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Assimilation efficiency for sediment-sorbed benzo(a)pyrene by *Diporeia* spp.

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Two methods are currently available for determining contaminant assimilation efficiencies (AE) from ingested material in benthic invertebrates. These methods were compared using the Great Lakes amphipod *Diporeia* spp. and [^{14}C]benzo(a)pyrene (BaP) sorbed to Florissant sediment (< 63 μm). The first approach, the direct measurement method, uses total organic carbon as a tracer and yielded AE values ranging from 45.9–60.4%. The second approach, the dual-labeled method, uses ^{51}Cr as a non-assimilated tracer and did not yield AE values for our data. The inability of the dual-labeled approach to estimate AEs was due, in part, to the selective feeding by *Diporeia* resulting in a failure of the non-assimilated tracer (^{51}Cr) to track with the assimilated tracer ([^{14}C]BaP). The failure of the dual-labeled approach was not a result of an uneven distribution of the labels among particle size classes, but more likely resulted from differential sorption of the two isotopically labeled materials to particles of differing composition. The [^{14}C]BaP apparently sorbs to organic particles that are selectively ingested, while the ^{51}Cr apparently sorbs to particles which are selectively excluded by *Diporeia*. The dual-labeled approach would be a viable and easier experimental approach for determining AE values if the characteristics that govern selective feeding can be determined.

Key words: Assimilation efficiency; Sediment; BaP; *Diporeia*; Direct measurement method; Dual-labeled method

INTRODUCTION

Sediments act as a sink for many lipophilic compounds, including chlorinated hydrocarbons, halogenated phenols, and polynuclear aromatic hydrocarbons (Adams et al., 1985; Knezovich and Harrison, 1988; Lydy et al., 1990a,b; Baker et al., 1991). A model for accumulation of these xenobiotics from sediment by benthic organisms proposed that accumulation occurs via two routes: ingestion of contaminated particles, and desorption into interstitial water and accumulation across the respiratory membranes (Landrum and Robbins, 1990) (Fig. 1). The relative importance of

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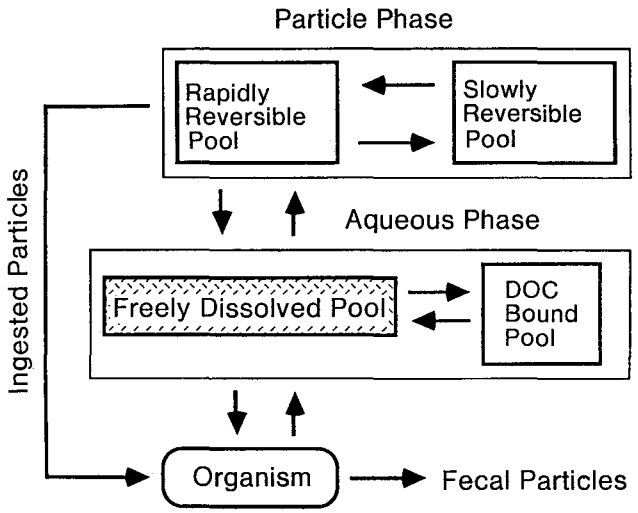

Fig. 1. Conceptual model for accumulation of sediment-associated contaminants.

the two routes for accumulation remains under discussion. However, before evaluation can be completed, values for assimilation efficiency (AE) with respect to ingested particles must be gathered. These data are required to parameterize the above model and are not currently available in the literature.

This study was designed to examine the role of ingestion in bioaccumulation of sediment-sorbed organic contaminants. Two methods are presently available to measure AEs of ingested material by invertebrates. The first method measures AE directly using the sediment organic carbon as a tracer, and determines the relative organic-carbon-normalized concentrations of ^{14}C-labeled contaminant in the sediment (food) and fecal material. This approach requires a measure or estimation of the organic carbon AE and the feeding selectivity of the organism (Lee et al., 1990). From this approach, the AE of hexachlorobenzene in the clam *Macoma nasuta* ranged from 39–57% (Lee et al., 1990).

A second method uses two radioactive isotopes – one that is assimilated (^{14}C-labeled compound) and one that is not (^{51}Cr) (Wightman, 1975; Klump et al., 1987, 1991; Lopez and Elmgren, 1989). The AEs calculated from this method for oligochaetes ingesting sediment dosed with 2,4,5,2′,4′,5′-hexachlorobiphenyl ranged from 15–36% and declined as the rate of sediment throughput increased. The AEs for the above two compounds, with respect to ingested sediments, were in the same range as those from ingested food for other chlorinated compounds in fish (Gobas et al., 1988). However, general extrapolation to the use of the AEs from fish for ingested sediment requires additional verification with a wider range of compounds and organisms. Further research examining the applicability of the above two methods to a

variety of organisms under variable environmental conditions is required to determine the best approach for measuring the AEs required to reliably parameterize toxicokinetic models.

AE determination may be complicated, in the case of benthic invertebrates, because of selective ingestion of sediment particles of specific size and composition. For example, oligochaetes preferentially select the finer-grained organic-rich fraction of the bulk sediment that contains comparatively high contaminant concentrations (McMurthy et al., 1983), thus increasing the contaminant exposure for the oligochaetes (Klump et al., 1987). The preferential selection of the fine-grained sediment fraction by benthos was confirmed as the major source of contaminants for Great Lakes benthos including *Diporeia* (formerly *Pontoporeia hoyi* (Bousfield, 1989)) (Eadie et al., 1985). Thus, substantial errors in calculating contaminant AEs from sediments can occur if concentrations are based on the bulk sediment rather than on the high concentrations on the ingested fine-grained fraction. Thus, additional effort is required to account for sorption to the fine-grained sediment fractions and to develop the appropriate normalizing factors so exposure of benthos can be properly evaluated.

This research measured (1) AEs for benzo(a)pyrene (BaP) sorbed to sediment for the Great Lakes amphipod *Diporeia* spp; (2) compared the direct carbon-tracer and dual-labeled approaches for determining AEs; and (3) examined the potential effects of varying contaminant concentrations on AEs.

MATERIALS AND METHODS

Experimental organisms and sediments

Diporeia spp. were collected from Lake Michigan approx. 5 km southwest of Grand Haven, MI at a depth of 24–29 m using a PONAR grab sampler. 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. In the laboratory, *Diporeia* were held in shallow aquaria containing approx. 3 cm of Lake Michigan sediment and 10 cm of lake water at 4°C (Landrum, 1982a). *Diporeia* is an excellent organism for examining assimilation from ingested sediment because it actively feeds on sediment (Dermott and Corning, 1988; Lopez and Elmgren, 1989) and because its fecal material is encapsulated in a peritrophic membrane that allows easy identification and separation from the sediment (Quigley, 1988).

Florissant sediment, a soil obtained from the National Fisheries Contaminant Research Center, Columbia, MO, was used for all assimilation experiments. Florissant soil is a fine-grained silt loam soil with a moderate amount (1%) of organic matter. It has been employed as a reference material for whole-sediment aquatic exposures because it contains only a few anthropogenic contaminants at very low concentrations (Ingersoll and Nelson, 1990). This material was additionally useful for the AE experiments because its low carbonate content (< 0.01%) permitted a simplification of the total organic carbon (TOC) analyses of the sediment and fecal material. The

contribution of TOC from the peritrophic membrane to the fecal material was considered insignificant in our experiments.

Sediment preparation

The sediment was wet sieved to a particle size of less than 63 μm prior to use in the assimilation experiments. The sieved sediment was dosed with [^{14}C]BaP (8.38 mCi·mmol^{-1}, lot No. 890114, Sigma) and $^{51}Cr^{3+}$ (^{51}Cr, initial specific activity of 37 mBq·ml^{-1}, lot No. 167, Amersham Corporation). The Bap was dissolved in an acetone carrier and had a radiopurity of greater than 97% when tested by TLC (hexane/benzene, 8:2 v/v) and liquid scintillation counting (LSC). Dissolved ^{51}Cr was present as a solution of chromic chloride in 0.1 M HCl. The total amount of the acetone and HCl carriers was < 250 μl and was not expected to affect the assimilation experiments (Landrum, 1982b). Wet sediment, corresponding to 50 g dry weight, was placed into a 400-ml beaker, and 200 ml of filtered Lake Michigan water was added. The sediment slurry was vigorously stirred at room temperature, while the [^{14}C]BaP and ^{51}Cr were slowly added dropwise. The mixture was stirred at room temperature for an additional 4 h and kept at 4°C, thereafter (Landrum and Faust, 1991). All analytical procedures were performed under gold fluorescent light (λ > 500 nm) to minimize the photodegradation of BaP.

Experimental system

The experiments were designed to obtain data for both the dual-labeled and direct measurement approaches simultaneously. Thus, a direct comparison could be made between the methods. Two experiments were conducted with differing sediment-BaP concentrations. Activities of both radiotracers were greatly increased in the second experiment. Each experiment consisted of ten 50 ml centrifuge tubes; seven tubes contained three *Diporeia* each, and three control tubes contained no *Diporeia*. Two days after dosing the sediment, the overlying water was decanted, removing most of the acetone and HCl carriers. The sediment was stirred for homogeneity, and approx. 1.5 g of wet sediment was transferred to each of the 50 ml centrifuge tubes. The concentrations of the radiolabels in the sediment were measured at the beginning, middle and end of the distribution of sediment to the centrifuge tubes to examine the homogeneity of the radiolabeled tracers within the bulk-sediment matrix. Filtered Lake Michigan water was then carefully added to each centrifuge tube, minimizing the disturbance of the sediment. On the following day, an initial water sample was collected and *Diporeia* were added to each of the treatment tubes. The tubes were then covered with nylon window screen and placed gently into a 40-l aquarium containing filtered, aerated lake water at 4°C. The duration of the experiments was 7 days.

At the end of 7 days, overlying water, sediment, *Diporeia*, and fecal pellets were sampled and [^{14}C]BaP and ^{51}Cr activities were determined for each of these samples. The [^{14}C]BaP and ^{51}Cr activities were not detected in overlying water samples. Wet-to-dry weight values for separate aliquots of the sediment were measured. Each of the

samples was weighed and placed into 12 ml of scintillation cocktail (RPI 3a70B). Each of the samples was intensely agitated for 1 to 2 min using a Tekmar 375-watt ultrasonic processor (Tekmar Co., Cincinnati, OH) (extraction recovery = 87 ± 5% for [^{14}C]BaP and 80 ± 7% for ^{51}Cr (n = 57)). Mass balance was maintained within the system with the total amount of radioactivity added to the system remaining constant over the time-course of the experiments. The total accountability at the end of the exposure was 93% for Exp. 1 and 87% for Exp. 2. After sonication, a 2-day waiting period ensured ample time for radiotracer extraction into the scintillation cocktail. The [^{14}C]BaP and ^{51}Cr tracers were counted simultaneously on a LKB 1217 liquid scintillation counter using dual-label counting with the ^{3}H window for the ^{51}Cr and the ^{14}C window for the [^{14}C]BaP (counting efficiency = 75 ± 3% for [^{14}C]BaP and 41 ± 5% for ^{51}Cr). This represents the conventional ^{3}H/^{14}C dual-label configuration for the counter. The data were corrected for quench using the external-standards ratio method after correcting for background, which was determined using scintillation cocktail alone. Quench curves were established separately for the ^{51}Cr and [^{14}C]BaP, and standards were routinely assayed throughout the experiments. The ^{51}Cr samples were also corrected for decay. Interference of ^{51}Cr activity on [^{14}C]BaP counting was checked by allowing the ^{51}Cr (half-life = 27.7 days) to decay to background levels (approx. 9 half-lives) and then recounting (Klump et al., 1987). No differences were noted in the [^{14}C]BaP activity among samples with and without ^{51}Cr activity; therefore, ^{51}Cr interference was not considered to be an important factor. Interference of [^{14}C]BaP activity on ^{51}Cr counting was checked by counting ^{51}Cr activity among selected samples with and without [^{14}C]BaP activity. From these samples, the [^{14}C]BaP activity did not appear to be interfering with the ^{51}Cr activity with the dual label correction. The TOC content from subsamples of the sediments and fecal pellets was determined on a Perkin-Elmer 2400 CHN Elemental Analyzer after drying to constant weight at 60°C. The TOC content of the bulk sediment was 1.3 ± 0.1%. Total lipid content of the *Diporeia* was 33.3 ± 3.5% (dry weight) measured by gravimetric analysis (Gardner et al., 1985).

Preliminary experiments were conducted to check whether purging the gut contents from *Diporeia* prior to scintillation counting would affect the relative concentrations of the radiotracers within *Diporeia*. The gut contents were purged by allowing the *Diporeia* to ingest unlabeled control Florissant sediment for a specified amount of time (approx. one gut throughput = 4.5 h). The results of these preliminary experiments indicated that this procedure did not yield significantly different radioactivity levels within the *Diporeia* than the non-purging technique. Therefore, the purging procedure was not considered necessary.

Assimilation efficiency calculations

The direct measurement method measures AEs by determining the relative-carbon-normalized concentrations of ^{14}C-labeled contaminant in the sediment (food) and fecal material. In this method, fecal pellets and sediment samples are collected from

exposed organisms. The total organic carbon (TOC) and the ^{14}C-contaminant concentration are determined for the fecal pellets and sediment. Before an AE can be calculated, a selectivity index (SI) is required (Lee et al., 1990). This index represents the extent of organic enrichment in the ingested particles over the bulk sediment. The SI is necessary for organisms that feed selectively, and it is calculated as follows:

$$SI = \frac{\left[\frac{TOCf}{(1-RC)}\right]}{TOCs} \tag{1}$$

Where: SI = selectivity index; TOCf = fraction of TOC in feces; TOCs = fraction of TOC in aggregate sediment; and 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 gut passage, is required to calculate the SI. RC values have not been determined for ingested sediments in *Diporeia*. However, RC estimates found in the literature range from 0–22% for other deposit feeding invertebrates (Hargrave, 1970, 1972; Lopez and Levinton, 1978; Cammen, 1980). SI values were calculated using RC values ranging from 0 to 22%.

After the SI is calculated, AE values are calculated as follows:

$$AE = \frac{[(CPS \cdot SI) - CPSf]}{(CPS \cdot SI)} \tag{2}$$

Where: AE = assimilation efficiency for contaminants sorbed onto ingested sediments; CPS = concentration of contaminant in aggregate sediment; CPSf = concentration of contaminant in feces; SI = selectivity index for sediment being studied.

An alternative method for calculating AEs uses two radioactive isotopes, one of which is assimilated (^{14}C) and one which is not (^{51}Cr) (Wightman, 1975; Cammen, 1977; Klump et al., 1987, 1991; Lopez and Elmgren, 1989). Sediment and fecal samples are collected in a manner similar to that described above. The AE is then calculated from the ratio of the activities of the two tracers in the feces and in the food (sediment) on a dry-weight basis, as follows:

$$AE = 1 - \frac{\left[\frac{^{14}C}{^{51}Cr}\right] \text{feces}}{\left[\frac{^{14}C}{^{51}Cr}\right] \text{sediment}} \tag{3}$$

If the activities are normalized to carbon, and RC values are included, AEs would be corrected for selective feeding.

Particle-size distribution

The sediment particle-size distribution on which the two radiotracers are sorbed was determined using a settling tube technique (Royce, 1970; Fochtman, 1978;

Plumb, 1981). In this approach, the particle density of the Florissant sediment needed to be measured. To determine the particle density of the sediment, a pycometer was filled with a sediment-water mixture, and then the volume and mass of the solids and water in the pycometer were determined. From these measurements, the particle density of the florissant sediment was calculated and then used in Stokes Law Equation to determine the particle velocities for each particle-size class. Approx. 20 g wet sediment was mixed with 1 l of distilled water in a 1-l graduated cylinder at room temperature. Replicate 25 ml water samples were taken at a 20 cm depth at 0, 120, 240 and 600 s, after the initial mixing. In addition, water samples were also taken at a depth of 10 cm at 1200 and 4600 s. From each water sample taken, three 2-ml aliquots were analyzed via LSC. The remaining portion of the sample (19 ml) was dried to constant weight at 60°C for mass and TOC determinations. From these analyses, it is possible to determine the total amount of radioactivity in each particle-size class.

RESULTS

Distribution of radiotracers

A homogeneous distribution of xenobiotics within the exposure matrix is important to ensure even exposure of the organisms to the contaminants among replicates. On the basis of samples collected for analysis at the beginning, middle and end of the distribution among the exposure tubes, the radiotracers were evenly distributed throughout the bulk sediment based on a coefficient of variation of 13% and 15% for ^{14}C and 7% and 3% for ^{51}Cr for the two experiments, respectively. Further, the increased sediment concentration in the second experiment (Table 1) did not apparently

TABLE 1
[^{14}C]BaP and ^{51}Cr activities for sediment, *Diporeia* and fecal pellets. Activities are dpm mg^{-1} dry material for the sediment and fecal material, while the *Diporeia* are on a wet weight basis. All values represent the mean of ten replicates (± SD).

Expt.	Sediment activity		Fecal activity		*Diporeia* activity	
	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}Cr
1	26.7 ± 11[a]	271 ± 19[b]	75 ± 12[a]	308 ± 56[b]	88 ± 6	BG
2	798 ± 137[a]	8636 ± 813[b]	2426 ± 369[a]	9804 ± 604[b]	1711 ± 256	BG

[a] ^{14}C activity in fecal material was significantly greater than the ^{14}C activity in the sediment for both experiment, *t*-test, $P < 0.05$.
[b] ^{51}Cr activity in fecal material was not significantly different than the ^{51}Cr in the sediment for both experiments, *t*-test, $P > 0.05$.
BG = background.

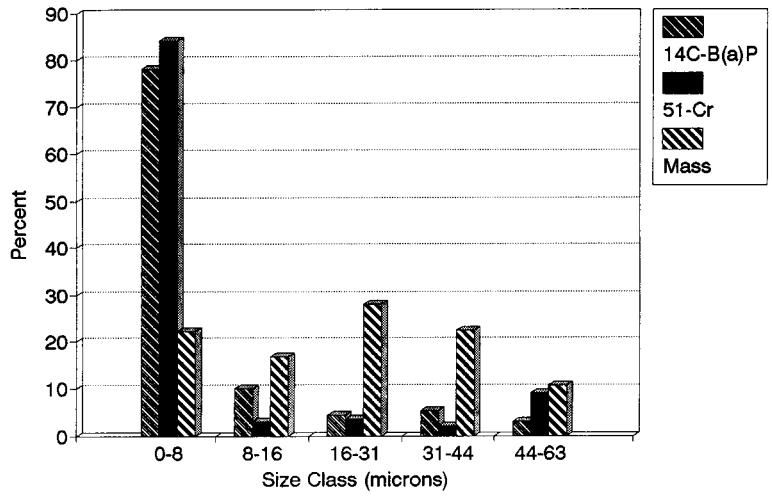

Fig. 2. Percent of radiotracers ([^{14}C]BaP and ^{51}Cr) and mass of particles within each of the particle size classes in the Florissant sediment.

affect the homogenity. The majority of both radiotracers were found in the 0–8 μm size class, and the radiotracer distributions were relatively similar among the all the size classes (Fig. 2). Because *Diporeia* presumably feed on the 0–8 μm size class and because the majority of both radiotracers were found in this size class, distribution within the sediment matrix should not have affected the two methods for determining AE, particularly the dual label method.

Assimilation efficiency determinations

The direct measurement method for determining AE relies on total organic carbon as a tracer and yielded AE values ranging from 45.9–60.4% (Table 2). The dual-labeled method relies on a ratio of assimilated to non-assimilated tracer and yielded AE values exceeding 100% (247% and 268% for the two experiments, respectively) suggesting a failure of the approach.

A precondition for the dual-label method to work is a demonstration that the ^{51}Cr is not assimilated. In a series of preliminary experiments, less than 1% of the ^{51}Cr was assimilated (Lydy, unpublished data). Thus, ^{51}Cr could be used as a conservative tracer for *Diporeia*. This finding is not unique, because ^{51}Cr is generally not assimilated by a variety of organisms ranging from invertebrates to mammals (Cammen et al., 1977; Klump et al., 1987).

Selective feeding for *Diporeia* was postulated based on the relative TOC in sediment and fecal material (Table 2) and the finding of calculated AE values exceeding 100% for the dual-label method. Furthermore, the [^{14}C]BaP activity in the fecal material

was enhanced 2.8–3.0-fold over the [^{14}C]BaP activity in the sediment during both experiments (Table 1), while a 5.2–6.0-fold increase in TOC was observed between the sediment and fecal pellets. These enhancements in TOC and ^{14}C activities along with the enhanced ^{14}C/^{51}Cr ratio in the fecal material indicate that selective feeding occurred and must be considered before the AEs can be calculated.

Concentration effects on AE determinations

Sediment concentrations as represented by the activities of both radiotracers were larger in the second experiment. Effects of increased concentrations on AEs for *Diporeia* were not different over a BaP concentration range of 1.45 to 43.3 nmol/g (Table 2).

DISCUSSION

The direct measurement approach for determining AE utilizes differences in OC-normalized contaminant levels between the ingested sediment and the fecal material. We calculated AEs using a range of SI values based on RC values from the literature (Hargrave, 1970, 1972; Lopez and Levinton, 1978; Cammen, 1980). While a 36% AE for carbon was found for *Diporeia* ingesting algae (Fitzgerald and Gardner, 1992), this value is much higher than that expected for benthos ingesting detritus from sediment. Further, no RC values were available for *Diporeia* ingesting sediment. This lack of a RC value for *Diporeia* does not represent a major problem, since the direct measurement approach is not highly sensitive to RC values (Table 2). RC values ranging from 0–22% were used in the AE calculations.

Although there are no literature AE values for BaP assimilation, the AE values determined by this method (45.7–60.4%) (Table 2) were similar to those for *Macoma*

TABLE 2
Sediment and fecal pellet total organic carbon (TOC) values and ranges of selectivity indices (SI) and assimilation efficiency (AE) values for *Diporeia* exposed to Florissant sediment. TOC values represent mean of ten replicates (± SD).

Expt.	Percent TOC		SI	Percent AE	
	sediment	fecal		direct[a]	dual label
1	1.35 ± 0.05[b]	7.0 ± 1.0[b]	5.2–6.7	46–58	247
2	1.32 ± 0.11[b]	7.9 ± 1.3[b]	6.0–7.7	49–60	268

[a]SI and AE values were calculated using the direct measurement approach and a range of reduction in carbon (RC) values (RC = 0 to 22%).
[b]Means are not significantly different between the two experiments when compared using a *t*-test ($P < 0.05$).

nasuta (39–57%) feeding on hexachlorobenzene dosed sediment (Lee et al., 1990) and for oligochaetes (15–36%) (Klump et al., 1987) and *Mysis relicta* (53%) ingesting hexachlorobiphenyl dosed sediment (Klump et al., 1991). The similarity among the AE values for the different animals, different compounds, and different sediments may occur for several reasons. The similarity among the AE values may simply be coincidence. However, the hexachlorobenzene and hexachlorobiphenyl have octanol/water partition coefficients (K_{OW}) similar in magnitude to BaP and thus may associate similarly with the organic matter. It is expected that all of these hydrophobic organic compounds partition to the organic matter in the sediment, which is the primary ingested portion. Thus, the ability to assimilate compounds from the organic matter may well be similar for compounds of similar K_{OW}. Overall, the direct measurement approach was successful for determining AE because it accounted for selective feeding.

The dual-labeled method uses the ratio of assimilated and non-assimilated radiotracers measured in the feces and food source to obtain AEs. This approach was first used to estimate AEs in freshwater pulmonates (Calow and Fletcher, 1972), and further advancements allowed ^{51}Cr and ^{14}C isotopes to be measured simultaneously using a Beta scintillation counter (Wightman, 1975). However, selective feeding by deposit-feeding mollusks and amphipods was shown to violate the assumptions of the dual-labeled approach, thereby preventing calculation of AEs (Lopez and Cheng, 1983; Lopez and Elmgren, 1989). In the present study, dual-labeled method yielded unreasonably high AE values, greater than 100%. These results suggest that the ^{51}Cr is not tracking well with the [^{14}C]BaP based on bulk sediment or that selective feeding does not permit accurate calculation of the food source ratio for these determinations (Table 2).

Many benthic invertebrates selectively ingest smaller organic and contaminant-rich sediment particles (Van Duyn-Henderson and Lasenby, 1986; Brown, 1986; Dermott and Corning, 1988; Landrum and Robbins, 1990). *Diporeia* are extremely selective particle feeders and some of this selectivity is size dependent – i.e., *Pontoporeia* spp. selectively ingests < 10 μm size sediment particles (Ankar, 1977; Moore, 1976).

Selective feeding by *Diporeia* appears to have prohibited the direct calculation of AEs by the dual-labeled approach in the manner of Klump et al. (1987, 1991) because the bulk sediment ratio of the two isotopes does not reflect the likely ratio of the ingested food source. Three possible hypotheses may explain the failure of the bulk sediment ratio to reflect the food source. First, it is possible that the radiotracers might have been unevenly distributed among the various particle-size classes. It is critical to have an even distribution of both radiotracers in each size class within the sediment matrix in order for the dual-labeled approach to work (Lopez and Elmgren, 1989). If the distribution were uneven, the *Diporeia* would have been exposed to higher concentrations of one of the radiotracers over the other during the feeding process. Our measure of the radiotracer distribution suggested that the distribution was similar among the various particle size classes (Fig. 2).

A second hypothesis for the failure of the dual-labeled method to produce direct estimates of AEs was that *Diporeia* selectively feed on particles containing [^{14}C]BaP over those containing the ^{51}Cr, thus changing the food source ratio over the bulk sediment ratio. It is possible that the two radiotracers sorbed to particles with different chemical characteristics. Thus, the ^{51}Cr may have been associated with the FeO coating on clay particles (Forstner, 1990), whereas the [^{14}C]BaP may have been associated with the organic particles (Landrum and Robbins, 1990). If *Diporeia* selectively fed more actively on the [^{14}C]BaP bound organic particles rather than the ^{51}Cr bound clay particles, the observed distribution between the different labels could be expected.

The third possible reason for the failure of the dual-labeled method may be attributed to preferential uptake of [^{14}C]BaP over ^{51}Cr from interstitial water (Fig. 1). It should be pointed out that interstitial water concentrations could not be determined for the two radiotracers because of the small quantity of sediment used. It is possible that the [^{14}C]BaP was additionally accumulated from the interstitial water resulting in a second source that would contribute to the concentrations of [^{14}C]BaP in the fecal material. The fecal route is considered to be the main route for elimination of hydrophobic contaminants by *Diporeia* (Landrum and Robbins, 1990). The potential of this route contributing substantially to a difference in the relative contribution of BaP in the fecal pellets should be minimal. BaP binds tightly to particles and has been suggested to accumulate primarily from ingested particles (Landrum, 1989; Landrum and Robbins, 1990). Second the elimination rate for BaP is extremely slow (Landrum, 1988), thus very little elimination would be expected within the time frame of this experiment and major changes in the radiotracer ratio over ingested values would not be expected.

A secondary objective was examination of possible contaminant concentration effects on AE. AE values appear to be independent of concentration for the range of 1.45 to 43.3 nmol g^{-1}. The independence of AE values occurs despite a relative increase in the first-order clearance of BaP from sediment at the higher concentration. A one compartment model (Eqn. 4) for accumulation was assumed based on longer term BaP contaminated sediment exposures for *Diporeia* (Landrum, 1989).

$$C_a = k_s C_s t \tag{4}$$

Where C_a is the concentration in the organism (nmol g^{-1}), k_s uptake clearance constant from sediment (g sed g^{-1} organism h^{-1}), C_s is the dry sediment concentration (nmol g^{-1}) and t is time (h). Elimination is assumed to be unimportant over the time-course of the experiment base on previous exposures (Landrum 1988, 1989).

The overall first-order clearance of BaP from sediment was 0.0018 g g^{-1} h^{-1} for the lower sediment concentration and 0.0029 g g^{-1} h^{-1} at the higher concentration. Such changes in contaminant clearance from sediment with increases in contaminant concentration have been observed previously in *Diporeia* (Landrum et al., 1991). If the

flux into the organism $k_s C_s$ is assumed to be dominated by feeding, then the flux could be redefined as f_r AE C_s, where f_r is the feeding rate and AE remains the assimilation efficiency. Therefore, because our calculated AE was constant, an increase in the overall flux would have to be supported by an increased feeding rate in proportion to the increased flux. We found that the feeding rate increased from 0.0016 ± 0.0005 mg sediment mg^{-1} organism h^{-1} in the lower BaP concentration to 0.0029 ± 0.0010 mg mg^{-1} h^{-1} at the higher concentration and was in proportion to the increased flux. Unlike oligochaetes that demonstrated reduced AE with increased feeding rate (Klump et al., 1987), within the range measured here, the AE for *Diporeia* was essentially constant. Further, the enhanced flux with increased concentration was previously speculated to result from enhanced feeding (Landrum et al., 1991) and our measurements demonstrate that this is a very plausible mechanism for *Diporeia*. Finally, at the feeding rates calculated in this study, 41% and 47% of the total accumulation of the contaminants in *Diporeia* can be attributed to feeding for the two experiments, respectively, based on a average AE value of 45.7%. These estimates are lower than suggested by previous modeling efforts (Landrum, 1989; Landrum and Robbins, 1990) but are comparable to more recent experimental data (Lydy and Landrum, unpublished data).

Future research efforts should focus on finding alternative non-assimilated tracers that will better track organic contaminants in the sediment environment for selective feeding organisms. Because dual-label approaches would make AE determinations simpler and because RC values are not readily available, finding alternative non-assimilated tracers for organic contaminants should be investigated. The following radionuclides have also been suggested as non-assimilated tracers: ^{134}Cs, ^{137}Cs, and ^{144}Ce; however, their use would probably have the same problem as the ^{51}Cr for tracking organic xenobiotics. Alternatively, radiolabeled latex and plastic beads have been suggested as possible non-assimilated tracers. For example, 15-μm plastic beads have been used successfully as a non-assimilated tracer in experiments with selenium and the benthic bivalve *Macoma* (Luoma et al., 1992). Latex and glass beads have also been used successfully in particle-size distribution experiments with *Mysis relicta* (Bigelow and Lasenby, 1991) and *Hydrobia* (Lopez and Levinton, 1978). A most promising alternative appears to be the use of extremely hydrophobic organic contaminants (log $K_{OW} > 7$) that bind tightly to organic matter and are poorly assimilated. Decachlorobiphenyl has been suggested for use, and this compound has shown great promise as a non-assimilated tracer (Lee, H., US Environmental Protection Agency, Newport, OR, personal communication).

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