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VARIATION IN THE BIOAVAILABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS TO THE AMPHIPOD *DIPOREIA* (SPP.) WITH SEDIMENT AGING

PETER F. LANDRUM,* BRIAN J. EADIE and WARREN R. FAUST
Great Lakes Environmental Research Laboratory, NOAA,
2205 Commonwealth Boulevard, Ann Arbor, Michigan 48105

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Abstract—The accumulation kinetics of the amphipod *Diporeia* sp. for sediment-sorbed polycyclic aromatic hydrocarbon (PAH) congeners were measured where the contact time between the PAH congeners and the sediments varied between 3 and 150 d. *Diporeia* were exposed to a reference sediment dosed with two radiolabeled PAHs (phenanthrene and pyrene) and to sediments dosed with the two radiotracers and a mixture of PAHs at 272.3 ± 10.9 nmol g^{-1} dry sediment, as the sum of the nine nonlabeled PAHs. The sediments were prepared by dosing aliquots of a single collection of sediment with PAHs at different times, producing a series of contact times. The exposures were all performed at the same time from a single collection of organisms. *Diporeia* were sampled for mortality and toxicokinetics for up to 28 d. Some sediment avoidance was observed during the first 12 d of exposure. The partitioning between sediment particles and interstitial water increased significantly for both phenanthrene and pyrene as contact time increased. The uptake rate coefficient decreased for both phenanthrene and pyrene when the contact with the sediment was increased from 3 to 60 d. When the contaminant-sediment contact was increased from 60 to 150 d, the phenanthrene uptake rate coefficient remained constant whereas that for pyrene increased somewhat. Similar uptake results were observed with the nonradiolabeled compounds.

Keywords—Bioavailability *Diporeia* Polycyclic aromatic hydrocarbons (PAHs) Sediment storage Sediment aging

INTRODUCTION

Bioavailability of sediment-associated contaminants has been observed to decline with increased contact time between the sediment and the contaminant [1,2]. This reduced bioavailability has been observed for laboratory-dosed sediments held for differing lengths of time [1] and for benzo[*a*]pyrene (BaP) accumulation from field-collected sediments compared with BaP laboratory-dosed to a separate aliquot of the field-collected material [2]. Even though reductions in accumulation have been observed, the potential impact of this process has not generally been recognized.

Alteration of chemical behavior parallels that observed for bioavailability. When the contact time between the sediment and contaminant is increased, the ability to chemically extract organic contaminants declines [3-6]. When the bioavailability and chemical extractability are compared, decreases in chemical extractability occur more slowly than do decreases in bioavailability [1]. Compounds with moderately large log octanol/water partition coefficients ($\log K_{ow}$) such as BaP, $\log K_{ow}$ 5.98, do not exhibit a decline in bioavailability for laboratory-dosed sediments, whereas compounds with smaller $\log K_{ow}$ values such as pyrene (PY), $\log K_{ow}$ 5.2, show significant alteration [1]. Over the longer contact time that sediments reside in the field, bioavailability apparently declines even for compounds such as BaP [2]. This suggests that the rate processes for this reduction are inversely proportional to $\log K_{ow}$. A similar inverse proportionality has been observed for decreases in chemical extractability [4].

The environmental significance of this bioavailability reduction may be large when comparing laboratory and field exposures. However, the kinetics are sufficiently fast for compounds such as PY and phenanthrene (PHE) that even comparisons between laboratory studies can be affected by variation in the contact time between the sediment and contaminant.

To better describe how bioavailability changes with contact duration between the sediment and contaminant (sediment aging), the amphipod

*To whom correspondence may be addressed.
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Diporeia (spp.), a dominant Great Lakes macroinvertebrate that accounts for most of the benthic biomass [7], was exposed to laboratory-dosed sediments containing a mixture of polycyclic aromatic hydrocarbons (PAHs) that had different contact times between sediment particles and contaminants. The differences in bioavailability were assessed as differences in the accumulation kinetics for PAH congeners. Organism health was determined by examining mortality and lipid content of the organisms relative to organisms exposed in a reference sediment to help ensure that any differential accumulation was not a result of differentially stressed organisms. The partition coefficient between sediment and interstitial water was measured to examine the hypothesis that observed reductions in bioavailability were due to increased sorption to the sediment.

MATERIALS AND METHODS

Collection of environmental samples

Diporeia (spp.) were collected from Lake Michigan off Grand Haven, Michigan, at a depth of 24 to 29 m with a Ponar grab sampler. (Note: The genus *Diporeia* (spp.) is a new name given to the organisms previously called *Pontoporeia hoyi* [8].) The *Diporeia* were gently removed from the sediment with a screen, placed in clean lake water, and kept cold with ice during transport to the laboratory. *Diporeia* were kept in aquaria containing 2 to 3 cm of sediment and 7 to 10 cm of lake water at 4°C [9]. Water used throughout the work was Lake Michigan surface water (collected about 1 m below the surface) and stored at 4°C. Lake Michigan sediment was obtained by PONAR grab approximately five miles off Grand Haven, Michigan, at 45 m depth. The sediment was sieved at 1 mm to remove animals and large pieces of debris. The sieved sediment was stored at 4°C before use.

Preparation of sediment

Aliquots of sediment were dosed 150, 60, and 3 d before beginning exposure of *Diporeia*. Each sediment aliquot was dosed with two radioactive tracers, [^{14}C]PHE (universally labeled, specific activity 14 mCi mmol^{-1}) and [^{3}H]PY (specific activity 34 Ci mmol^{-1}). Additionally, nine nonlabeled PAHs—anthracene (Anth), fluorene (FL), fluoranthene (FlA), chrysene (Chr), BaP, benzo[*e*]pyrene (BeP), perylene (Per), benzo[*b*]fluoranthene (BbF), and benzo[*ghi*]perylene (BghiP)—were dosed to all of the sediments except a fourth aliquot, which served as a reference sediment that contained only the two radiolabeled compounds dosed 3 d before exposing organisms.

To dose the sediments, a solution of PAH was prepared by dissolving 30 mg of each nonlabeled compound in 50 ml acetone. The appropriate amount of this mixture (8.33 ml) was diluted to make a dosing solution of 25 ml in an acetone carrier. The dosing solution was then added to approximately 3 L of a 1:4 sediment to water slurry (w/v), as described below.

The 1-mm sieved sediment was mixed until it appeared to be homogeneous. An amount of wet sediment corresponding to 680 g dry weight was placed in a 4-L beaker, and 2,500 ml lake water was added. The following two solutions were added drop by drop while the mixture was stirred vigorously at room temperature: 1.4 ml methanol containing [^{14}C]PHE (2.76 μCi/680 g dry sediment) and [^{3}H]PY (13.5 μCi/680 g dry sediment), followed by 25 ml acetone containing the PAH solution. The amount of acetone and methanol carriers added to each sediment mixture was held constant. The mixture was stirred at room temperature for 4 h and kept at 4°C thereafter. The small amount of water-soluble carrier should have a minimal impact on the partitioning to the sediments [10]. After 2 d of settling, the overlying water was decanted, and fresh lake water was added. During the storage, the sediments were kept covered with 2 to 3 cm of lake water, and fresh lake water was added as necessary.

Sediment dry weight for all samples was determined by determining the wet:dry weight ratio of subsamples. The dry weight was obtained by drying sediments to a constant weight at 60°C. The percentage of volatile solids was determined from the difference in weight between the 60°C dried sediment and the same sample after ashing at 500°C for 4 h as a percentage of dry weight.

Preexposure procedures

Two days after dosing the last aliquot of sediment, the overlying water was decanted from each aged sediment, the sediment was stirred for homogeneity, and 45 g wet sediment was transferred to each of 15 beakers (400 ml); 250 ml lake water was added to each beaker [11]. The estimated amounts of acetone (<1.2 μl ml^{-1}) and methanol (<0.07 μl ml^{-1}) remaining were not expected to affect the toxicokinetics [12]. The estimate of the amount of carrier assumes that (a) 80% of the water was decanted (2,500 ml of the 2,840 to 3,180 ml total), (b) there was no loss of acetone or methanol due to

volatilization, and (c) the carriers dispersed into the fresh overlying water.

Samples for PAH concentration and sediment characteristics were taken during the distribution of sediment to the exposure beakers. Triplicate samples were taken from each set of aged sediments at the start, middle, and end of the distribution. These samples included 2 g sediment for analysis of nonlabeled PAHs by GC, 2 g sediment for wet and dry weight determination, and 5 g sediment desiccated with sodium sulfate and extracted with ethyl acetate for determination of radioisotope activity. Lake water (250 ml) was then carefully added to each beaker, minimizing the disturbance of the sediment. The following day, three water samples (25 ml) were randomly taken from beakers representing each aging level, to determine the distribution of the radiotracers between bound and freely dissolved concentrations in the overlying water.

Distribution of the radiolabeled compounds in the overlying water, collected above, was determined from the initial 25-ml water samples from each aged set. Total activity was determined on a 2-ml subsample; 20 ml was filtered for particle-bound contaminant, with a backup filter which was counted to correct for sorption to the filter and 2 ml of filtrate counted; 15 ml of the filtrate was passed through a C_{18} Sep-Pak® (Waters, Mississauga, Ontario, Canada); and a 2-ml subsample of the eluate was counted for dissolved organic matter- (DOM-) bound PHE and PY. The amount of freely dissolved compound in the water column was determined by the difference between the total activity and the sum of the DOM- and particle-bound activities on a per-milliliter basis [13,14].

Experimental design

Diporeia were exposed in groups of 20 organisms to each aged sediment in triplicate per exposure duration—2, 6, 12, 19, and 28 d. Thus, the total number of groups of exposed organisms was 15 per duration of aging (3, 60, or 150 d). The organisms were kept at 4°C under constant illumination from a 7.5-W red darkroom light. The sediments containing only the two radiotracers served as the reference for the accumulation studies and as a control for mortality and sediment avoidance. In previous studies [11,15; P.F. Landrum, unpublished data], water oxygen concentration and water quality, hardness, alkalinity, and pH were not found to change under similar exposure conditions and were not measured in this study.

At each sample time (2, 6, 12, 19, and 28 d), three beakers from each aged sediment were sampled at random. To quantify the avoidance behavior, the number of test organisms found on the sediment surface or swimming in the overlying water was recorded for each of the triplicate beakers. *Diporeia* were removed from the sediment by passing the sediment through a 1-mm screen. The extent of mortality was determined by recording the number of organisms surviving for one of the triplicate samples. Organisms were sampled for both nonlabeled PAHs and radioisotope analyses. Organisms were also recovered for subsequent analysis from the remaining two samples as the sediment was transferred for interstitial water recovery. However, the organisms could not be recovered quantitatively from the two samples used for recovery of interstitial water while minimizing the disturbance of the sediment for the interstitial water isolation; thus, a mortality assessment could not be determined for these samples. For each replicate sample, (a) the radioisotope concentration was measured for two individual *Diporeia* (wet weight basis); (b) the individual lipid content of two organisms was determined by microgravimetric analysis [16]; and (c) the remaining live organisms were combined, weighed, and extracted by homogenizing in a tissue grinder with methylene chloride for GC determination of their PAH content.

Three subsamples of sediment from each beaker were used to determine the wet:dry weight ratio, the PAH content determined by GC analysis, and the radioisotope activity after desiccation and extraction with ethyl acetate. Radiopurities of the radiotracers were examined for sediment extracts taken at the beginning and end of the exposures.

The interstitial water was isolated from two of the beakers by transferring the excess sediment that was not needed for chemical analysis to a centrifuge tube and centrifuging at 1,000 *g* for 10 min. Total, bound, and free PHE and PY were determined in the same manner as that for the overlying water described above.

The partition coefficient between the sediment and the interstitial water was calculated from the freely dissolved concentration of the PY or PHE determined for the interstitial water and the concentration in the sediment on a dry weight basis [17]. Thus,

$$K_p = C_s/C_{iw} \quad (1)$$

where K_p is the partition coefficient, C_s is the concentration in the sediment on a dry weight basis,

and C_{iw} is the freely dissolved concentration in the interstitial water.

Analyses

To prepare the sediments for PAH analysis by GC, wet sediments (approximately 5 g [2 g dry weight]) were Soxhlet extracted for 4 h in 100 ml methanol; 200 ml methylene chloride was then added, and the extraction continued overnight. The extracted samples—sediments and *Diporeia*—were washed twice with hexane-extracted distilled water in equal amount with the sample. The washed extract was rotary evaporated to a few milliliters, and 50 ml pentane was added; the sample was further concentrated to approximately 1 ml on the rotary evaporator. Each extract, now in pentane, was introduced to an activated silica gel column (12 × 1 cm), and the hydrocarbons were eluted with 12 ml of pentane. The PAHs were eluted with 12 ml of pentane:methylene chloride (5:1). The PAH portion of each sample was rotary evaporated to approximately 1 ml, and approximately 2 ml of hexane was added. The resultant mixture was then evaporated to 1 ml under a gentle stream of nitrogen for GC analysis. Extraction yields were calculated by using the ^{14}C- and ^{3}H-labeled PAHs. Extraction recoveries of spiked sediments ranged from 92 to 116% [11].

Capillary GC analysis was performed on a Hewlett-Packard 5880 (Avondale, PA) equipped with automated dual injection ports (280°C) and two model PI-52 photoionization detectors (10.2-eV lamps at 280°C) (HNU Systems Inc., Newton, MA) using 25-m BP-1 and BP-5 columns. The analysis was run from 120 to 270°C at 4°C/min with a carrier (H_2) flow of approximately 1 ml/min. Compound identification required that retention times on both columns be within 0.5% of standard runs for the same day. (Average sediment analytical method yields for the various PAHs were FL 83%, Anth 64%, FlA 62%, Chr 50%, BbF 54%, BeP 66%, BaP 46%, Per 138% and BghiP 48%, based on a series of spiked samples.) Individual *Diporeia* analytical method yields ranged from 49 to 89%, estimated from the recoveries of the radiolabeled compounds. NBS Standard 1647 (National Bureau of Standards, Washington, DC) was run as a calibration check for quantitation approximately every 10th sample.

The radiopurities of the dosed compounds and compounds extracted from the sediments were determined by TLC and liquid scintillation counting. The extracts were chromatographed in hexane:benzene (8:2 v/v). The regions corresponding to the parent compound and all potential metabolites (including the origin) were scraped from the plate and placed in vials containing scintillation cocktail, for determination of the radioactivity. The percentage of radiopurity is the ratio of the amount of activity found at the retention position of the parent compound divided by the total activity measured for the sample.

Radioactivity was determined by liquid scintillation counting on an LKB 1217, using scintillation cocktail 3a70B (Research Products International, Mt. Prospect, IL). The data were corrected for quench, using the external standards ratio method, after correcting for background.

Kinetics model

The uptake coefficient was calculated from the data in Tables 1 and 2, using the following general model [1]:

$$dC_a/dt = k_s \cdot C_s \cdot e^{-\lambda t} - k_d \cdot C_a \qquad (2)$$

where k_s is the uptake coefficient (g dry sediment g^{-1} organism h^{-1}), C_s is the concentration in the sediment (nmol g^{-1} dry sediment), λ is the rate constant (h^{-1}) for reduction in the bioavailable concentration of the specific PAH congener, k_d is the elimination rate constant (h^{-1}), and C_a is the concentration in the animal (nmol g^{-1}). For some of the congeners, the k_d value had not previously been determined and was estimated from a regression between k_d and $\log K_{ow}$ [18]. The data for compounds with a $\log K_{ow} < 5$ were fit to the integrated form of Equation 1 by using the nonlinear regression program, NLIN, in SAS® [19], with the average elimination rate constant [18] supplied. For compounds with a $\log K_{ow} > 5$, the model could be simplified to a one- or two-compartment donor-dependent model, with λ equal to zero [1].

Statistics

Differences between two means or slopes from regression lines were compared by using Student's *t* test. Differences were considered statistically significant when $p < 0.05$. Linear regression and correlation coefficients were determined by using the linear regression program in SAS® [19].

RESULTS

Sediments

Sediments collected from the same site had previously been characterized for organic carbon content (1.0 ± 0.2%) and volatile solids content (1.9 ± 0.2%) [11]. The sediment volatile solids content for

Table 1. Initial concentration of radiolabeled PAHs and mean concentration of nonlabeled PAHs (nanomoles per gram) in exposure sediment

		Dose level				
		Reference[a]		Experiment[b]		
PAH	log K_{ow}[c]	Aged 3 d	Aged 3 d	Aged 60 d	Aged 150 d	% Recovery[d]
Fluorene	4.18		24.4	15.9	41.6	61
^{14}C Phenanthrene	4.57	0.70	0.70	0.17	0.22	230[e]
Anthracene	4.54		28.1	19.8	17.2	52
Fluoranthene	5.2		39.7	48.6	38.5	113
^{3}H Pyrene	5.2	0.41[f]	0.42[f]	0.61[f]	0.45[f]	81
Chrysene	5.7		17.4	16.2	12.6	48
Benzo[*b*]fluoranthene	5.98[g]		27.6	28.5	25.8	93
Benzo[*e*]pyrene	5.98[g]		49.4	39.1	44.3	152
Benzo[*a*]pyrene	5.98		27.4	20.5	21.4	77
Perylene	6.5		33.4	57.1	68.0	177

[a]Reference sediment—only radiotracers added to sediment. This sediment was not analyzed for nonlabeled contaminants.
[b]Experimental sediments were dosed with both radiotracers and the nine nonlabeled PAHs and were aged differently.
[c]From [33].
[d]Mean recoveries of PAHs based on expected nominal concentrations dosed to the sediments.
[e]The recovery for phenanthrene was unusually high because the amount of label added to the 3-d aged sediment was much greater than anticipated.
[f]Concentration in picomoles per gram.
[g]Log K_{ow} estimated from a regression of molecular weight vs. log K_{ow}.

this study was 2.3 ± 0.2%. Thus, even though organic carbon was not measured directly, it should not have been significantly different from the previously characterized material. The contaminant homogeneity in the sediments was examined by analyzing the radiotracers from samples taken during the sediment distribution to the exposure beakers. The C.V.s were low, 9.3 ± 3.4% (mean C.V. ± SD), for the PHE and 6.9 ± 4.5% for the PY for all the aged sediments. Due to the low C.V.s, the sediments were assumed to be homogeneously dosed on a bulk sediment basis.

Table 2. The mean concentration[a] of PAH congeners in *Diporeia* (nanomoles per gram)

Age (days)	Sample time (days)	FL	Anth	FlA	Chr	BbF	BeP	BaP	Per	BghiP
3	2	319	236	223	136	147	56	32	159	301
3	6	578	376	366	259	250	175	111	353	518
3	12	277	325	405	325	484	389	306	540	391
3	19	252	386	386	232	413	341	270	404	362
3	28	72	185	465	254	504	381	302	496	351
60	2	145	185	312	53	171	44	20	163	282
60	6	114	241	401	101	143	107	71	286	304
60	12	90	230	446	154	175	147	103	278	239
60	19	271	247	396	193	254	194	147	325	108
60	28	145	191	332	224	238	257	202	429	318
150	2	120	152	282	61	71	52	24	119	94
150	6	157	247	371	140	159	179	99	206	275
150	12	151	314	426	202	218	246	175	313	272
150	19	60	163	436	171	222	222	155	250	250
150	28	18	79	520	162	222	226	151	373	326

Fluorene (FL), anthracene (Anth), fluoranthene (FlA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*e*]pyrene (BeP), benzo[*a*]pyrene (BaP), perylene (Per), and benzo[*ghi*]perylene (BghiP).
[a]$n = 3$ for each sample time for each sediment.

The amount of the radiolabeled compounds measured in the sediment (Table 1), compared to the nominally dosed concentrations, averaged 102% for both the 60- and the 150-d contact and 370% for the 3-d contact for PHE, and 81% for PY. These mass balances between amount dosed and amount measured are similar to those found for other studies [1,11,20]. The relative amounts of the nonlabeled PAHs measured in the sediments were similar to that of the PY, based on the nominal amount dosed to the sediments (Table 1). The large amount of PHE found in the 3-d aged sediments was probably due to an error in the total dosed amount. The extractable radiotracer concentrations in the sediments declined somewhat between the initial measurement before exposure and at the end of the exposures for both PY and PHE. The PY concentration declined 15.5 ± 2.3%, whereas the PHE declined 51.5 ± 9.7%. No declines in PAH concentrations were detectable for the nonlabeled compounds. The declines in concentration were accounted for during the determination of the kinetic coefficients.

The radiopurities of the labeled compounds were determined to ensure that no degradation had taken place during storage or over the course of the exposures. Both compounds had high radiopurities, even after storage for 150 d. At the start of the experiment, the radiopurities were 98 ± 0.7% for PHE and 95 ± 1.1% for PY. There was little or no degradation by the end of the exposure period (radiopurity = 96 ± 1.8% for PHE and 92 ± 1.4% for PY). Thus, the amount of the labeled contaminants could be determined from the radioactivity and the concentration calculated from the specific activity of the respective compound.

Partition coefficients

The partition coefficients between the interstitial water and the sediment particles were determined at each sample time from two of the triplicate beakers containing exposed organisms for the two radiolabeled compounds. Additionally, on the last day of the experiment, partitioning was determined for duplicate controls—samples that contained no organisms. In the samples with the nonlabeled PAHs, the partition coefficients increased significantly for PY and PHE with longer durations of aging (Fig. 1). The rates of increase in the partition coefficients were not different for the two compounds, based on the slopes of the regression lines. The partition coefficients for the samples taken on day 19 of the organism exposures were approximately three times larger than any other measures

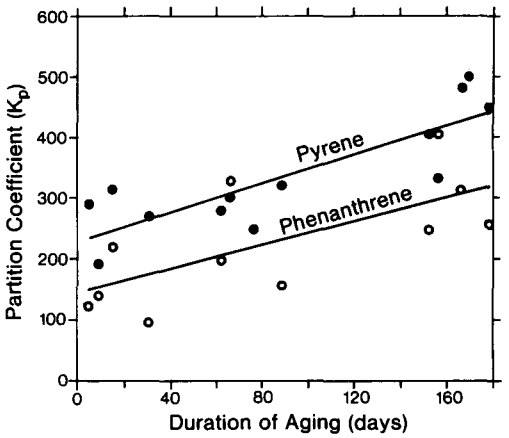

Fig. 1. Increasing partition coefficients between interstitial water and sediment particles for pyrene and phenanthrene as the contact time between the compounds and the sediment increased [pyrene $K_p = 227.7 \pm 25.5 + (1.19 \pm 0.23) \cdot \text{time}$, $r^2 = 0.678$, $p < 0.01$, $n = 13$; phenanthrene $K_p = 143.8 \pm 34.1 + (0.956 \pm 0.33) \cdot \text{time}$, $r^2 = 0.41$, $p = 0.016$, $n = 12$].

for all durations of aging and were excluded from further consideration.

In the absence of the nonlabeled PAHs in the reference sediment, a similar trend toward larger partition coefficients with longer aging was observed for both PY and PHE. However, the variability was large and no significant increase was found due to the small number of samples and the short duration of aging from 3 to 31 d, that is, from the beginning of the *Diporeia* exposure to the end of the exposures.

The partition coefficients for PY and PHE were similar to those determined previously with the same length of aging [11]. The partition coefficients were not significantly different between sediments with organisms and controls (no organisms) for samples taken at the end of the experiment for all aging times with both compounds (Table 3). The partition coefficients in the absence of organisms also increased with increased aging. The slopes of the regression lines for the partition coefficients vs. contact time, in the absence of organisms, were not significantly different from those containing organisms. However, the intercepts were somewhat lower compared to the overall regressions for PHE and PY (Table 3 and Fig. 1).

Toxicity and bioaccumulation

The sediment PAH concentration employed for this study was similar to the highest dose that ex-

Table 3. Partitioning between the interstitial water and the sediment particles for phenanthrene and pyrene in the presence and absence of *Diporeia*

Aging time (days)	K_p (PHE)		K_p (PY)	
	No *Diporeia*	*Diporeia*	No *Diporeia*	*Diporeia*
31	133	94.8	235	269
88	205	153	294	318
178	250	251	503	447
31[a]	317	279	507	526

Regression lines for PHE, no *Diporeia*, $K_p = 119.8 \pm 21.5 + (0.769 \pm 0.21)\cdot$time, $r^2 = 0.93$; PHE, with *Diporeia*, $K_p = 60.8 \pm 1.9 + (1.06 \pm 0.002)\cdot$time, $r^2 = 0.99$; PY, no *Diporeia*, $K_p = 159.1 \pm 36.4 + (1.86 \pm 0.35)\cdot$time, $r^2 = 0.97$; PY, with *Diporeia*, $K_p = 222.8 \pm 16 + (1.23 \pm 0.15)\cdot$time, $r^2 = 0.98$.
[a]These samples contained no added nonlabeled PAHs.

hibited a minimal mortality in a previous *Diporeia* study [11]. This dose was chosen to avoid overt responses in the organisms while making the analysis of the nonlabeled PAHs as easy as possible. Although some sediment avoidance was observed early in the experiment, 25 ± 13%, there were no significant differences among the reference, the sediment containing only the radiotracers, and the other sediments containing the nonlabeled PAHs. Most of the avoidance was observed during the first 6 d of exposure and had dropped to 15 ± 10% by day 12. Little mortality (0–10%) was found, except in one sample from the reference sediment at the last sample time (80%) and the 150-d aged sediment at the last sample time (40%). The mortality in these samples is extremely unusual [11] and was not reflected by the other samples. The condition of the *Diporeia*, as determined by the lipid content, exhibited a significant decline for the 3- and 60-d aged sediments, but there was no significant decline for the reference and the 150-day aged sediments, based on linear regression of lipid content vs. exposure duration (Table 4).

The k_s values for the radiotracers (PHE and PY) in the reference sediment (Table 5) were similar to values previously determined [1]. Further, the k_s values for the PAH congeners, both radiolabeled and nonlabeled, with a $\log K_{ow}$ of ≤ 5.2 and aged 3 d were similar to those of previous studies in which the total sediment PAH concentration was similar [11]. However, for PAHs with $\log K_{ow} > 5.2$, the k_s values were larger than those previously observed [11]. As a result, the slope of the regression line between $\log K_{ow}$ and $\log k_s$ is approximately half that previously observed [1,11] (Fig. 2).

After 60 d contact, the regression line between $\log K_{ow}$ and $\log k_s$ had the same slope as the regression from the 3-d contact, but the intercept declined by 0.69 log units (a factor of approximately five) (Fig. 3). By the time the contact time had been extended to 150 d, the slope of the regression line between $\log K_{ow}$ and $\log k_s$ was about half that of both the 3- and the 60-d contact times, and the intercept was 0.24 log units less than the intercept for the 60-d contact time relationship (Fig. 3). The lower slope results primarily from an increase in the measured k_s values for the compounds with the larger $\log K_{ow}$ values, $\log K_{ow} > 5.2$, whereas the k_s values remained similar to those after 60 d contact for compounds with smaller $\log K_{ow}$ values, $\log K_{ow} < 5.2$ (Table 5).

Table 4. Percentages of lipid for *Diporeia* over the course of the 28-d exposure

Sediment aging (days)	% lipid[a] (mean ± SD)				
	6 d Exposure	12 d Exposure	19 d Exposure	28 d Exposure	p[b]
Ref.[c]	18.9 ± 5.0 ($n = 5$)	16.8 ± 3.4 ($n = 6$)	11.4 ± 4.3 ($n = 5$)	17.5 ± 7.8 ($n = 4$)	0.21
3	15.0 ± 4.8 ($n = 6$)	14.6 ± 6.2 ($n = 6$)	13.3 ± 5.6 ($n = 6$)	7.6 ± 3.4 ($n = 5$)	<0.01
60	14.5 ± 3.2 ($n = 6$)	16.2 ± 5.8 ($n = 5$)	8.5 ± 2.8 ($n = 6$)	9.0 ± 3.7 ($n = 6$)	<0.01
150	14.0 ± 5.1 ($n = 6$)	12.2 ± 8.5 ($n = 6$)	16.8 ± 3.6 ($n = 6$)	11.8 ± 8.7 ($n = 6$)	0.11

[a]Percentages of lipid on a dry weight basis. Initial lipid content 17.2 ± 5.6% ($n = 17$).
[b]Achieved significance level.
[c]This row represents 3 d of contact, with only the labeled PAHs added to the sediment.

Table 5. Uptake clearance (k_s) values for PAH congeners with various durations of sediment aging

PAH congener	k_s (g dry sediment g^{-1} h^{-1})			
	Aged 3 d[a]	Aged 3 d	Aged 60 d	Aged 150 d
Fluorene	ND	0.464 (0.13)[b]	0.079 (0.046)	0.083 (0.015)
Phenanthrene	0.038 (0.009)	0.213 (0.039)	0.092 (0.033)	0.094 (0.013)
Anthracene	ND	0.21 (0.034)	0.145 (0.037)	0.239 (0.046)
Fluoranthene	ND	0.082 (0.021)	0.11 (0.025)	0.086 (0.024)
Pyrene	0.023 (0.001)	0.124 (0.013)	0.034 (0.003)	0.064 (0.012)
Chrysene	ND	0.180 (0.036)	0.051 (0.004)	0.126 (0.012)
Benzo[*b*]fluoranthene	ND	0.095 (0.02)	0.052 (0.02)	0.059 (0.004)
Benzo[*e*]pyrene	ND	0.043 (0.013)	0.019 (0.003)	0.047 (0.01)
Benzo[*a*]pyrene	ND	0.053 (0.02)	0.022 (0.002)	0.054 (0.02)

ND = not determined.
[a]Reference concentration—only radiotracers added to the sediment.
[b]Numbers in parentheses represent the standard error of the regression fit for the data to the appropriate kinetic model.

DISCUSSION

The sediments were generally homogeneously dosed on a bulk sediment basis, as represented by the low coefficients of variation for the radiolabeled compounds. These coefficients of variation were not different among sediments with different aging. Further, the radiolabeled compounds did not degrade during the preexposure storage. Thus, the radiolabel could be used as a tracer of the respective parent compounds.

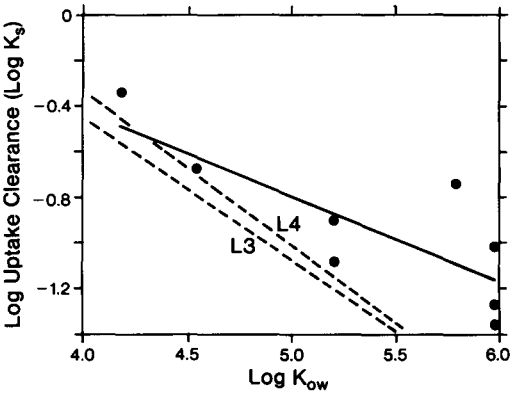

Fig. 2. Relationship between the uptake clearance (k_s) and the octanol-water partition coefficient (K_{ow}) for PAHs sorbed to sediments for 3 d. The solid line is for this study with sediments that had been stored for 150 d [$\log k_s = 1.102 \pm 0.198 - (0.38 \pm 0.098) \cdot \log K_{ow}$, $r^2 = 0.68$, $n = 9$, $p < 0.01$]; the two dashed lines are for fresh sediments from previous studies [11] at total PAH concentrations slightly less than those used for this study (L3) [$\log k_s = 2.12 \pm 0.71 - (0.64 \pm 0.13 \cdot \log K_{ow})$ and slightly greater than those used for this study (L4) [$\log k_s = 2.38 \pm 0.78 - (0.68 \pm 0.15) \cdot \log K_{ow}$].

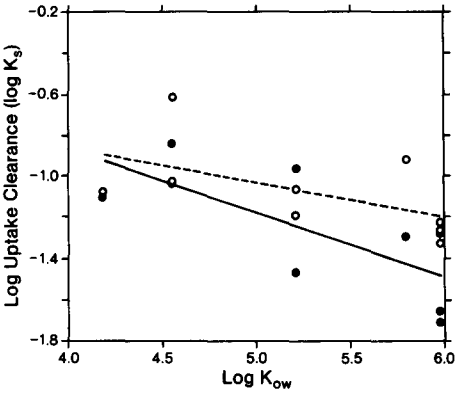

Fig. 3. Relationship between the uptake clearance (k_s) and the octanol-water partition coefficient (K_{ow}) for PAHs sorbed to sediments for 60 d (closed circles and solid line) and 150 d (open circles and dashed line) [60 d: $\log k_s = 0.408 \pm 0.22 - (0.317 \pm 0.11) \cdot \log K_{ow}$, $r^2 = 0.54$, $n = 9$, $p < 0.05$; 150 d: $\log k_s = -0.17 \pm 0.19 - (0.173 \pm 0.094) \cdot \log K_{ow}$, $r^2 = 0.33$, $n = 9$, $p < 0.10$].

The PAH concentrations measured in the sediments were approximately 20% lower than nominally dosed. This is similar to the relative amount of PAHs found in other experiments where sediments were dosed in the laboratory [1,11,20]. The one exception in this work was the PHE added to the 3-d aged sediments. For these sediments, the added amount of PHE was apparently much larger than the planned amount. For all the compounds, the kinetics and partitioning were based on measured sediment concentrations, not the nominal doses. Because the sediments contained radiolabeled compounds, losses that occur through the analytical methodology can be corrected by comparing the amount of radiotracer in the extract prepared for GC with extracts prepared for radiotracer analysis that are analyzed directly and do not pass through the cleanup and volume reduction steps.

The sediment-associated PAHs behaved differently from those of previous studies. The concentrations of both PHE and PY declined significantly over the course of the exposures for all aging durations. Previously, the decline in the PHE and PY concentrations have been small and generally observed only for PHE [1,11]. The reasons for the enhanced losses in these stored sediments are not known. However, the influence of the declines in contaminant concentration was minimized for the toxicokinetics by using the model in Equation 1, which allows for loss of bioavailable contaminant concentration over time.

Both radiolabeled compounds exhibited increased partitioning between interstitial water and sediment particles as aging increased (Fig. 1). However, even after 150 d of aging, the partition coefficients for PY and PHE were lower by a factor of approximately four than those measured for field-collected sediments [21]. These increases in partition coefficients apparently did not result from some organism action as the partition coefficients also increased in the absence of *Diporeia*. The increasing partitioning suggests that the compounds are becoming more tightly bound with increased contact time. Such increased binding has been described as movement from a reversible to a resistant pool of bound compound in other partitioning experiments [4,5]. This increased partitioning is likely reflected, at least in part, in the bioavailability decreases of the PAHs as aging increases.

The *Diporeia* exhibited some early avoidance of the sediment, but the avoidance was not different for the differently aged sediments. Thus, decreases in the uptake coefficients from the different aged sediments could not be attributed to selective avoidance. The decline in lipid content was significant but similar for organisms exposed to the 3- and 60-d aged sediments containing the nonlabeled PAHs. Because the largest change in bioavailability occurred between the 3- and 60-d aged sediments and the organisms for these two exposures exhibited similar changes in lipid content, organism health did not seem to account for the decreased bioavailability of the sediment-sorbed PAHs.

Two changes in the uptake clearance coefficients (k_s) were observed with the stored sediments. First, with only 3 d of aging (the same duration as that of previous experiments), the k_s values were similar to previous values for the reference sediment containing only the radiotracers. However, for the same length of aging with the addition of the nonlabeled PAHs, PAH congeners with $\log K_{ow}$ values >5.2 had larger than expected k_s values, compared to those of a previous study containing a similar total PAH concentration [11]. This increase in the k_s values for the larger $\log K_{ow}$ components represents increased bioavailability.

The differences in bioavailability between the previous k_s values from fresh sediments and this study with stored sediments were not likely due to differences in the composition of the originally collected material, because k_s values measured for freshly collected sediment were similar among various collections made over the course of five years [1]. Nor was the PAH concentration likely responsible for the change in bioavailability, as the slope of the regression lines between the $\log k_s$ and the $\log K_{ow}$ remained the same regardless of the concentration of added PAH in a previous study [11].

Two hypotheses may account for the differential bioavailability of compounds with the larger $\log K_{ow}$ values: (a) changes in the sediment composition with storage result in changes in partitioning and bioavailability, and/or (b) changes in behavior, specifically ingestion rate, may differentially result in increased accumulation of the more strongly sorbed compounds.

Storage of field-collected sediments can affect the toxicity of some contaminants [22], presumably reflecting changes in contaminant bioavailability, but storage does not always result in altered toxicity [23,24]. Additionally, the composition of the interstitial water recovered from field-collected sediments can change with sediment storage [25,26], which will potentially alter bioavailability and toxicity. Whereas alteration of toxicity has been observed with sediment storage, alteration of sediment composition as a potential contributor to the changed toxicity has not been well examined. Further, the compounds with the larger $\log K_{ow}$

values were present in nonlabeled form, thus it was not possible to examine their partitioning in the 150-d stored sediment compared to previously measured partitioning in fresh sediment [15] to evaluate changes in chemical behavior of these PAHs. Although changes in sediment composition would likely have the greatest effect on the compound-sediment interactions and therefore the bioavailability of compounds with the larger log K_{ow} values, whether sediment storage results in differences in sediment composition that are manifest in differences in the bioavailability remains to be proven.

Alternatively, the differences between the rates of accumulation from the stored sediment and fresh sediment may be due to an alteration in the dominant route of accumulation. Values of k_s as measures of bioavailability are integrated measures of exposure and do not distinguish between the various routes of accumulation such as from interstitial water or particle ingestion. However, factors that influence ingestion or respiration may differentially affect the measured bioavailability. Because ingestion is apparently the dominant route for accumulation of compounds with large log K_{ow} values (approximately ≥ 6 [1,27]), the observed increases in bioavailability for these compounds may reflect changes in ingestion. The organisms in the stored sediment, aged for 3 d, exhibited a decline in lipid content over the course of the experiment. Such stress during early stages of toxicity has resulted in increased feeding for aquatic oligochaetes [28,29] and might have accounted for the overall change in k_s at different sediment-PAH doses [11]. If the stress, as reflected by the lipid decline, results in an increased ingestion rate, then the compounds with the larger log K_{ow} values may differentially exhibit larger k_s values. Such increases in k_s for the compounds with the larger log K_{ow} values might also be augmented by compositional changes, as suggested above. Although both processes may account for all or part of the observed difference between the bioavailability in fresh vs. stored sediment, both are speculative and remain to be verified.

The second observed effect on k_s with the stored sediments was a decline in the bioavailability of the PAH congeners with increased aging between 3 and 60 d. This decline between the two lengths of aging is reflected approximately equally across all log K_{ow} values (Table 5) and results in a smaller intercept for the 60-d regression line between log k_s and log K_{ow}, compared to the 3-d regression line, whereas the slopes for the regression lines are similar. The organism health for these two aged sediments was similar, exhibiting minimal mortality and similar declines in lipid content. Thus, the decline in the bioavailability cannot be attributed to differences in organism health. This decline occurs as the partition coefficients between the sediment and the interstitial water for PY and PHE are increasing.

When the aging was increased from 60 to 150 d, the k_s values did not decline further for most compounds, although the partition coefficients continued to increase for PHE and PY. For the 150-d aged sediment, the bioavailability did not continue to track the observed increases in the partitioning between interstitial water and sediment particles. The regression line for log k_s vs. log K_{ow} for 150 d of aging had a shallower slope than that for the 3- and 60-d regression lines, indicating that the compounds that have larger log K_{ow} values had increased their bioavailability relative to the compounds with smaller log K_{ow} values. Thus, compositional changes that were suggested for sediments stored 150 d but dosed 3 d before exposure may be enhanced with 150 d of contact.

The cause of the bioavailability changes with increased contact time between the PAHs and the sediment particles is not known, although diffusion into particles rather than surface sorption has been suggested to account for changes in chemical extractability with time [4,5]. This mechanism also has been suggested to affect the bioavailability of PAHs [1]. As above, changes in the sediment composition, particularly the organic fraction of the sediment, may alter the chemical extractability and bioavailability of organic contaminants. The volatile solids concentration at the beginning of the experiment was similar to that of sediments collected previously from the same site. Unfortunately, the concentration of the organic carbon was not measured at the end of the experiment. Yet these data, along with those discussed above, suggest that changes in bioavailability for stored sediment could be due in part to sediment-compositional changes that occur during storage.

Differential rates of ingestion as an explanation for the enhanced bioavailability between the 60- and 150-d aged k_s values for the larger molecular weight compounds might also apply. However, the organisms in the longer aged sediment were not apparently as stressed as those in the 60-d aged sediment, as they did not exhibit a significant change in lipid content over the course of the exposure. Thus, the hypothesis formulated above suggesting stimulation of feeding with increased stress may not be appropriate for this case. Because ingestion

was not measured in any of the cases, it is impossible to determine if there was differential feeding under any of the conditions.

In the environment, the aging of contaminants on sediment particles is generally long, compared to most of the laboratory procedures employed for introducing contaminants to sediments [1,30,31]. The longer aging results in reduced bioavailability of the contaminants relative to laboratory-dosed sediments [2]. Additionally, such reductions may depend on sediment composition, as suggested above. Overall, the effect of aging is an important variable, in conjunction with organic matter concentration and composition, for consideration when developing standardized protocols for bioassays with laboratory-dosed sediments.

One approach for establishing sediment quality criteria is to use laboratory-spiked sediments for establishing toxicity [32]. Different researchers prepare sediments for testing with procedures that result in varying sediment aging from the initiation of sediment dosing to organism exposure. These aging differences may result in different toxic responses between laboratories due to alteration of bioavailability and could confound the setting of criteria. A standardized contact time between sediments and contaminants should be established to ensure that data collected between different investigators will be reasonably comparable for this variable. However, establishing standard contact times will not address the effects of different sediment compositions nor the differences between laboratory and field exposures on bioavailability.

CONCLUSION

Increases in the length of contact between the sediment and the contaminant generally reduced bioavailability compared to 3 d of aging, but after 60 d, the bioavailability appeared to stabilize. Additionally, the apparent increased bioavailability of compounds with large log K_{ow} values aged for 3 d on 150-d stored vs. fresh sediments suggests compositional changes of laboratory-dosed sediment during storage. Proof of such an effect of composition on bioavailability remains to be confirmed. However, for laboratory bioassay experiments, the length of contact time between the sediment and the contaminant affects bioavailability both for different laboratory-dosed systems and for comparisons with field samples. Thus, the length of aging should be standardized to permit comparison between laboratories, although the optimal duration for this contact has yet to be established.

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