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Measuring assimilation efficiencies for sediment-bound PAH and PCB congeners by benthic organisms

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Abstract

Assimilation efficiencies (AEs) of sediment-sorbed ³H-benzo(a)pyrene (BaP) and ¹⁴C-2,2',4,4',5,5'-hexachlorobiphenyl (HCBP) were measured in *Diporeia* spp. (Amphipoda) and the AE for BaP was determined in *Lumbriculus variegatus* (Oligochaeta). For *Diporeia*, three methods of determining AEs were compared and for *L. variegatus* AEs were measured by two methods. The first method used organic carbon as a tracer based on a feeding selectivity index (SI) and the relative concentrations in the sediment and fecal material. After 10-day exposure, the AEs in *Diporeia* for BaP and HCBP were 45% to 57% and 46% to 58%, respectively. The AEs in *L. variegatus* for BaP were 0% to 26% throughout the 5-day exposure. The second method estimated assimilation from ingestion based on the feeding rate and the SI for organic carbon. The AEs in *Diporeia* for BaP and HCBP were 11 to 15% and 36% to 52%, respectively. For the third approach, a dual-label technique was used with ¹⁴C-polydimethylsiloxane as a non-assimilated tracer for estimating AE based on the relative ratio of the assimilated and non-assimilated tracers in the food and fecal material. This technique gave a BaP-AE of 56% for *Diporeia* after 10 days. The BaP-AE in *L. variegatus* ranged from 23% to 26% for the first day of exposure, then decreased to 10% by the end of the 5-day exposure period. Differences in the AEs for *Diporeia* exposed to BaP determined from these techniques result, in part, from the differential distribution of the xenobiotics among the sediment particles and the selective feeding by *Diporeia*.

Keywords: Assimilation efficiency; Polychlorinated biphenyls; Polycyclic aromatic hydrocarbons; *Diporeia* spp.; *Lumbriculus variegatus*

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1. Introduction

Non-polar organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), are bound by particles in the water and accumulate in sediments. However, benthic organisms living in sediment and ingesting sediment particles may accumulate high body burdens and thus return sediment-associated compounds to active circulation in food webs. Accumulation of sediment-associated contaminants may occur either via the aqueous phase, i.e., via pore water, or through ingestion of contaminated sediment particles. The relative importance of these routes depends on the ecology and feeding behavior of the organism and characteristics of the sediment and chemicals. Further, accumulation via particle ingestion depends on the feeding rate of the organism, assimilation efficiency, feeding selectivity, and contaminant concentration on the ingested food particles, which may be significantly different from the concentration in the bulk sediment (Lee et al., 1990).

Recent modeling efforts to demonstrate the role of sediment in the food web transfer of organic contaminants indicate that improved measures of the assimilation efficiency (AE) for ingested materials are required to improve the model accuracy (Thomann et al., 1992). Thus, the AE with respect to ingested particles must be measured to obtain reliable information about the relative role of different accumulation routes.

Assimilation is defined as 'absorption of material from the gut' (Johannes and Satomi, 1967) and consequently, assimilation efficiency is the portion of compound retained from the food by the organism. The simplest method to determine bioavailability or assimilation of a contaminant is by measuring the reduction in its concentration between ingested sediment particles and egested feces. Unfortunately, this simple approach cannot be applied to benthic invertebrates, because they do not ingest whole sediment. Rather, they selectively ingest sediment particles of specific size and composition. Currently, there is no method to physically segregate the particles selected for ingestion from the whole sediment to obtain an accurate measure of contaminant concentration. For example, oligochaetes preferentially select the fine-grained, organic-rich particles (McMurthy et al., 1983), which can contain comparatively high contaminant concentrations and thus increase the contaminant exposure (Klump et al., 1987). This leads to the potential use of organic carbon normalization of contaminant concentrations as a surrogate for the ingested contaminant concentrations. However, *Diporeia* spp., a benthic amphipod in the Great Lakes that accumulates the majority of its PAHs from fine-grained sediments (Eadie et al., 1985), is an extremely selective feeder and can selectively ingest particles enriched in one hydrophobic contaminant over another relative to the particle's organic carbon content (Harkey et al., 1994).

There are few methods available to measure AE of ingested material by benthic organisms. The major difficulty, as exemplified by the two examples above, is determining or tracing the amount of contaminant actually ingested, since measures of the bulk sediment concentration are obviously inappropriate for reference. The first method measures AE directly, using the sediment organic carbon as a tracer. This method requires a measure of the organic carbon AE and the feeding selectivity of the

organism (Lee et al., 1990). The AE is then calculated by comparing the relative concentration in the sediment to that in the fecal material on a carbon-normalized basis, incorporating the selectivity of the organism for the organic carbon fraction of the sediment. Using this method, the AE of hexachlorobenzene in the clam, *Macoma nasuta*, was determined to range from 39% to 57% (Lee et al., 1990), while the AE for benzo(a)pyrene (BaP) in *Diporeia* ranged from 46% to 60% (Landrum and Lydy, 1993).

A second method measures the organism body burden and determines the importance of different accumulation routes (feeding versus water) (Harkey et al., 1994). This method requires measures of the feeding rate of individual organisms and also employs the feeding selectivity on organic carbon to adjust the relative concentration of ingested versus whole sediment concentrations. This method is readily used with *Diporeia*, which are intermittent feeders (Quigley, 1988), because the fecal pellets are packaged in a peritrophic membrane, and they do not feed on their own fecal pellets (Lydy and Landrum, 1993). Those animals which are not actively feeding during the exposure presumably accumulate contaminants through body surfaces from pore and overlying water and through direct contact with contaminated particles. The feeding organisms additionally accumulate contaminants from ingestion. Thus, their body burden would increase relative to their feeding rate.

The third method uses two radioactive isotopes, one that is assimilated and one that is not. ^{51}Cr has been commonly used as a non-assimilated tracer (Calow and Fletcher, 1972; Wightman, 1975; Klump et al., 1987; Lopez and Elmgren, 1989; Lydy and Landrum, 1993). If the non-assimilated tracer is bound to the same particles as the contaminant or is indiscriminately ingested in the same ratio as the contaminant, it will serve as a tracer of the amount of accumulation. The AEs measured with this method for oligochaetes ingesting sediment dosed with 2,2',4,4',5,5'-hexachlorobiphenyl ranged from 15% to 36% (Klump et al., 1987). On the other hand, AEs for *Diporeia* ingesting ^{14}C -benzo(a)pyrene could not be measured by this method because the feeding selectivity of this organism interfered with the assumption that the tracer was ingested in proportion to the contaminant (Lydy and Landrum, 1993). To attempt to define the mechanism for the dual-tracer method failure, the relative distribution of the assimilated and non-assimilated tracers among the sediment particles was measured. However, no distribution differences were observed with the particle-separation method employed, and thus the mechanism for the dual-tracer method failure remained open to speculation. The most probable cause for the dual-tracer method to fail is differential sorption of the two tracers to particles of different composition that are ingested selectively.

The use of an organic non-assimilated tracer that would likely sorb similarly to an organic contaminant among the different particles might solve the problems of uneven distribution and failure of the tracer to be ingested at the same rate as the contaminant. Polydimethylsiloxanes (PDMS), which are synthetic molecules noted to be extremely hydrophobic and essentially insoluble in water, might serve as an appropriate tracer. Because of their molecular structure, they have unique surface/interfacial properties and reside at or on the interface between polar and apolar media, e.g., water and air or water and particles such as sewage sludge or sediment particles

(Jarvie, 1986). Further, the toxicity of PDMS to different aquatic organisms is low (Hobbs et al., 1975; Aubert et al., 1985) and these polymers are not significantly accumulated either by oligochaetes from the sediment (Kukkonen and Landrum, 1994) or by fish from aqueous or dietary exposures (Opperhuizen et al., 1987).

Our objectives were (1) to measure assimilation efficiencies (AEs) for two benthic invertebrates, (2) to examine the use of an organic non-assimilated tracer, PDMS, and (3) to compare the direct organic carbon and dual-labelled approaches to determine AEs.

2. Materials and methods

2.1. Organisms and sediments

Diporeia spp. were collected from surficial sediment at a water depth of 25 to 29 m in Lake Michigan (43.02°N, 86.29°W) using a PONAR grab sampler. Organisms were gently screened from the sediment, transported to the laboratory in lake water with ice, and kept in the dark in 3 to 4 cm lake sediment overlaid with 10 cm lake water at 4°C.

Lumbriculus variegatus was reared in 37-l glass aquaria containing well water at 23 ± 2 °C. Shredded and presoaked, unbleached paper towels were used as a substrate in the aquaria. A flow of 8 to 10 l per day of freshwater was passed through the aquaria. The oligochaetes were fed with salmon starter (Zeigler Bros., Gardners, PA) three times per week.

Lake Michigan sediment (organic carbon ≈ 0.5% of sediment dry weight) was obtained by PONAR grab approximately 8 km southwest from Grand Haven, MI (43.03°N, 86.37°W) at 45-m depth. The sediment samples used in this experiment were from two different sample collections. The sediment was sieved at 1 mm to remove animals and large debris and held in the dark at 4°C. Water used throughout the work was Lake Michigan surface water stored in the dark at 4°C.

2.2. Chemicals

³H-benzo(a)pyrene (BaP, specific activity 69 Ci mmol⁻¹, Amersham Corp.) and ¹⁴C-2,2',4,4',5,5'-hexachlorobiphenyl (HCBP, 12.2 mCi mmol⁻¹, Sigma Chemical Co.) were dissolved in acetone and radiopurity was determined using thin layer chromatography (TLC) and liquid scintillation counting (LSC) (Landrum, 1989). The purity of both compounds was >98%.

¹⁴C-polydimethylsiloxane (PDMS, specific activity 302 μCi g⁻¹) was obtained from Dow Corning Corporation, Midland, MI, USA. The PDMS fluid had a viscosity of 200 centistokes. The siloxane liquid was dissolved in chloroform–acetone (50:50, v:v) to dose the sediment.

2.3. Sediment dosing

Two different sediment samples were prepared. One sample (1450 g wet weight

with 1000 ml lake water) was dosed with 13 μCi (43 mg) of ^{14}C -PDMS in 50 μl chloroform–acetone and with 50 μCi of ^3H -BaP in 80 μl acetone. The other sample (600 g wet weight with 500 ml lake water) was dosed with 30 μCi of ^3H -BaP and 4.8 μCi of ^{14}C -HCBP, both in 40 μl of acetone. The dosing solutions were added dropwise to the sediment slurry in a 4-l beaker while the mixture was stirred vigorously at room temperature.

The water–sediment mixtures were stirred at room temperature for 4 h and kept at 4°C overnight. The overlying water was assayed and decanted for mass balance. The sediments were mixed with fresh lake water again and allowed to stand in the dark at 4°C under lake water for 1 month (BaP + HCBP) or 3 months (BaP + PDMS). The small amount of remaining water-soluble carrier used should have only a minimal effect on the partitioning of the compounds to the sediment (Nkedi-Kissa et al., 1985).

2.4. *Diporeia* assay

This experiment was designed to obtain data for both the dual-labelled and direct-measurement approaches. The sediment was stirred for homogeneity and 5 g wet weight were transferred to each of ten 50-ml centrifuge tubes. Lake Michigan water was then carefully added to each tube and tubes were allowed to stand overnight at 4°C. On the following day, one *Diporeia* was transferred to each tube; the tubes were covered with nylon window screen and placed into a 40-l aquarium containing aerated lake water at 4°C for 10 days. Besides dosed sediments, undosed Lake Michigan sediment was placed into 40 tubes with one organism each and the tubes were incubated for 10 days to determine the organic carbon selectivity index of *Diporeia* in this sediment. After the exposure, animals were removed from the tubes and fecal pellets were individually removed from the sediment with a micropipet under a dissecting microscope. The pellets were transferred into tared aluminum foil boats, dried in a desiccator for 7 days (constant weight), weighed and analyzed for ^3H and ^{14}C activity.

2.5. *Lumbriculus* assay

This experiment was designed to evaluate both the dual-tracer and organic carbon selectivity approaches for estimating AE. The overlying water was decanted, the sediment was mixed for homogeneity, and 40 g of wet sediment was distributed to six 50-ml exposure beakers. Lake water was then carefully added to each beaker with minimal sediment disturbance. On the following day, ten test organisms were carefully added by pipeting to each beaker. These groups of ten *L. variegatus* were exposed at $23 \pm 1^\circ\text{C}$ to BaP- and PDMS-dosed sediment only. The overlying water was changed once or twice a day in the beakers. Some of the fecal material deposited on the top of the sediment was carefully pipeted out after 12, 24, 36, 48, 60, 72, 96, and 120 h of exposure, collected on the tared glassfiber filters (Whatman CF/C), dried in the desiccator for 7 days (constant weight), weighed and analyzed for radioactivity. Besides dosed sediment, undosed Lake Michigan sediment was used to determine the organic carbon-based selectivity index of *L. variegatus* in this sediment.

2.6. Analyses

At the end of the exposures, animals were gently sieved from the sediment, rinsed in filtered lake water, blotted dry, weighed (Cahn 4700 electrobalance), and placed in scintillation cocktail (Research Products International, 3a70B). Tritium and carbon-14 activity were counted after 2 days with a LKB 1217 liquid scintillation counter. The data were corrected for quench using the external standards ratio method after correcting for background. Dosed wet sediment (80 to 180 mg) samples were taken in triplicate for determination of contaminant concentration, dry:wet weight ratios, and total organic carbon (TOC). The dry:wet weight ratios were obtained by weighing a wet sediment sample and drying it at 90°C to constant weight. Contaminant concentration in sediment was determined by placing approximately 100 mg wet sediment into 12 ml scintillation cocktail, sonicating (Tekmar high intensity sonic disrupter) for 2 min to maximize the extraction of compounds, and measuring ^3H and ^{14}C activity 2 days after sonication.

Sediment particle size distribution and the distribution of chemicals among the particles were determined by a modified sedimentation technique (Royse, 1970; Siebert, 1977). Approximately 40 g wet sediment was first wet-sieved using filtered (0.3 μm ; Gelman Sciences, glassfiber, type A/E) Lake Michigan water through 420-, 105- and 63- μm standard sieves. Materials remaining on the sieves were collected in beakers. Triplicate samples were taken for LSC and the remainder was dried to constant weight at 90°C for mass analyses. Material passing the 63- μm sieve was mixed with 1.0 l filtered Lake Michigan water in a graduated cylinder at room temperature. Samples (25 ml) from the sediment suspension were taken at 20-cm depth at 0, 120, 240, and 600 seconds after mixing. After 1200 and 4600 seconds, suspension samples were taken at a depth of 10 cm. The sampling times and depths were calculated from Stoke's law using 2.6 g ml $^{-1}$ as the specific gravity of the particles (Royse, 1970) to yield specific particle size classes. From each sample taken, three 2-ml aliquots were analyzed via LSC. The rest of the sample (19 ml) was dried to constant weight at 90°C for mass determinations.

The TOC contents of the sediment and fecal samples were determined by drying the samples, treating the dry sample with 1 N HCl to remove carbonates, redrying and analyzing organic carbon on a Perkin-Elmer 2400 CHN Elemental Analyzer.

2.7. Assimilation efficiency calculations

The direct measurement method produces AEs by determining the relative carbon normalized concentrations of radiolabeled contaminant in the food (sediment) and fecal material. Before an AE can be calculated, a selectivity index (SI) is required (Lee et al., 1990; Lydy and Landrum, 1993). This index represents the extent of organic enrichment in the ingested particles over the bulk sediment. Determination of the SI is necessary for organisms that feed selectively, and it is calculated as follows:

$$SI = \frac{\left[\frac{TOC_f}{(1 - RC)} \right]}{TOC_s} \quad (1)$$

where: SI = selectivity index

TOC_f = total organic carbon in feces

TOC_s = total organic carbon in sediment

RC = fractional loss of carbon during gut passage.

An estimate of the reduction in carbon (RC) value, which represents the fractional loss of carbon during the gut passage, is required to calculate the SI. RC values have not been measured for ingested sediments in *Diporeia* or *L. variegatus*. However, RC estimates found in the literature for other sediment-ingesting organisms range from zero to 22% (Hargrave, 1970; Lopez and Levinton, 1978; Cammen, 1980; Rasmussen, 1984). Some higher values are reported (up to 50%) for algae and microbial cells or cellulose (Foulds and Mann, 1978; Johnson et al., 1989; Lopez and Elmgren, 1989). SI values were calculated using RC values ranging from 0 to 22% and used to determine a range AE estimates.

After SI is calculated, AE can be calculated as follows:

$$AE = \frac{[(C_s \cdot SI) - C_f]}{C_s \cdot SI} \quad (2)$$

where AE = assimilation efficiency for contaminant sorbed to ingested particles

C_s = contaminant concentration in the bulk sediment (DPM g⁻¹ dry sed.)

C_f = contaminant concentration in feces (DPM g⁻¹ dry fec.)

SI = selectivity index for the sediment and organism being studied.

The amount of contaminant was expressed in terms of the amount of radioactivity (DPMs), because in these calculations it does not matter whether concentrations are expressed in DPM g⁻¹ or ng g⁻¹ units, since these units cancel in the above equations. The term 'TOC method' will be used throughout the text when referring to this method.

If it is possible to estimate the portion of organism body burden (C_a) that results from particle ingestion (C_{af}), the AE can be calculated as follows (Harkey et al., 1994):

$$AE = \frac{C_{af}}{FR \cdot SI \cdot t \cdot C_s} \quad (3)$$

where AE = assimilation efficiency

C_{af} = contaminant concentration in the organisms due to feeding (DPM g⁻¹ organism)

FR = feeding rate (g dry sed. g⁻¹ organism h⁻¹)

SI = selectivity index

t = exposure time (h)

C_s = contaminant concentration in the bulk sediment (DPM g⁻¹ dry sed.)

This method is referred to as the 'feeding method' in the text. The method was only used with *Diporeia*, because the extent of feeding for each organism was determined and because the organisms will not reingest their own fecal pellets. C_{af} was calculated:

$$C_{af} = C_{at} - C_{aw} \quad (4)$$

where C_{af} = contaminant concentration in the organisms due to feeding (DPM g⁻¹ organism)

C_{at} = measured total contaminant concentration in organisms (DPM g⁻¹ organism)

C_{aw} = estimated concentration accumulated from pore water (DPM g⁻¹ organism)

C_{aw} was estimated by plotting C_{at} versus fecal pellet mass/organism mass (e.g., Fig. 1). The y-axis intercept would represent the accumulation from all non-feeding sources (Harkey et al., 1994). This assumes that accumulation from all other sources except feeding is the same whether the organisms fed or not.

Feeding rate was calculated for individual feeding organisms (*Diporeia*) or for groups of ten organisms (*Lumbriculus*) as follows:

$$FR = \frac{\text{dry weight of fecal material}}{\text{time} \cdot \text{weight of organism}} \quad (5)$$

The third method to calculate AE uses two radiolabeled compounds, one of which is

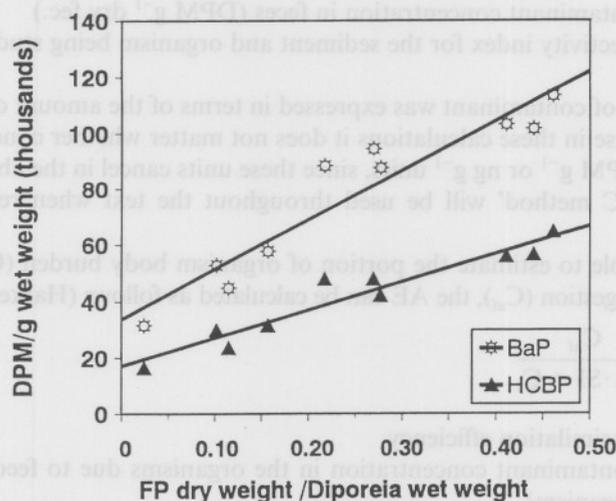


Fig. 1. A plot of body burden, benzo(a)pyrene (BaP), and 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP) in individual animals (C_{at}) versus fecal pellet production (FP) used in the feeding method to estimate the body burden in the organisms due to feeding for *Diporeia*. The y-intercept is the accumulation from all sources other than feeding, C_{aw} .

assimilated (^3H -BaP) and one which is not (^{14}C -PDMS). Sediment and fecal samples were collected in a manner similar to that described above. The AE is calculated from the ratio of the activities of the two tracers in the feces and in the sediment on a dry-weight basis, as follows:

$$\text{AE} = 1 - \frac{\left[\frac{^3\text{H}}{^{14}\text{C}} \right]_{\text{feces}}}{\left[\frac{^3\text{H}}{^{14}\text{C}} \right]_{\text{sediment}}} \quad (6)$$

This method is referred to as the 'dual-label method.' The main assumptions of this method are (1) the non-assimilated tracer (PDMS in this study) is ingested at the same ratio as the assimilated compound, and (2) the non-assimilated tracer is not accumulated.

3. Results

3.1. Distribution of xenobiotics

Lake Michigan sediment is dominated by particle sizes ranging from 420 μm to 43 μm . These fractions make up 80% of the total dry weight of the sediment (Fig. 2). There was a slight difference in particle mass distributions between the two sediment samples. In the sample dosed with BaP and HCBP, the 63–43- μm fraction comprised about 32% of the total dry mass, while the 43–31- μm fraction comprised only 6%. In the sample dosed with BaP and PDMS, these two fractions comprised about the same amount (15%) of the total dry mass. The distribution of xenobiotics among the different sediment particle size classes was different from the mass distribution and each xenobiotic had its own pattern (Fig. 2). Particles from 63 μm to 31 μm tended to bind most of the compound. This distribution pattern is rather similar to the pattern of pyrene in sediment from the same location (Kukkonen and Landrum, 1995).

In the two samples dosed with BaP, the distribution differed mainly due to differences in particle mass distribution, because the relative concentration of BaP (concentration in the fraction divided by the concentration in the bulk sediment) in the different fractions is similar for both samples. The relative BaP concentrations were larger on the very small particles, e.g., 4 to 5 times greater in <10- μm particles than in the bulk sediment (Fig. 3). There was only a small difference in the distribution patterns between BaP and HCBP. HCBP tended to bind more to the middle-size particles than did BaP, a difference shown by the relative concentrations of HCBP, which peaked at the 20–10- μm fraction and then decreased slightly in the smaller particles (Fig. 3). However, in this sediment no clear difference was observed between BaP and HCBP distributions as was observed with measures of the distributions in Florissant soil used as a reference sediment (Harkey et al., 1994). The PDMS also tended to sorb more on the middle-size particles and its relative concentration clearly

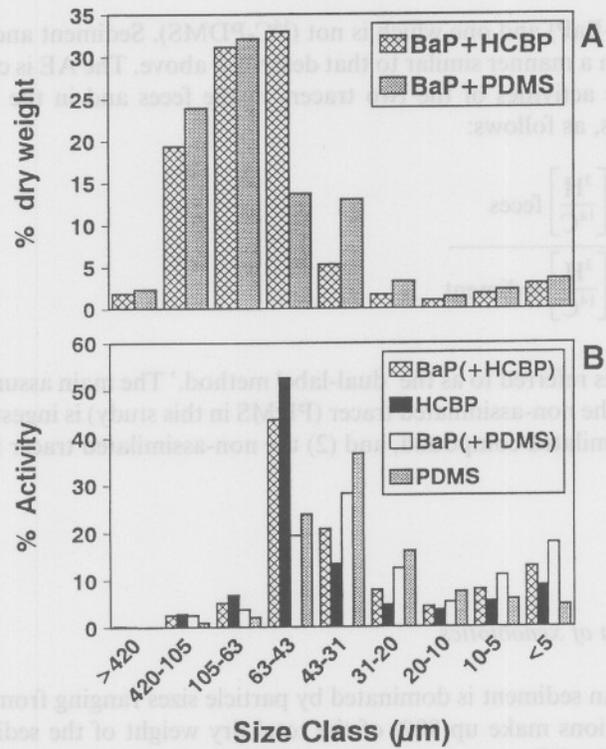


Fig. 2. Particle mass (A) and xenobiotic distribution (B) in the sediment samples. The values shown represent the mean of two replicates.

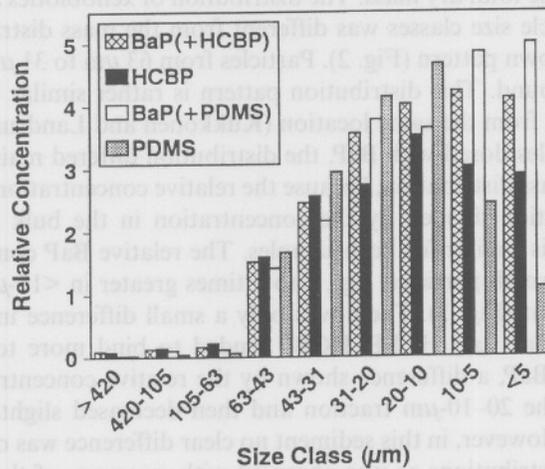


Fig. 3. The relative concentrations of the xenobiotics in the sediment samples. Relative concentration is the concentration in the fraction divided by the concentration in the bulk sediment.

declined for the <10- μm particles (Fig. 3). The coarse particles (>63 μm) did not bind much of the compounds (Fig. 3).

3.2. Selectivity index and feeding of the organisms

The measured organic carbon-based selectivity indices varied from 6.8 to 8.7 for *Diporeia* and from 1.1 to 1.5 for *L. variegatus*, depending on which carbon reduction value is used (Table 1). The selectivity index measured here for *Diporeia* is very close to the values (5.2 to 7.7) measured previously in a different sediment (Lydy and Landrum, 1993). SI values for *L. variegatus* were not found in the literature. The feeding rate ranged from 0.49 ± 0.35 ($n = 10$) to 1.06 ± 0.65 ($n = 10$) μg dry sediment $\cdot \text{mg}^{-1}$ organism $\cdot \text{h}^{-1}$ for *Diporeia* at 4°C over 10 days. The feeding rate for *L. variegatus* was not measured during this exposure, but was measured earlier for organisms from the same culture in the same sediment. In that experiment, the feeding rate varied from 85 ± 20 ($n = 12$) to 166 ± 13 ($n = 3$) μg dry sediment $\cdot \text{mg}^{-1}$ organism $\cdot \text{h}^{-1}$ at 23°C (Kukkonen and Landrum, 1994). These feeding rates are comparable to those reported for the same or similar organisms (Appleby and Brinkhurst, 1970; Klump et al., 1987; Lydy and Landrum, 1993; Harkey et al., 1994). Even though the experiments were made at two different temperatures and temperature has a significant effect on the feeding rate of benthic organisms (Appleby and Brinkhurst, 1970; Hargrave, 1972), the organic carbon-based SI values and feeding rate determinations show clear feeding behavior differences between these two species.

For *L. variegatus*, the estimated SI range is comparable to the concentration ratio of BaP and PDMS in feces and in the bulk sediment at every time during the exposure (Table 2). However, for *Diporeia*, the estimated SI range does not agree well with the concentration ratios for the xenobiotics between feces or bulk sediment particularly for PDMS (Table 3). These data suggest that in the case of an extremely selective feeder, e.g., *Diporeia*, TOC may not be an accurate tracer.

3.3. Assimilation efficiency determinations

The TOC and dual-tracer methods for determining BaP-AE in *L. variegatus* yield-

Table 1
Sediment and fecal pellet total organic carbon (TOC) content (mean \pm s.d. (n)) and ranges of selectivity indices (SI) for *Diporeia* sp. and *Lumbriculus variegatus* exposed to Lake Michigan sediment

	<i>Diporeia</i>	<i>L. variegatus</i>
TOC% sediment	0.528 ± 0.054 (8)	0.430 ± 0.040 (16)
TOC% feces	3.598 ± 0.260 (5)	0.490 ± 0.041 (28)
SI range ^a	6.81–8.74	1.14–1.46

^a SI values were calculated using a range of reduction in carbon (RC) values from zero to 22% (Equation 1).

Table 2

Benzo(a)pyrene (BaP) and polydimethylsiloxane (PDMS) concentrations in the feces (DPM/g dry material, mean (\pm s.d.), $n = 6$) and concentration ratio between feces and the bulk sediment for *Lumbriculus variegatus*. Sediment concentrations were $109\,018 \pm 8108$ DPM/g dry sediment for BaP ($n = 17$) and $34\,737 \pm 3872$ DPM/g dry sediment for PDMS ($n = 17$)

Time (h)	BaP _{fec}	PDMS _{fec}	BaP _{fec/sed}	PDMS _{fec/sed}
12	124 944 (11 983)	51 876 (3095)	1.15	1.49
24	121 080 (14 831)	53 090 (7580)	1.11	1.53
36	121 753 (4881)	43 698 (2144)	1.12	1.26
48	123 466 (8436)	46 022 (8002)	1.13	1.32
60	122 849 (6927)	44 633 (4261)	1.13	1.28
72	123 164 (5539)	45 239 (1980)	1.13	1.30
96	118 149 (10 306)	43 399 (3433)	1.08	1.25
120	129 256 (12 271)	48 301 (9357)	1.19	1.39

Table 3

Benzo(a)pyrene (BaP) and polydimethylsiloxane (PDMS) or 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP) concentrations in the sediment (DPM/g dry sediment, mean (\pm s.d.), $n = 9$) and feces (DPM/g dry material, mean (\pm s.d.), $n = 10$) and the concentration ratio between feces and the bulk sediment for *Diporeia*

Compound	Sediment (DPM/g)	Feces (DPM/g)	Concentration ratio
BaP _{PDMS}	123 099 (5599)	637 705 (97 404)	5.18
PDMS	32 798 (2085)	394 895 (84 974)	12.04
BaP _{HCBP}	189 427 (12 464)	709 115 (123 636)	3.74
HCBP	28 928 (1943)	106 932 (31 741)	3.70

Table 4

Assimilation efficiencies (%) for benzo(a)pyrene by *Lumbriculus variegatus* calculated by two methods ($n = 6$)

Time (h)	TOC method ^a	Dual-tracer method ^b
12	0-21.5	22.9 \pm 7.5
24	2.6-23.9	25.6 \pm 15.2
36	2.0-23.5	10.9 \pm 3.5
48	0.7-22.4	13.0 \pm 10.1
60	1.2-22.8	11.7 \pm 5.5
72	0.9-22.6	12.9 \pm 4.5
96	4.9-25.8	13.0 \pm 3.6
120	0-18.8	12.9 \pm 11.0

^aThe method using sediment total organic carbon as a tracer provides a range of AE values because a range of SI values calculated from RC = 0 to 22% were used to calculate AE (Equation 2).

^bThe method using polydimethylsiloxane (PDMS) as a tracer yields a mean value because an estimation could be obtained for each sample (Equation 6).

ed similar values (Table 4). The AE measured by the TOC method showed a range from zero to 26%; AE values measured by the dual-tracer method fall within this range except at the first two sampling times, where the AE value is at the upper end of the range. The observed decrease in AE values over the course of the experiment is similar to that of *Stylodrilius heringainus* for HCBP (Klump et al., 1987).

The body burden of HCBP and BaP in *Diporeia* depended on the amount of sediment ingested, specifically the more fecal pellets produced, the higher the body burden (Fig. 1). The regression equations describing the relationship between fecal pellet production and contaminant accumulation in *Diporeia* are $C_{at} = 29772(\pm 6900) + 1.98(\pm 0.24) \times 10^5 \times FP$ ($r^2 = 0.89$, $P < 0.001$, $n = 10$) and $C_{at} = 18973(\pm 6900) + 1.98(\pm 0.24) \times 10^5 \times FP$ ($r^2 = 0.93$, $P < 0.001$, $n = 10$) for BaP and HCBP, respectively, where FP is the amount of fecal pellets produced (mg fecal pellet dry weight · mg organism). The similar regression for BaP with PDMS was $C_{at} = 19339(\pm 2211) + 8.06(\pm 1.59) \times 10^4 \times FP$ ($r^2 = 0.79$, $P = 0.001$, $n = 10$; data not shown). In these regressions, the y-intercept provides an estimate of the body burden accumulated from non-feeding sources.

The feeding method determinations yielded similar BaP-AE values for *Diporeia* in both exposures (Table 5). With PDMS, the BaP-AE range was from 11% to 14%, and with HCBP the BaP-AE ranged from 12% to 15%. The BaP-AE values calculated by the TOC method for *Diporeia* ranged from 24% to 41% in the BaP/PDMS-dosed sediment and from 45% to 57% in the BaP/HCBP-dosed sediment (Table 5). The dual-tracer method yielded the highest BaP-AE value for *Diporeia*. The TOC method and the feeding method which were used to calculate HCBP-AE values for *Diporeia* gave reasonably similar results (Table 5).

Table 5
Assimilation efficiencies (%) for benzo(a)pyrene (BaP) and 2,2',4,4',5,5'-hexachlorobiphenyl (HCPB) by *Diporeia* calculated by different methods. The exposure time was 10 days ($n = 10$)

Exposure	TOC method ^a	Feeding method ^b	Dual-tracer method ^c
for BaP:			
BaP + PDMS	23.9-40.7	11.1-14.2	55.6 ± 8.9
BaP + HCBP	45.0-57.2	11.7-15.0	not measured
for HCPB:			
BaP + HCBP	45.7-57.7	36.3-52.1	not measured

^aThe method using sediment total organic carbon as a tracer provides a range of AE values because a range of SI values calculated from RC = 0 to 22% were used to calculate AE (Equation 2).

^bThe method using body burden due to feeding provides a range of AE values because a range of SI values calculated from RC = 0 to 22% were used to calculate AE (Equation 3).

^cThe method using polydimethylsiloxane (PDMS) as a tracer yields a mean value because an estimation could be obtained for each sample (Equation 6).

4. Discussion

The effects of feeding behavior and especially selective feeding by benthic organisms on the accumulation of organic xenobiotics is not yet well understood. There is evidence that assimilation from ingested material can be a significant accumulation route for lipophilic compounds (Landrum, 1989; Boese et al., 1990; Harkey et al., 1994). To understand these processes, we need to know more of the basic ecology, physiology, and behavior of the organisms used in the experiments.

Appleby and Brinkhurst (1970), McMurtry et al. (1983), Kaster et al. (1984) and Klump et al. (1987) have studied the defecation rates and substrate and particle selection of oligochaetes. It has been shown that the feeding activity of oligochaetes, which are conveyor belt feeders, can affect both the distribution and burial rate of sediment-bound xenobiotics in the surface sediments (Karickhoff and Morris, 1985; Keilty et al., 1988a,b). These organisms are known to selectively feed on fine organic-rich materials (McMurthy et al., 1983) and this selectivity is necessary to account for mixing in sediment cores from the Great Lakes (Robbins, 1986). While their selectivity is for fine material, the organic carbon SI measured for *L. variegatus* essentially indicates no selective feeding upon specific organic-rich particles; all fine particles are ingested equally.

Diporeia, however, feeds intermittently, using only a fraction of available food (Dermott and Corning, 1988; Quigley, 1988; Evans et al., 1990). This intermittent feeding supports a strong connection between the seasonal diatom blooms and the nutritional requirements of *Diporeia* (Gardner et al., 1985). Further, the apparent preference for specific foods is reinforced by measures of the selectivity index, both in Lake Michigan sediment (this work) and in a reference sediment, Florissant soil (Lydy and Landrum, 1993). In both cases, *Diporeia* had organic carbon SI values near six. This preference for specific organic particles makes measures of AE particularly difficult and explains the inability to use ^{51}Cr as a tracer of ingested particles in this organism (Lydy and Landrum, 1993), although this isotopically-tagged material works well as a tracer in oligochaetes (Klump et al., 1987).

Because the organisms have different feeding habits, rates, and selectivity indices (SI) (Table 1), an interesting comparison can be made between these two species and the methods used to measure AE. Based on the feeding rates and selectivity indices, we can assume that *L. variegatus* is a general feeder that processes the heterogeneous sediment quickly, while *Diporeia* is highly selective. The AEs of *L. variegatus* obtained by two different methods at several time points (Table 4) demonstrate that both of these approaches are equally useful for determining AEs for organic non-polar compounds by oligochaetes or other organisms which have a low selectivity index. Although there are no previously published AE values for BaP assimilation by *L. variegatus*, the AE values measured by these two methods were similar to those determined for other oligochaetes (15% to 36%) feeding on HCBP-dosed sediment (Klump et al., 1987). The decrease in the AE values over time measured by the dual-label method may be due to one of two likely mechanisms. If the feeding rate increased during the exposure, the residence time in the gut would decrease and the AE would likely decline. An increase in feeding rate may have occurred if the quality of the organic

matter in the sediment declined over the course of the experiment. Alternatively, the concentration in the organisms may simply have approached steady-state; thus the elimination of the compound through the feces increased, such that the concentration ingested would have equaled the concentration excreted (Kukkonen and Landrum, 1994). Both mechanisms may have contributed to the observed decline in the measured AE values. Thus, measures for AE determinations should be made well before the organisms approach steady state or deplete the sediment of high quality food in the experimental containers.

The HCBP-AE values of *Diporeia* were rather similar when calculated by the feeding and TOC methods (36% to 58%). This range is similar to HCBP-AE of *Mysis relicta* (Klump et al., 1991), another selective feeder (Van Duyn-Henderson and Lasenby, 1986). Lee et al. (1990) measured the AE to be from 38% to 56% for hexachlorobenzene by another selective feeder, *Macoma nasuta* (TOC-based selectivity index of 4.4 in the sediment studied). Comparisons with AE values estimated for other organisms, even with different hydrophobic chlorinated hydrocarbons, indicate that the AE values determined by these methods are within a reasonable range, given the feeding selectivity and characteristics of the compound.

With the PAH, BaP, the feeding method gave similar AE values in two different assays (11% to 15%, Table 5). These values are within the range (6% to 30%) obtained earlier by the same method, but with *Diporeia* ingesting BaP-dosed Florissant soil (Harkey et al., 1994). These feeding method BaP-AE values differ from the BaP-AE values obtained by the TOC method (45% to 57%). The range of BaP-AEs for Lake Michigan sediment measured by the TOC method is similar to values (46% to 60%) measured by the same method in Florissant soil (Lydy and Landrum, 1993). The BaP-AE values determined by the TOC method in the BaP- and PDMS-dosed sediment remained somewhat lower (24% to 41%). The dual-label method gave the highest estimate of BaP assimilation by *Diporeia* ($55.6 \pm 8.9\%$). The dual-label method using PDMS was successful in estimating the BaP-AE compared to the failure with use of ^{51}Cr as the tracer (Lydy and Landrum, 1993). The success of the dual-label method, in this work, might be a result of the type of non-assimilated tracer employed. We used an organic compound, while traditionally inorganic ^{51}Cr is used. Thus, the distribution of the non-assimilated and assimilated compounds on particles of differing size and composition might be closer than the distribution of an organic molecule and a heavy metal. Differences in the relative distributions combined with the extreme feeding selectivity of *Diporeia* could result in the differential success of the dual-labeled method in the two studies.

It is difficult to determine which BaP-AE value is correct. Selective feeding by *Diporeia* appears to complicate the AE calculation, particularly for BaP. When the AE is calculated in the feeding method, the accumulation is based on body burden after correcting for accumulation from other sources and compared to the amount ingested. This method does not rely on the relative distribution of compounds but does rely on an accurate estimate of feeding selectivity. In the other two methods, the compound concentration relative to a tracer (PDMS in the dual-tracer method and organic carbon after correcting for tracer selectivity in the TOC method) in the sediment and the fecal material are used for the calculations. For either of these methods

to work, the distribution of both the compound studied and the tracer in the sediment must be similar. It appears that both the TOC and PDMS failed to trace BaP accurately. The difference in the distribution pattern of BaP and PDMS is obvious (Figs. 2 and 3). The difference in the AE values of BaP calculated by the TOC method in two *Diporeia* exposures gives evidence that the method failed. Although the distribution of organic carbon was not measured in the sediment to support this conclusion, it was previously measured in sediment collected from this same station (Kukkonen and Landrum, 1994). In that sample, the relative distribution of pyrene was not the same as the distribution of organic carbon. If the carbon distribution data from the previous study are used, the BaP distribution in the current study differs from the organic carbon distribution. It is also possible that PDMS slightly changes the bioavailability and distribution of BaP (Kukkonen and Landrum, 1994) and that the TOC method may not account for this change.

Overall, the choice of method of AE determination in oligochaetes does not appear to be critical. However, because of the rapid kinetics, it is important to assess the AE value early in the experiment before either depletion of the sediment quality or approach of steady state. For selective feeders such as *Diporeia*, none of the tracer methods including, organic carbon (this work, Lydy and Landrum, 1993), ^{51}Cr (Lydy and Landrum, 1993) or PDMS (this work), seems to provide a successful tracer of the ingestion process relative to the contaminants. The differences in the AE values for compounds of similar hydrophobicities, BaP and HCBP (this work, Harkey et al., 1994), indicate that different tracers may be required for different compound classes and this is supported by the differential distribution of the compounds and organic carbon within sediments (Harkey et al., 1994; Kukkonen and Landrum, 1994).

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