{ow} values for compounds were obtained from [28]. If a value was not available from this first source, then values were obtained from [29].

The relationship between uptake or depuration of compounds by oligochaetes over time was evaluated using a regression line to plot the curve that best described the data (i.e., the maximum r^2 value using Regression Wizard in SigmaPlot [30]). Steady-state BSAFs for individual compounds measured in *L. variegatus* were estimated from the plateau of these regression lines developed during the 56-d sediment uptake study (regression plots individual chemicals not shown). If there was no plateau in the uptake curve, a pseudo–steady-state concentration was estimated between the mean concentrations measured on days 28 and 56. We initially evaluated the uptake curves using a first-order kinetic models [1] but found these models could not adequately describe the observed pattern of accumulation (particularly for the low-K_{ow} PAHs).

RESULTS

Chemical characteristics of sediments

In the control sediment, concentrations of OCs, PCBs, and PAHs at the start of the exposures were below the detection limits (0.003 μg/g dry wt for OCs and PCBs and 0.03 μg/g dry wt for PAHs; Table 1). In the Huntsville sediment, dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), and DDT were the only OCs typically elevated above about 0.2 μg/g (dry wt). In the Little Scioto sediment, only concentrations of PAHs were above detection limits. Concentrations of OCs, PCBs, and PAHs in samples of oligochaetes were typically below the detection limits of about 0.1 to 0.6 μg/g (dry wt) for all of the sediment exposures except for concentrations of DDT and its metabolites in the oligochaetes exposed to Huntsville sediment and PAHs in oligochaetes exposed to Little Scioto sediment. Therefore, the analysis is limited to the uptake and depuration of DDT and its metabolites for the exposure of oligochaetes to Huntsville sediment and PAHs for the exposure of oligochaetes to Little Scioto sediment.

In the Huntsville sediment, the concentrations of DDE and DDD were relatively consistent from the start of the exposure to days 28 and 56 of the sediment exposure (Table 1). In contrast, the measured concentration of 2,4′-DDT was about 50 to 70% lower on days 28 and 56 compared with the start of the exposure. In the Little Scioto sediment, slightly higher concentrations of PAHs were observed on days 28 and 56 compared with the start of the exposure. We presume these apparent increases may be the result of variability in the analytical method or the result of heterogeneity in the subsamples collected for analysis.

Table 1. Physical and chemical characteristics of the control (CS), Huntsville ([HS] Huntsville, AL, USA), and Little Scioto ([LS] Dayton, OH, USA) sediment samples[a]

Analyte	log K_{ow}	Day 0			Day 28				Day 56			
		CS	HS	LS	HS	%[b]	LS	%[b]	HS	%[b]	LS	%[b]
Physical characteristics (%)												
Moisture		59	40	41	ND		ND		ND		ND	
Particle size												
Sand		73	63	74	ND		ND		ND		ND	
Silt		11	18	13	ND		ND		ND		ND	
Clay		16	19	13	ND		ND		ND		ND	
Total organic carbon		6.4	1.1	8.7	ND		ND		0.9	−18	9.2	6
Chemical concentrations (μg/g; dry wt)												
4,4′-DDD	6.1	*	2.2	*	2.1	−4	*		2.7	20	*	
2,4′-DDD	6.1	*	0.20	*	0.17	−13	*		0.21	8	*	
4,4′-DDT	6.5	*	1.0	*	0.94	−4	*		0.84	−14	*	
2,4′-DDT	6.5	*	0.17	*	0.052	−69	*		0.085	−49	*	
4,4′-DDE	6.8	*	3.1	*	3.1	−1	*		3.2	3	*	
2,4′-DDE	6.8	*	0.17	*	0.14	−20	*		0.17	0	*	
Low-K_{ow} PAHs												
Naphthalene	3.4	**	**	15	**		24	61	**		17	13
C1-naphthalenes	3.7	**	**	10	**		13	35	**		15	53
Fluorene	3.9	**	**	45	**		47	4	**		54	20
1-Methylnaphthalene	3.9	**	**	6.4	**		10	55	**		10	49
Biphenyl	3.9	**	**	1.3	**		2.0	53	**		4.1	222
Acenaphthene	3.9	**	**	38	**		37	−2	**		42	12
2-Methylnaphthalene	4.0	**	**	3.2	**		3.1	−4	**		5.1	61
Acenaphthylene	4.1	**	**	25	**		27	9	**		29	16
Dibenzothiophene	4.2	**	**	7.6	**		8.1	7	**		8.1	7
C2-naphthalenes	4.3	**	**	13	**		17	31	**		18	38
2,6-Dimethylnaphthalene	4.3	**	**	5.4	**		5.1	−5	**		4.7	−13
C1-fluorenes	4.4	**	**	13	**		18	34	**		17	31
Phenanthrene	4.6	**	0.061	120	0.068		120	0	0.065		130	8
Anthracene	4.6	**	**	82	**		95	15	**		110	34
C1-dibenzothiophenes	4.7	**	**	3.6	**		4.9	34	**		6.7	84
C3-naphthalenes	4.8	**	**	26	**		32	23	**		32	26
1,6,7-Trimethylnaphthalene	4.8	**	**	7.7	**		7.3	−5	**		8.9	15
C2-fluorenes	4.9	**	**	14	**		10	−24	**		21	52
C1-phenanthrene/anthracenes	5.0	**	0.022	48	0.029		58	20	0.026		60	25
Pyrene	5.1	0.033	0.19	190	0.21		230	21	0.20		240	26
Fluoranthene	5.1	0.035	0.19	220	0.23		280	27	0.19		290	32
1-Methylphenanthrene	5.1	**	**	21	**		26.0	23	**		16	−25
C2-dibenzothiophenes	5.3	**	**	1.2	0.019		8.7	641	**		11	839
C1-fluoranthene/pyrenes	5.3	**	0.062	160	0.070		190	19	0.064		210	31
C4-naphthalenes	5.3	**	**	10	**		28	178	**		32	218
C3-fluorenes	5.4	**	**	15	0.024		**	ND	**		**	ND
C2-phenanthrene/anthracenes	5.5	**	0.028	26	0.028		32	25	0.027		32	26
High-K_{ow} PAHs												
Chrysene	5.6	**	0.159	230	0.15		290	26	0.13		350	52
Benzo[*a*]anthracene	5.7	**	0.085	160	0.11		180	13	0.10		200	25
C3-dibenzothiophenes	5.8	**	0.023	1.0	0.031		1.8	82	0.020		6.5	546
C3-phenanthrene/anthracenes	6.1	**	0.034	17	0.035		24	43	0.028		22	31
C1-chrysenes	6.1	**	0.061	110	0.071		130	18	0.056		150	36
Benzo[*a*]pyrene	6.1	**	0.13	280	0.15		290	4	0.13		350	25
Benzo[*b*]fluoranthene	6.2	**	0.15	240	0.17		250	4	0.15		300	25
Benzo[*k*]fluoranthene	6.2	**	0.15	240	0.17		250	4	0.15		300	25
Perylene	6.3	0.131	0.060	91	0.065		110	21	0.059		120	32
Benzo[*e*]pyrene	6.4	**	0.12	200	0.14		210	5	0.12		250	25
C4-phenanthrene anthracenes	6.5	**	0.021	2.4	0.021		11	344	0.049		13	430
C2-chrysenes	6.6	**	0.026	31	0.030		38	24	0.024		42	37
Indeno[123*cd*]pyrene	6.7	**	0.12	210	0.14		210	0	0.10		260	24
Dibenz[*a,h*]anthracene	6.7	**	0.017	44	0.022		48	8	**		57	31
Benzo[*ghi*]perylene	6.7	**	0.11	220	0.14		210	−4	0.087		260	18
C3-chrysenes	7.2	**	**	3.4	**		6.0	74	**		5.2	51
C4-chrysenes	7.7	**	**	30	0.019		42	43	**		50	68

[a] Concentrations are mean of triplicate CS samples and the mean for duplicate HS and LS samples. ND = not determined; DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene; PAH = polycyclic aromatic hydrocarbon. One asterisk indicates a detection limit of 0.003 and two asterisks indicates a detection limit of 0.03 (μg/g dry wt).

[b] Percent change in measured concentrations from day 0 to day 28 or from day 0 to day 56.

Table 2. Wet weight (g) and lipid content (% as dry wt) of *Lumbriculus variegatus* at the start of the exposures, isolated during the 56-d uptake exposure to control, Huntsville (Huntsville, AL, USA) or Little Scioto (Dayton, OH, USA) sediments, and isolated during the 7-d depuration period in control sediment or in water

	Sediment exposures						Depuration									
							In sediment						In water			
Treatment	Day	Replicates	Weight (g)	±SD	Lipids (%)	±SD	Day	Replicates	Weight (g)	±SD	Lipids (%)	±SD	Weight (g)	±SD	Lipids (%)	±SD
Culture organisms	0	3	1.2	0.12	1.5	0.5										
Control (Pequaywam Lake)	28	3	1.9	0.35	1.6	0.5										
	56	3	1.9	0.23	2.2	0.5										
Huntsville (DDT, DDD, DDE)[a]	1	3	0.81	0.06	2.1	0.9										
	3	3	0.96	0.07	2.2	0.4	0.5	2	0.74	0.03	2.4	0.4	0.81	0.06	4.5	2.1
	7	3	0.77	0.02	1.6	0.9	1	2	0.68	0.16	3.3	0.2	0.74	0.01	2.1	1.6
	14	3	0.91	0.05	1.1	0.6	2	2	0.76	0.17	7.1	1.0	0.75	0.05	3.1	1.6
	28	5	0.88	0.07	0.6	0.4	3	2	0.93	0.18	2.4	0.6	0.68	0.02	4.4	3.3
	56	3	0.53	0.02	2.6	1.5	7	2	0.99	0.13	2.7	1.2	0.65	0.08	3.6	1.9
Little Sciota (PAHs)[a]	1	3	0.91	0.12	3.0	0.9										
	3	3	0.83	0.16	4.1	0.8	0.5	2/1[b]	0.29	0.18	3.6		0.30	0.01	55[c]	
	7	3/1[b]	0.43	0.10	1.7		1	2/1	0.22	0.09	4.5		0.28	0.06	11	
	14	3/1	0.38	0.05	4.7		2	2/1	0.27	0.04	5.9		0.35	0.06	7.4	
	28	5/1	0.31	0.11	6.2		3	2/1	0.24	0.01	11[c]		0.30	0.05	16[c]	
	56	3/1	0.14	0.05	8.7		7	2/1	0.30	0.01	7.6		0.16	0.01	25[c]	

[a] DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene; PAH = polycyclic aromatic hydrocarbons.
[b] Replicates for wet weight/replicates for lipids.
[c] Less than 0.07 mg (wet wt) of oligochaetes used for analysis of lipid concentrations, which may have contributed to this high variability in the measure of lipids.

Uptake and depuration of DDT and its metabolites by oligochaetes from Huntsville sediment

Within 15 min of introduction into the exposure beakers, the oligochaetes in the control and Huntsville sediments burrowed into the sediment and were observed processing the top 2 cm of sediment. By day 28, the upper 1 cm of sediments had been visibly processed and oligochaetes were observed burrowing into the upper 3 cm of the sediment. Oligochaetes isolated from the control sediment on day 28 were larger and more active than oligochaetes isolated from the Huntsville sediment. By the end of the 56-d exposure, all of the sediment to the bottom of the control and Huntsville beakers had been visibly processed by the oligochaetes (to a depth of about 5 cm).

Table 2 summarizes the mass of the oligochaetes isolated on days 1, 3, 7, 14, 28, and 56 of the uptake exposure with control and Huntsville sediments and during the 7-d depuration in control sediment or in water. The exposures started with about 1.0 to 1.2 g of oligochaetes/replicate, and on day 1, about 75% of the mass of oligochaetes was isolated from the exposure beakers. In the control sediment, there was about a 50% increase in the mass of oligochaetes isolated on days 28 and 56 of the exposure relative to the initial mass. In contrast, the mass of oligochaetes isolated from Huntsville sediment remained constant from days 1 to 28 and decreased about 40% between days 28 and 56. During the 7-d depuration period in control sediment or in water, the mass of oligochaetes isolated on day 28 from the exposure to Huntsville sediment remained relatively constant (Table 2). During the 56-d exposure to control or Huntsville sediment, concentrations of lipids in oligochaetes remained relatively constant (ranging from 0.6 to 2.6% as dry wt; Table 2). Similarly, concentrations of lipids in oligochaetes during the 7-d depuration period in control sediment or in water also remained relatively constant (ranging from 2.1 to 7.1% as dry wt).

Uptake of DDT, DDD, and DDE on days 1, 3, 7, 14, 28, and 56 by *L. variegatus* is summarized in Figure 1A. Depuration of these compounds starting on day 28 is summarized in Figure 2. Concentrations of 2,4′-DDT were at or below the detection limit of about 0.2 to 0.4 μg/g (dry wt) and are not included in Figure 1A. Concentrations of DDT, DDD, and DDE in oligochaetes at the start of the exposures and at days 28 and 56 of the exposure to control sediment were all below the detection limits.

The BSAFs for DDT and its metabolites measured in the oligochaetes reached a plateau by day 14 of the exposure to Huntsville sediment (Fig. 1A). An exception to the pattern was 2,4′-DDE that continued to increase throughout the 56-d exposure. Concentrations of 4,4′-DDT were below the detection limit until day 14, when there was a peak in the concentration of this analyte followed by a gradual decline at days 28 and 56. The overall regression line for these data indicates a steady-state BSAF of about 3 was reached by day 14 of the exposure; however, BSAFs for the five compounds range from about 1 to 8 from day 14 to 56 (Fig. 1A).

Depuration of DDT and its metabolites tended to be slower in water compared with sediment (Fig. 2A), though there was considerable variability in these measurements. The contribution of sediment in the gut of the oligochaetes to total body burden was estimated by assuming sediment contributed 10% to the dry weight of the oligochaetes [31]. This estimation also assumes that gut contents were similar to bulk sediment (i.e., that oligochaetes do not selectively consume any particular size class of sediment particles [10,32]). Based on this assumption, an average of only 3.9% (standard deviation [SD] of 2.5) of the DDT, DDD, or DDE measured in the oligochaetes on day 28 would be attributed to sediment in the gut. Based on these estimates, the concentrations of these compounds measured on day 28 were probably associated with tissue rather than the gut contents of oligochaetes.

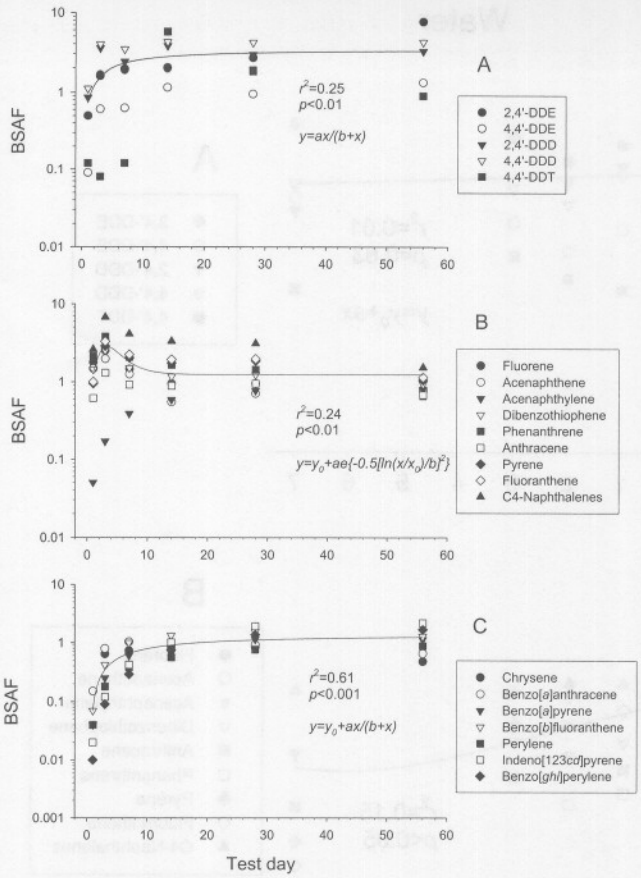

Fig. 1. Uptake of DDT, dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE; **A**), low-K_{ow} polycyclic aromatic hydrocarbons (PAHs; **B**), and high-K_{ow} PAHs (**C**) by *Lumbriculus variegatus* exposed to Huntsville (Huntsville, AL, USA) or Little Scioto (Dayton, OH, USA) sediments. The line represents a regression across all of the compounds for a given panel. The biota-sediment accumulation factors (BSAFs) were calculated as the ratio of the concentration of a compound measured in the oligochaetes normalized to lipid to the concentration of the compound measured in the sediment normalized to organic carbon.

Concentrations of DDT, DDD, and DDE measured in native oligochaetes that were collected at the same time that sediment was collected from the field were similar to the steady-state concentrations estimated from the laboratory exposures with *L. variegatus* (Fig. 3A, Table 3). For example, the concentration of 4,4′-DDD was 10 μg/g in native oligochaetes and about 12 μg/g in *L. variegatus* estimated at steady state in the laboratory exposure (Table 3). Similarly, the concentrations of DDT, DDD, and DDE in native oligochaetes sampled from the test sediments on day 28 of the exposure were generally similar to the steady-state concentrations estimated for *L. variegatus* (Fig. 3B).

Uptake and depuration of PAHs by oligochaetes from Little Scioto sediment

In contrast with the control and Huntsville samples, oligochaetes in the Little Scioto sediment did not immediately burrow into the sediment after being added to the exposure beakers. For the first 24 h, most of the oligochaetes remained in a tight mass on the surface of the sediment. Some oligochaetes were observed crawling on the sediment surface or up the side of the beaker to congregate on the overflow stopper. By the second day of the exposure, most of the oligochaetes had burrowed into the Little Scioto sediment; however, over the next 3 d, oligochaetes were occasionally observed twitching on the surface of the sediment. Throughout the exposure to Little Scioto sediment, oligochaetes processed sediments nearer the surface compared with the oligochaetes in the control and Huntsville sediments. At the end of the 56-d exposure, only about the upper 1 cm of the Little Scioto sediment had been visibly processed to any extent (compared with the control and Huntsville sediments, which had been visibly processed to the bottom of the beaker by day 56).

The mass of oligochaetes isolated from Little Scioto sediment decreased markedly from day 1 to day 56 of the sediment uptake exposure (Table 2). By day 56, the mass of oligochaetes had decreased by about 85% of the mass on day 1. This is in contrast with the control sediment, where there was a doubling in the mass by days 28 and 56 of the exposure, and the Huntsville sediment, where the mass remained constant from day 1 to day 28 and decreased by only about 40% from day 28 to day 56. During the 7-d depuration period in control sediment or in water, the mass of oligochaetes isolated on day 28 from the exposure to Little Scioto sediment remained relatively constant (Table 2). Concentrations of lipids measured in oligochaetes exposed to Little Scioto sediment were more variable throughout the 56-d sediment uptake exposure and during the 7-d depuration period compared with oligochaetes exposed to the control and Huntsville sediments (Table 2). The low mass of oligochaetes isolated from the Little Scioto sediment may have contributed to this high variability in the measurement of lipid concentration (Table 2).

Different patterns of accumulation were observed between low- and high-K_{ow} PAHs. Therefore, Figure 1B includes data for the selected PAHs with a low log K_{ow} (<5.6) and Figure 1C includes data for the selected PAHs with a high log K_{ow} (>5.6). These groups of PAHs were selected to be representative of the various PAHs listed in Table 1. Naphthalene, biphenyl, and 2-methylnaphthalene were not included in these figures because concentrations of these compounds were frequently below the detection limit during the uptake or depuration periods. Concentrations of PAHs in oligochaetes at the start of the exposures and at days 28 and 56 of the exposure to control sediment were routinely below the detection limits (0.1 μg/g dry wt).

Accumulation of most low-K_{ow} PAHs peaked at day 3, followed by a lower plateau between days 7 and 56 of the uptake exposure from sediment (Fig. 1B). One exception to this pattern was a steady increase in concentration of acenaphthylene over the course of the 56-d exposure. The overall regression line for the low-K_{ow} PAHs indicates a steady-state BSAF of about 1 was reached by day 14 of the exposure; however, BSAFs for individual compounds range from about 0.7 to 3 from day 14 to 56 (Fig. 1B). Accumulation of high-K_{ow} PAHs typically exhibited a steady increase from day 1 to day 14 or day 28 (Fig. 1C). The overall regression line for the high-K_{ow} PAHs indicate a steady-state BSAF of about one was reached by day 14 of the exposure; however, BSAFs for individual compounds range from about 0.4 to 2 from day 14 to 56 (Fig. 1C).

Depuration of PAHs by oligochaetes held in control sediment and depuration of low-K_{ow} PAHs by oligochaetes held in water followed a pattern of gradual decline over the 7-d period (Fig. 2B). In contrast, very little of the high-K_{ow} PAHs was depurated by oligochaetes held in water (Fig. 2C). By the end of the 7-d depuration period, concentrations of high-K_{ow}

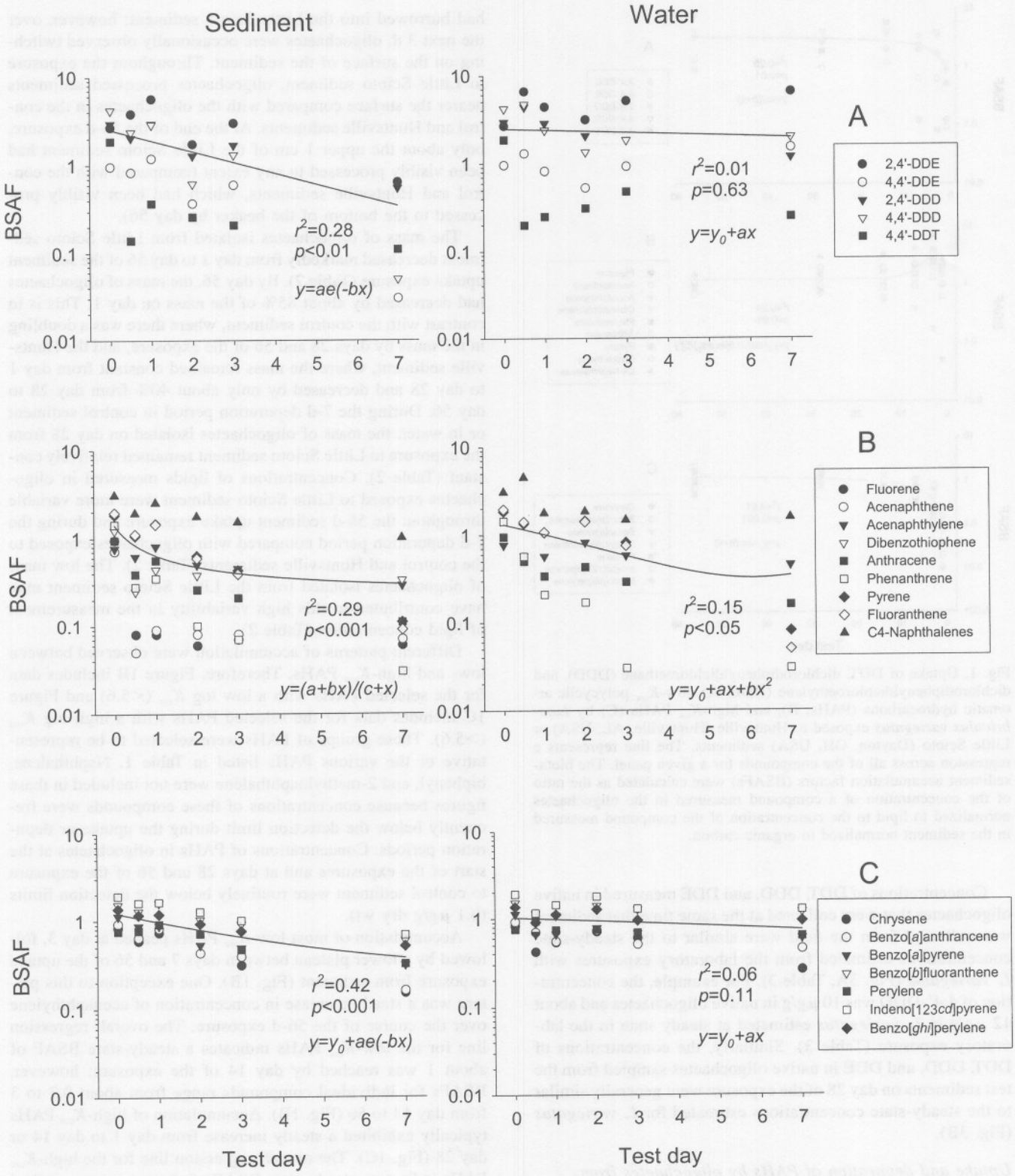

Fig. 2. Depuration of DDT, dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE; **A**), low-K_{ow} polycyclic aromatic hydrocarbons (PAHs; **B**), and high-K_{ow} PAHs (**C**) by *Lumbriculus variegatus* in control sediment or in water. See Figure 1 for additional detail.

PAHs were generally higher in oligochaetes held in water compared with oligochaetes held in sediment. Assuming that sediment contributed to 10% of the dry weight of the oligochaetes [31], the estimated contribution of sediment in the gut of the oligochaetes on day 28 averaged 37% (SD = 27) for low-K_{ow} PAHs and 27% (SD = 11) for high-K_{ow} PAHs. During the first 12 h of depuration in water or sediment, PAH concentrations in oligochaetes were reduced by an average of 53% (SD = 23) and 23% (SD = 28), respectively, for low-K_{ow} PAHs and 18% (SD = 11) and 23% (SD = 25), respectively, for high-K_{ow} PAHs. Therefore, the decrease in the concentrations of PAH measured in oligochaetes during the 12-h depuration pe-

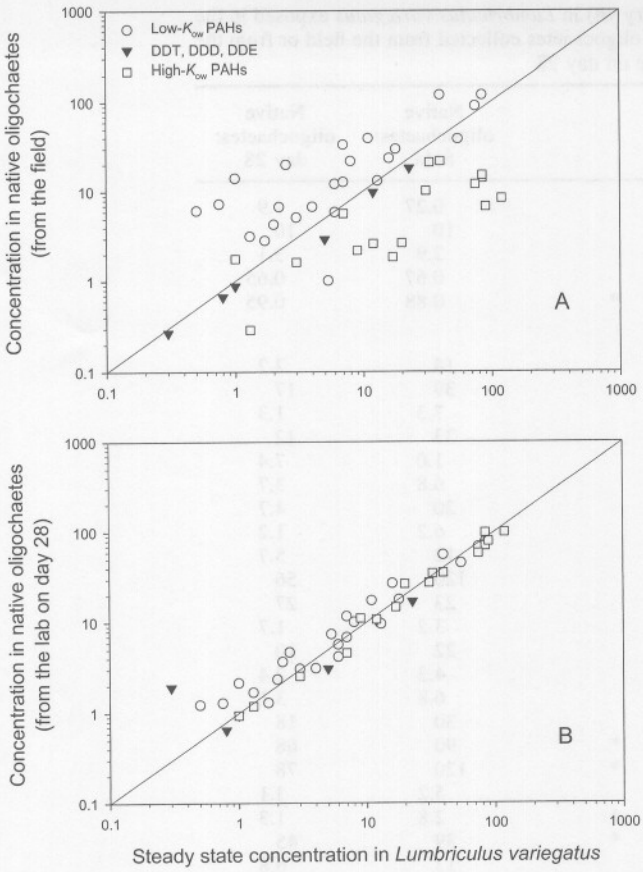

Fig. 3. Concentration of DDT, dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE), or polycyclic aromatic hydrocarbons (PAHs) measured in native oligochaetes collected from the field (**A**) or from day 28 of the laboratory exposures (**B**) compared with the estimated steady-state concentration for *Lumbriculus variegatus* (μg/g dry wt).

riod in sediment or in water is consistent with that expected from the clearance of contaminated sediment from the gut rather than elimination of compounds from tissue.

Overall, concentrations of PAHs measured in native oligochaetes collected at the same time that sediment was collected from the field were similar. However, concentrations of low-K_{ow} PAHs in native oligochaetes were biased higher than the steady-state concentrations estimated from the laboratory exposures with *L. variegatus* (Fig. 3A, Table 3), while high-K_{ow} PAHs were biased lower. Native oligochaetes recovered from the test sediments on day 28 showed even closer correspondence to those measured in *L. variegatus* and showed no systematic differences between low- and high-K_{ow} PAHs (Fig. 3B, Table 3).

DISCUSSION

Bioaccumulation methodology

Exposure to the Little Scioto sediment contaminated with high concentrations of PAHs was stressful to *L. variegatus*. Oligochaetes avoided this sediment at the start of the exposure and there was a reduction in the mass of oligochaetes isolated from this sediment during the 56-d exposure (Table 2). Van Hoof et al. [16] also reported toxicity and avoidance of a sample of Little Scioto sediment by *L. variegatus* during a 14-d bioaccumulation exposure. Oligochaetes, including *L. variegatus*, have been observed to avoid sublethal concentrations of contaminants in sediment [9,33]. The concentrations of PAHs in Little Scioto sediment in the present study were a factor of 64 above the probable effect concentration of 22.8 μg/g for total PAHs [34]. The probable effect concentration is intended to represent the concentration of individual contaminants in sediment above which toxicity is frequently observed in field-collected samples.

Results of these exposures with Little Scioto sediments prompted ASTM [1] and the U.S. EPA [2] to recommend conducting a 4-d screening toxicity test with a sediment before the start of a definitive bioaccumulation test with *L. variegatus*. In the screening test, the number of *L. variegatus* should not be reduced in the test sediment relative to the control sediment and test organisms should burrow into the sediment. While avoidance or toxicity of a sediment sample does not negate the results of a bioaccumulation exposure, it does compromise the interpretation of the results [1,2].

Neither avoidance nor overt lethality were observed with oligochaetes exposed to Huntsville sediment; however, the oligochaetes exposed to Huntsville sediment appeared to be smaller and less active compared with the oligochaetes exposed to control sediment. The concentration of total DDE in the Huntsville sediment was above the probable effect concentration for total DDE of 0.031 μg/g by a factor of 105 [34], which also indicates that this sediment was severely contaminated. Therefore, results of the exposures to both Huntsville and Little Scioto sediments should be interpreted with some caution when extrapolating BSAFs in the present study to less contaminated sediments. Specifically, BSAFs in the present study may be lower due to reduced feeding or exposure to sediment. It would be interesting to test dilutions of these two sediments to determine if bioaccumulation is similar when the oligochaetes are exposed under potentially less toxic conditions.

Both the ASTM [1] and U.S. EPA [2] recommend testing a minimum of 1 g and preferably 5 g of oligochaetes (wet wt) in each replicate. With 1 g of tissue (wet wt), a detection limit of about 0.01 μg/g (dry wt or 0.1 μg/g wet wt) was achieved for PAHs, OCs, and PCBs. With less than 0.1 g of tissue, the detection limit increased to about 0.2 μg/g (dry wt or 0.6 μg/g wet wt). While it would have been desirable to have 5 g of tissue/replicate, this would have substantially increased the logistical challenges of conducting this test (i.e., collection and processing five times more sediment for each replicate [5 L vs 1 L of sediment tested in the present study]).

Because chemicals with a high K_{ow} often require extended periods of time to reach steady state between tissues and the exposure medium, growth or weight loss should be accounted for in assessing the uptake rate and time to reach steady state [1]. In our case, the validity of the use of this mass-change approach is dependent on whether the observed reduction in the composite mass of organisms is due to a reduction in the mass of individuals, a reduction in the number of survivors with little reduction in individual mass, or a combination of both factors. Because of the labor involved and the limited sample size, we did not measure the mass of individual oligochaetes. Thus, this question would need to be addressed in future studies.

Patterns of uptake of PAHs and DDT from sediment

Van Hoof et al. [16] conducted 14-d bioaccumulation exposures with a sediment sample collected from the Little Scioto River and observed similar patterns of accumulation of low-

Table 3. Estimated steady-state concentration (μg/g; dry wt) in *Lumbriculus variegatus* exposed in the laboratory and concentrations of compounds in native oligochaetes collected from the field or from the sediment exposure on day 28

Analyte[a]	Steady state[b]		Native oligochaetes: field	Native oligochaetes: day 28
4,4′-DDT	0.30		0.27	1.9
4,4′-DDD	12		10	10
4,4′-DDE	5.0		2.9	3.1
2,4′-DDD	0.80		0.67	0.65
2,4′-DDE	1.0	*	0.88	0.95
Low-K_{ow} PAHs				
C1-naphthalenes	1.0		14	2.2
Fluorene	11		39	17
1-Methylnaphthalene	0.75		7.3	1.3
Acenaphthene	7.0		33	12
Acenaphthylene	5.3		1.0	7.4
Dibenzothiophene	2.2		6.8	3.7
C2-naphthalenes	2.5		20	4.7
2,6-Dimethylnaphthalene	0.50		6.2	1.2
C1-fluorenes	6.0		12	5.7
Phenanthrene	40		120	56
Anthracene	16		23	27
C1-dibenzothiophenes	1.3		3.2	1.7
C3-naphthalenes	8.0		22	10
1,6,7-Trimethylnaphthalene	2.0		4.3	2.4
C2-fluorenes	4.0		6.8	3.1
C1-phenanthrene/anthracenes	18		30	18
Pyrene	75	*	90	68
Fluoranthene	85	*	120	78
1-Methylphenanthrene	3.0		5.2	3.1
C2-dibenzothiophenes	1.7	*	2.8	1.3
C1-fluoranthene pyrenes	55	*	39	45
C4-naphthalenes	7.0		13	6.8
C3-fluorenes	6.0	*	5.9	4.2
C2-phenanthrene/anthracenes	13	*	13	10
High-K_{ow} PAHs				
Chrysene	40	*	22	36
Benzo[*a*]anthracene	33	*	21	35
C3-dibenzothiophenes	1.0	*	1.8	0.9
C3-phenanthrene/anthracenes	7.0	*	5.7	4.6
C1-chrysenes	31	*	10	27
Benzo[*a*]pyrene	85		14	98
Benzo[*b*]fluoranthene	85	*	15	70
Benzo[*k*]fluoranthene	85	*	15	70
Perylene	20	*	2.7	27
Benzo[*e*]pyrene	75	*	12	58
C4-phenanthrene/anthracenes	3.0	*	1.6	2.5
C2-chrysenes	12	*	2.6	11
Indeno[1,2,3 *cd*]pyrene	120	*	8.4	100
Dibenz[*a,h*]anthracene	17	*	1.8	15
Benzo[*ghi*]perylene	90	*	6.8	79
C3-chrysenes	1.3	*	0.29	1.2
C4-chrysenes	9.0	*	2.2	11

[a] DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene; PAH = polycylic aromatic hydrocarbon.

[b] Estimated from the plateau of a regression line. If the curve did not plateau, a pseudo–steady-state concentration was estimated between the day 28 and day 56 data (designated with an asterisk).

K_{ow} (Fig. 1B) and high-K_{ow} (Fig. 1C) PAHs. The test conditions were similar between these two studies; however, Van Hoof et al. [16] tested smaller volumes of sediment and conducted exposures at 19°C versus the temperature of 23°C used in the present study. Van Hoof et al. [16] suggested that the peak in the concentrations of low-K_{ow} PAHs accumulated by *L. variegatus* after day 2 to 3 of the exposure might involve the reduction in the amount of the labile PAH fraction (freely dissolved and rapidly desorbed sediment fraction). Uptake from pore water would be expected to be an important route of exposure for low-K_{ow} PAHs, whereas ingestion of sediment is reportedly a major route of exposure for high-K_{ow} PAHs [13,16]. Hence, Van Hoof et al. [16] suggested that the pattern of accumulation of low-K_{ow} PAHs may result from a reduction in the amount of labile low-K_{ow} PAHs in the pore water. In contrast, a reduction in labile high-K_{ow} PAHs in the pore water would not be expected to have as much of an effect on accumulation of high-K_{ow} PAHs if ingestion of sediment is the major route of exposure for these compounds [16]. In the present study, it is unlikely that depletion of the labile PAH

fraction would have occurred so rapidly. The loading level of oligochaetes was very low relative to the amount of organic carbon in the sediment (greater than a 50:1 ratio of TOC in sediment-to-organism dry wt at the start of the sediment exposures). In addition, the oligochaetes had processed only a small fraction of the sediment in the beaker after the first 2 to 4 d of the exposure and even avoided the sediment for the first 24 h of the exposure.

Van Hoof et al. [16] also suggested that degradation of low-K_{ow} PAHs by bacteria in the dissolved phase might have contributed to the decline in the concentrations of these compounds measured in oligochaetes after day 2 to 3 of the sediment exposure (Fig. 1B). In contrast, more strongly sorbed high-K_{ow} PAHs would tend to be less available for microbial degradation; hence, the steady increase in accumulation of these compounds. Reduced feeding by oligochaetes [35] or changes in clearance rates were also hypothesized by Van Hoof et al. [16] as possibly contributing to the peaks in the uptake curves for the low-K_{ow} PAHs. Perhaps semipermeable membrane devices could be used in future studies to evaluate potential changes in the bioavailability of PAHs in sediment over time [36]. Importantly, the homogenization of the sediment samples at the start of the exposures may alter the bioavailability of the compounds and may explain the higher initial uptake of low-K_{ow} PAHs by oligochaetes.

An alternative explanation for the shape of the uptake curves may be the metabolism of low-K_{ow} PAHs relative to the metabolism of high-K_{ow} PAHs by *L. variegatus*. A peak in accumulation followed by a decline during sediment exposures has been attributed to an increase in the metabolic degradation rate of compounds by organisms after the start of the exposure (most commonly observed in exposures with low-K_{ow} PAHs [1,37]). A limited number of studies have evaluated the ability of *L. variegatus* to metabolize organic compounds. Leppanen and Kukkonen [14] reported, after 21-d sediment exposures with *L. variegatus*, a higher proportion of the low-K_{ow} PAH pyrene was metabolized relative to the proportion of the high-K_{ow} benzo[*a*]pyrene. In the present study, it is unlikely that the sediments were depleted of low-K_{ow} PAHs given that organisms would have processed only a small fraction of the sediment by day 2, suggesting that availability of PAHs was not limited. Therefore, a plausible explanation for the shape of the uptake curves may be differential metabolism of low-K_{ow} PAHs compared with high-K_{ow} PAHs by *L. variegatus*. Harkey et al. [38] reported negligible metabolism of pyrene or benzo[*a*]pyrene by *L. variegatus*; however, the exposures were conducted at 10°C, which might explain the lack of metabolism of these compounds. Alternatively, times to steady state may be less for low-K_{ow} PAHs compared with high-K_{ow} PAHs. Therefore, after an enzyme-induction period of 2 to 3 d, metabolism of tissue residues may be more apparent for low-K_{ow} PAHs compared with high-K_{ow} PAHs. Clearly, additional research is needed to further evaluate the ability of *L. variegatus* to metabolize PAHs if this organism is to be used routinely to assess the bioavailability of these chemicals. Perhaps analyzing for conjugates of PAHs would be useful to determine if the organisms are metabolizing PAHs.

Metabolism of DDT to DDD or DDE by *L. variegatus* might also help to explain the peak in 4,4′-DDT accumulation at day 14 followed by the plateau in DDD concentrations and the continued uptake of DDE throughout the 56-d exposure (Fig. 1A). Sediment concentrations of 4,4′-DDT, DDD, and DDE remained relatively constant throughout the 56-d exposure (Table 1). Therefore, the peak in accumulation of 4,4′-DDT could not be attributed to a change in bulk sediment concentrations of this compound during the exposure. Metabolism of DDT might also explain the tendency for DDD or DDE to increase during the depuration of oligochaetes (Fig. 2A). However, we identified no studies that have evaluated the ability of *L. variegatus* to metabolize DDT. Alternatively, breakdown of DDT to DDD and DDE may also result from the processing of samples through the gas chromatographic analysis [39].

Accumulation of low-K_{ow} and high-K_{ow} PAHs from field-collected sediment by *L. variegatus* in the present study was consistent with the findings of Van Hoof et al. [16] and Harkey et al. [17]. Accumulation of low-K_{ow} PAHs by *L. variegatus* was observed to peak at day 2 to 4, followed by a decline to about 33 to 50% of this maximum by day 14 [16] or by day 28 [17] (Table 4). Accumulation of high-K_{ow} PAHs either continued to increase to day 14 [16] or peaked at about Day 14 followed by a slight decline to day 28 [17] (Table 4).

Based on the literature, accumulation of low-K_{ow} and high-K_{ow} PAHs by *L. variegatus* from spiked sediment did not follow as consistent a pattern as that observed for the accumulation of these compounds from field-collected sediments (Table 4). For example, accumulation of pyrene continued to increase in spiked-sediment exposures conducted for 6 to 58 d [9,13–15]. In contrast, peak concentrations of pyrene were observed at day 2 to 4, followed by a decline in exposures conducted with field-collected sediments (Table 4) or with a spiked sediment [16]. Accumulation of higher K_{ow} benzo[*a*]pyrene in spiked-sediment exposures reached a plateau by day 4 to 7 [10] followed by a slight decline to the end of a 21-d exposure [14] or continued to increase in exposures conducted for 6 d [15]. In contrast, accumulation of benzo[*a*]pyrene by *L. variegatus* in field-collected sediments continued to increase to day 14 [16] and reached steady state by day 28 in the present study (Fig. 1C).

Loonen et al. [11] reported that *L. variegatus* reached a steady state with two polychlorinated dibenzo-*p*-dioxins (log $K_{ow} > 6$) after 3 to 11 d in 28-d spiked-sediment exposures (Table 4). Similar elimination of these two compounds was observed in clean sediment or in water. In contrast, Fisk et al. [12] reported that *L. variegatus* did not achieve steady state with three of four polychlorinated alkanes (log $K_{ow} > 5$) during 14-d spiked-sediment exposures (Table 4).

Differences in aging time may influence the time to achieve steady state in spiked-sediment exposures. For example, in studies with spiked sediment, a continued uptake of pyrene was observed in sediments that were held for less than two weeks before the start of the exposure [13,14]. In contrast, a peak in uptake of pyrene was observed followed by a slight increase if the spiked sediments were held for six weeks before the start of the exposure [9]. However, differences observed in uptake of PAHs cannot be attributed solely to differences in aging time of spiked sediment. Even with an extended aging time of four weeks, benzo[*a*]pyrene reached a peak concentration more rapidly in exposures conducted with the spiked sediment [13] compared with exposures conducted with field-collected sediment ([16,17, present study]). Moreover, Kraaij [40] reported that aging sediment for 2 to 959 days after spiking did not result in a pronounced reduction in bioavailability of PAHs to oligochaetes. Additional research is needed to

Table 4. Summary of studies that have evaluated the accumulation of compounds from sediment using exposures with *Lumbriculus variegatus*

Study	Compound[a]	Duration of exposure (days)	General patterns
Field-collected sediments			
Van Hoof et al. [16]	Low-K_{ow} PAHs	14	Peak at day 2 to 4 and decline to end of exposure
	High-K_{ow} PAHs		Continued uptake throughout the exposure or steady state by end of exposure.
Harkey et al. [17]	Low-K_{ow} PAHs	28	Peak at day 2 to 4 and decline to end of exposure.
	High-K_{ow} PAHs		Peak at about day 14 and slight decline by end of exposure.
Present study	Low-K_{ow} PAHs	56	Peak at day 3 followed by steady state by day 14.
	High-K_{ow} PAHs		Steady state by day 14 to 28, continued uptake throughout the exposure or slight decline from day 28 to day 56.
	DDT		Peak at day 14 followed by decline to end of exposure.
	DDD		Steady state by day 14.
	DDE		Continued uptake throughout the exposure
	Total DDT		Steady state by day 14.
Spiked sediments			
Kukkonen and Landrum [9]	Pyrene	58	Peak at day 4 to 7 and slight increase by end of exposure.
Kukkonen and Landrum [10]	Benzo[*a*]pyrene	7	Steady state by day 4 to 7.
Loonen et al. [11]	Polychlorinated dibenzo[*p*]dioxins	28	Steady state by day 3 to 11.
Fisk et al. [12]	Polychlorinated alkanes	14	Continued uptake throughout the exposure for three of four compounds.
Leppanen and Kukkonen [13]	Pyrene	28	Continued uptake throughout the exposure.
Leppanen and Kukkonen [14]	Pyrene and Benzo[*a*]pyrene	21	Continued uptake of pyrene throughout the exposure. Peak in benzo[*a*]pyrene by day 4 and slight decline to end of exposure.
Leppanen and Kukkonen [15]	Pyrene and benzo[*a*]pyrene	6	Continued uptake throughout the exposure.
Van Hoof et al. [16]	Low-K_{ow} PAHs	14	Peak at day 2 to 4 and decline to end of exposure.
	High-K_{ow} PAHs		Continued uptake throughout the exposure or steady state by end of exposure.

[a] DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene; PAH = polycylic aromatic hydrocarbon.

better evaluate differences in bioaccumulation between exposures conducted with spiked sediment and field-collected sediment.

While a plateau in the uptake curve was often reached or approached with a variety of compounds in exposures conducted for 3 to 14 d with *L. variegatus* (Table 4), data from exposures conducted in the present study for 28 d enabled more accurate estimates of steady-state concentrations for DDT and its metabolites (Fig. 1A) or for low-K_{ow} and high-K_{ow} PAHs (Fig. 1, B and C). In the present study, a 28-d exposure would generate a BSAF about 33 to 50% lower than a 3- to 7-d exposure for low-K_{ow} PAHs (Fig. 1B). Therefore, time-series sampling has been recommended by ASTM [1] if peak concentrations of low-K_{ow} PAHs or other rapidly metabolized compounds are of interest.

Depuration

Mount et al. [32] reported that gut purging of *L. variegatus* was essentially complete in 6 h after removal from sediment. After a 6-h purging period, compounds with log-K_{ow} values >3.85 were estimated to remain at >90% of their initial concentrations in the organisms, but after 24 h, only compounds with log-K_{ow} values >5.0 were estimated to be at >90% of the initial concentration in tissue [32]. For this reason, ASTM [1] and U.S. EPA [2] recommend purging of *L. variegatus* for 6 to 8 h in water. Longer purging periods (not to exceed 24 h) should be suitable if all target analytes have log-K_{ow} values >5.0. In the present study, oligochaetes were not intentionally depurated after exposure to sediment (except in the depuration study); however, it took about 3 h to remove organisms from sieved material and place them into water. Hence, there was probably a substantial amount of gut clearance (perhaps as much as 90% of the gut contents) in the oligochaetes at the end of all of the sediment exposures.

In the present study, depuration of *L. variegatus* after the 28-d exposure to Huntsville or to Little Scioto sediments was more rapid and less variable in control sediment compared with depuration in water. Similarly, depuration of pyrene and benzo[*a*]pyrene by *L. variegatus* was more rapid in sediment compared with water [9,10]. Mount et al. [32] found good correspondence between depuration predicted by the small fish bioaccumulation model by Gobas et al. [41] and literature data on depuration of organic chemicals from oligochaetes held in clean water. Based on that model and the K_{ow} values of the compounds evaluated in the present study, one would predict a 7-d depuration period to result in depuration of 1 to 6% of body burden for DDT and its metabolites, 24 to 91% for low-K_{ow} PAHs, and 2 to 15% for high-K_{ow} PAHs. These ranges are in general accord with water-only depuration observed in the present studies (Fig. 2), wherein substantial depuration was observed only for the low-K_{ow} PAHs. Depuration rates for DDT and its metabolites and high-K_{ow} PAHs were much higher in organisms held in clean sediment relative to both water-only depuration and model predictions. This suggests that depuration in clean sediment may artificially accelerate depuration of hydrophobic compounds, presumably from some combination of enhancing chemical loss through the gut [42] and inflating dry weight of the organisms (because of clean gut contents contributing to organism wt).

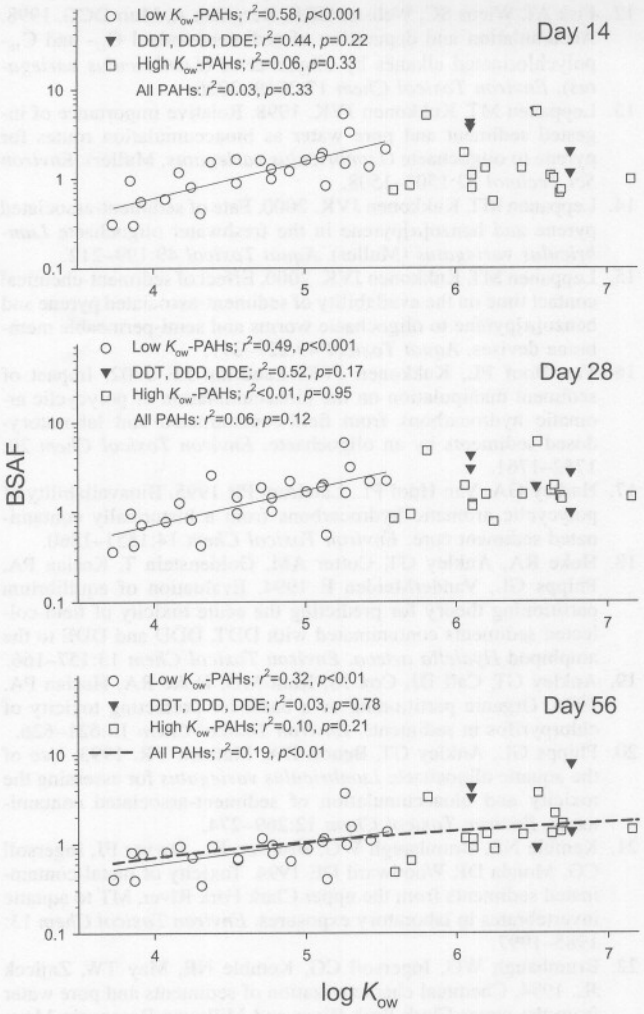

Fig. 4. Biota-sediment accumulation factors (BSAF) for *Lumbriculus variegatus* exposed to Huntsville (Huntsville, AL, USA) sediment DDT, dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE) or Little Scioto (Dayton, OH, USA) sediment polycyclic aromatic hydrocarbons (PAHs). A regression line is plotted only when there was a significant linear correlation ($p < 0.01$).

BSAFs for PAHs and DDT

The BSAFs in the present study were more variable at days 14 and 28 compared with day 56, ranging from 0.34 to 3.7 for PAHs on day 56 (mean 1.2) and ranging from 0.91 to 7.83 for DDT and its metabolites on day 56 (mean 3.5, Fig. 4). Brunson et al. [6] reported comparable PAH BSAFs for *L. variegatus* exposed to less contaminated sediments from the Upper Mississippi River (USA). Van Hoof et al. [16] also reported BSAFs for PAHs after 14-d exposures with *L. variegatus* to a separate sediment sample collected from the Little Scioto River. The BSAFs reported by Van Hoof et al. [16] were 9 to 40% lower than the BSAFs reported in the present study (i.e., fluorene 0.38 vs 0.95, phenanthrene 0.28 vs 1.6, anthracene 0.33 vs 0.89, fluoranthene 0.21 vs 1.9, pyrene 0.16 vs 1.7, benzo[*a*]anthracene 0.13 vs 0.84, and chrysene 0.12 vs 0.74). The differences in methods used to measure lipids may have contributed to these differences in BSAFs. Van Hoof et al. [16] used a microtechnique to measure lipids [43] and used a mean value of 7.0% lipid to calculate BSAFs, whereas lipids in the present study and in [6] were determined using a less accurate gravimetric method. Differences in the amount of soot in the sediment samples might possibly contribute to these differences in BSAFs (a higher concentration of soot in a sample could reduce the bioavailability of PAHs in sediment [44]).

Figure 4 illustrates the relationship between the BSAFs and log-K_{ow} values on days 14, 28, and 56 of the exposures. A significant linear correlation ($p < 0.01$) was observed between BSAF and K_{ow} values on days 14, 28, and 56 for low-K_{ow} PAHs and on day 56 for all PAHs (Fig. 4). No significant linear correlation was observed between BSAFs and log-K_{ow} values for DDT and its metabolites. Tracey and Hansen [45] compiled a database from eight laboratory or field studies containing 4,054 BSAFs for 27 species. Similar to the present study, BSAFs summarized by Tracey and Hansen [45] were lower for PAHs (mean 0.34) compared with organochlorine pesticides (mean 1.36). No relationship between log K_{ow} and BSAFs was observed across a broad range of log-K_{ow} values for PAHs (3.35–6.65) or organochlorine pesticides (2.75–6.95); however, BSAFs for PCBs decreased above and below a log-K_{ow} range of 5.99 to 7.27 [45]. There was only a weak positive correlation between BSAF and K_{ow} values for PAHs in the present study (Fig. 4). Importantly, no reduction in bioaccumulation was observed in the present study at higher K_{ow} values, as reported for OCs and oligochaetes [46,47] or for PAHs and amphipods [48].

Laboratory to field comparisons

Oligochaetes collected from the Little Scioto River tended to inhabit leaf litter and debris above the sediment, whereas oligochaetes collected from the Huntsville Spring Branch tended to inhabit the sediment. These differences in behavior of the oligochaetes may help to explain the stronger correspondence between laboratory and field populations of oligochaetes for the DDT/Huntsville data compared with the PAH/Little Scioto data (Fig. 3). Oligochaetes inhabiting the sediment–water interface of the Little Scioto River may have been exposed to higher concentrations of more soluble low-K_{ow} PAHs in the overlying water relative to less soluble high-K_{ow} PAHs, which seemingly reflects the differences between organisms exposed in the field and in the laboratory. The strong correspondence between native oligochaetes sampled on day 28 and *L. variegatus* suggests that the oligochaetes were not differentially metabolizing the PAHs in Little Scioto sediment (Fig. 3).

Two previous studies have compared results of laboratory bioaccumulation studies conducted with *L. variegatus* with residues from synoptically collected field populations of oligochaetes [4,6]. Ankley et al. [4] reported good agreement between concentrations of PCBs in the laboratory and field organisms, particularly for PCBs with K_{ow} values <7. Similarly, Brunson et al. [6] reported that about 90% of the paired PAH concentrations in laboratory-exposed *L. variegatus* and field-collected oligochaetes were within a factor of three of one another.

In conclusion, sediment exposures conducted for a minimum of 14 to 28 d better reflected steady-state concentrations for DDT and its metabolites and steady-state concentrations for PAHs. However, a time-series sampling should be performed if there is interest in determining the maximum accumulation of compounds such as low-K_{ow} PAHs in short-term exposures. Additional research is needed to determine the cause of the initial peak in the accumulation of 4,4′-DDT or low-K_{ow} PAHs followed by a decline during the exposures. It

is not clear if these patterns result from a change in the bioavailability of the compounds in the sediments, metabolism of the compounds by *L. variegatus* (or by bacteria in sediment), or a change in the feeding or respiratory behavior of oligochaetes during the exposure. Depuration rates for DDT and its metabolites and high-K_{ow} PAHs were much higher in organisms held in clean sediment relative to both water-only depuration and model predictions. This suggests that depuration in clean sediment may artificially accelerate depuration of hydrophobic compounds. Finally, the comparisons between laboratory and field exposures indicate that results of laboratory tests can be extrapolated to the field with a reasonable degree of certainty; however, differences in behavior may modify the bioaccumulation of contaminants by organisms in the field.

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