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COMPARISON OF IN VITRO DIGESTIVE FLUID EXTRACTION AND TRADITIONAL IN VIVO APPROACHES AS MEASURES OF POLYCYCLIC AROMATIC HYDROCARBON BIOAVAILABILITY FROM SEDIMENTS

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Abstract—The bioavailability of particle-associated contaminants was measured by a new approach that employs the digestive fluid of deposit feeders to solubilize contaminants in vitro. The proportion of contaminant solubilized by digestive fluid of the polychaete *Arenicola brasiliensis* was considered a measure of bioavailability and was contrasted with other, more traditional measures (i.e., uptake clearance, bioaccumulation factor, and absorption efficiency). There was generally good agreement among the four methods on the relative bioavailability of benzo[*a*]pyrene from six sandy sediments. Measures of phenanthrene bioavailability did not show strong correlations due to both a more limited data set and perhaps greater importance of uptake from the dissolved phase. The bioavailability of spiked polycyclic aromatic hydrocarbons (PAHs) differed from that of equivalent in situ–contaminated PAH but not in a predictable and consistent manner. By direct measurement of PAH content of recently ingested sediments collected from the foregut, we were able to quantify the importance of particle-selective feeding in increasing PAH content of ingested material relative to the bulk, ambient sediments. In most instances, the effect of selective feeding by *A. brasiliensis* was minimal, increasing PAH content of ingested material <20% above the ambient sediments. Absorption efficiencies of PAH during gut passage were determined by direct measurement of PAH concentration in sediments at various points along the digestive tract. Overall digestive absorption efficiencies were similar to the extent of in vitro solubilization by digestive fluids from the same sediments. These data suggest that extent of solubilization of sediment-bound contaminants during gut passage is a critical constraint on uptake and that absorption efficiency, with respect to the solubilized fraction, approaches 100%.

Keywords—Bioavailability Absorption efficiency Selective feeding Polycyclic aromatic hydrocarbons *Arenicola brasiliensis*

INTRODUCTION

Standard methods of chemical analysis involve an extraction step that is intended to extract all of the targeted contaminant from sediments using, for example, a strong acid or strong organic solvent. Such strong extractants are not designed to mimic the weaker, natural extractants that mediate bioaccumulation (e.g., water and digestive fluids), and thus are likely to overestimate the fraction of the contaminant that is bioavailable. Although there have been some attempts to develop chemically weaker extractions for metals that are intended to better quantify the bioavailable contaminant fraction [1], the validity of these claims remains uncertain, and no comparable partial extraction methods exist for organics that are capable of distinguishing between the bioavailable and nonbioavailable fractions of a compound. Lacking a chemical means to measure bioavailability, quantification of the parameter generally relies on biological measures.

There are four approaches to measuring bioavailability that have been commonly used. Toxicity is in part of function of bioavailability, but toxicity tests are complicated by a host of physiological and biochemical factors (e.g., detoxification mechanisms) that often make them unsuitable for even comparative bioavailability measurement [2]. Steady-state body burdens, when expressed as ratio between contaminant in the tissue and the surrounding media (i.e., sediment or water), are a measure of bioavailability but are also a function of contaminant biotransformation rates. In recent years, toxicokinetic measures, specifically uptake clearance, have been advocated as an absolute measure of bioavailability [2,3]. This parameter represents the rate of increase in tissue contaminant concentration normalized to the sediment concentration. Finally, absorption efficiency, as determined by direct measurement or indirect estimation of contaminant loss between ingested material and feces, can be used as a measure of bioavailability through the diet. However, for many deposit-feeding organisms, this approach is complicated, and sometimes compromised, by particle-selective feeding behavior [4,5].

A new approach to assessment of the bioavailability of ingested, particle-associated contaminants has recently been proposed that employs the digestive fluid of deposit feeders to solubilize contaminants [6,7]. The approach is essentially a chemical extraction, but with a biologically relevant extractant. Digestive fluid of a deposit-feeding organism is removed from the gut lumen, the sediments of concern are incubated with that fluid in vitro, and the amount of solubilized contaminant is then quantified. Although the approach does not address the subsequent absorption of the solubilized contaminant across the gut wall, the method at least places an upper limit on the proportion of contaminant that is likely to be made bioavailable during gut passage.

The intent of this study is to evaluate the utility of digestive

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fluid extraction as a measure of bioavailability and to compare the results with bioavailability measurements made by three other approaches: absorption efficiency, toxicokinetics, and steady-state body burdens.

MATERIALS AND METHODS

Experimental methods were identical to those described in a companion paper [7], in which more complete details are given. A brief overview is provided below.

Sediment collection and animal exposures

Six sediments collected from coastal areas of California, USA, were used in these experiments and are designated as: AR—home sediment of the test species, *Arenicola brasiliensis*, amended with wet scrubber sludge (containing approximately 5% polycyclic aromatic hydrocarbons [PAHs] by weight) from an aluminum refinery (0.04% sludge on wet weight basis); SS—sediments collected from Colorado Lagoon, the discharge point of a storm sewer serving Long Beach, California, USA; CO—beach sand from the southern California coast, visibly contaminated with spilled crude oil; SC—beach sand from the relatively pristine coast of Sonoma County, California, USA; BB—sediments from an intertidal area of Bodega Bay, California, USA; RR—a blend of sandy and muddy sediments, both from subtidal areas in central San Francisco Bay, California, USA.

The sediments were used in three independent exposure series: spiked [^{3}H]benzo[*a*]pyrene (57–78 Ci/mmol, Amersham Life Science, Arlington Heights, IL, USA); spiked [^{3}H]phenanthrene (267 mCi/mmol, National Cancer Institute, Bethesda, MD, USA; AR, SS, and CO sediments only); and in situ–contaminated, unlabeled phenanthrene and benzo[*a*]pyrene (AR, SS, and CO sediments only). The [^{3}H]PAHs, dissolved in acetone, were mixed into the sediments, and the labeled sediments were held at 4°C for 13 to 15 d prior to use. Sediments AR, SS, and CO contained 3 to 5 ppm of unlabeled PAH at the time of collection (or upon addition of scrubber sludge in the case of AR). Results based on spiked radiolabeled PAH are denoted by [^{3}H] (e.g., [^{3}H]benzo[*a*]pyrene), and the [^{3}H] prefix is omitted when discussing data from the unlabeled compounds.

Arenicola brasiliensis were collected from intertidal sands near San Francisco, California, USA, and dissected to expose the digestive tract. Digestive fluid was withdrawn from the midgut with a pipette, centrifuged to remove sediment particles, and held at −80°C until use. Digestive fluid from each *A. brasiliensis* individual was kept segregated for the [^{3}H]phenanthrene tests, whereas fluid from 30 individuals was composited for the [^{3}H]benzo[*a*]pyrene experiments. Digestive fluid extraction of sediment was done by placing 0.8 ml of fluid and 0.5 g wet weight of the sediment to be extracted (≈0.35–0.40 g dry weight) in glass centrifuge tubes. The digestive fluid and sediment mixture was vortexed and then placed on an orbital shaker for 4 h. After this time period the supernatant was recovered for PAH quantification by centrifugation at 2,100 *g* for 10 min.

Other *A. brasiliensis* individuals were used for exposure studies, and were placed in stainless steel trays containing the six sediments. Animals for short-term exposures (<72 h) were held individually in trays containing 2 kg of sediment (dry weight); trays for 10 to 19-d exposures contained four individuals with a total of 8 kg of sediment. Individuals were sampled after 1, 2, 3, 10, and 14 (or 19) d. At the time of sampling the digestive tract was excised, and sediment in the gut removed by dissection. The digestive tract of *A. brasiliensis* includes four morphologically distinct sections: the foregut, midgut, hindgut, and rectum. The foregut is a short, linear section comprising the first 10% of the total gut length. The foregut is delineated anteriorly by a pair of thick-walled ceca (function unknown) just behind the mouth. The midgut is an inflated, highly vascularized, and papillated area comprising 40% of the total gut length. A pair of digestive secretory ceca are located anteriorly at the juncture with the foregut. Enzymatic activity is greater in the midgut than in any other gut segment based on work with both *A. brasiliensis* (J. Judd, personal communication) and the congeneric *Arenicola marina* [8]. The midgut is the only section of the digestive tract containing substantial quantities of free digestive fluid. The midgut gradually tapers into the hindgut, becoming more slender in shape, with the loss of papillae on the exterior surface of the gut. The smooth-walled hindgut is approximately equal in length to the midgut. The rectum comprises the posteriormost 10% of the digestive tract, which is expanded to only slightly less than the diameter of the body, and often contains large amounts of sediment. The size and minimal surface area of the rectum suggests it serves as a storage area for sediment between defecation events.

As sediment was removed from the gut it was kept separate according to the gut segment where it was found. Typically, each gut section contained 0.1 to 0.7 g wet sediment, an amount sufficient for [^{3}H]PAH quantification and for determination of absorption efficiency by comparison of foregut to rectal [^{3}H]PAH concentrations. After removal of ingested sediment, the gut wall, the remainder of the body tissues, and all coelomic fluids were homogenized and frozen until later analysis.

Sample analysis

Activity in worm tissues associated with parent [^{3}H]PAHs was distinguished from activity of metabolites by hexane–sodium hydroxide partitioning [9]. Individual worms were homogenized and a subsample placed in tared centrifuge tubes to which 2 ml of hexane and 2 ml of sodium hydroxide (4 N in 50% ethanol) were added. The samples were allowed to stand overnight, then mixed vigorously and separated into organic and aqueous layers by centrifugation. The ^{3}H activity in the hexane phase was assumed to be associated with parent compound, and the activity in the sodium hydroxide layer was assumed to be associated with water-soluble metabolites. Ambient sediment and digestive fluids containing [^{3}H]PAHs were placed in Hionic-Fluor scintillation cocktail (Packard Instruments, Meriden, CT, USA). Sediment dissected from the gut was also placed directly in scintillation cocktail, except that midgut sediment was lightly blotted with an absorbent tissue to remove as much free digestive fluid as possible. Concentrations of [^{3}H]PAHs were determined from activity using either a Packard Tricarb 4640 (Packard Instruments, Meriden, CT, USA) or Beckman LS6500 (Beckman Instruments, Fullerton, CA, USA) liquid scintillation counter.

Nonradiolabeled PAH was extracted from digestive fluids using a single-phase dichloromethane:methanol extraction [6], with the dichloromethane fraction cleaned by passing through $NaSO_4$ and silicic acid columns. Analyses of cleaned extracts were conducted by reverse-phase high-performance liquid chromatography, using a Hitachi 7000 system (Hitachi Instruments, San Jose, CA, USA) with photodiode array (PDA) identification of peaks. If concentrations were sufficient, PDA was

Table 1. Physical and chemical characteristics of the sediments used[a]

Sediment	Silt and clay (%)	Organic carbon (%)	Nitrogen (%)	Phenanthrene (μg/kg) ^{3}H-labeled/unlabeled	Benzo[*a*]pyrene (μg/kg) ^{3}H-labeled/unlabeled
AR[b]	1.7	0.51	0.07	169/1,160	0.73/99
SS	4.3	1.39	0.07	119/213	0.97/358
CO	0.5	0.23	0.01	217/123	1.07/9
SC	0.0	0.06	0.01	—/—	0.36/—
BB	6.3	0.23	0.04	—/—	0.51/—
RR	15.9	0.21	0.03	—/—	0.49/—

[a] [^{3}H]phenanthrene was added to only AR, SS, and CO. No data are available on unlabeled polycyclic aromic hydrocarbons (PAH) in sediments SC, BB, and RR, but none of these sites are near known PAH point sources.

[b] AR = home sediments of *Arenicola brasiliensis* amended with wet scrubber sludge from an aluminum refinery; SS = sediments collected from Colorado Lagoon adjacent to a storm sewer outfall serving Long Beach, CA, USA; CO = well-sorted beach sand from the southern California coast, visibly contaminated with crude oil; SC = well-sorted beach sand from relatively pristine coastline of Sonoma County, CA, USA; BB = sediments from an intertidal area of Bodega Bay, CA, USA; RR = mix of sandy and muddy sediments, both from intertidal areas in central San Francisco Bay, CA, USA.

used for quantification, but at lower concentrations an in-line, programmable fluorescence detector was used. Detection limits were generally 5 to 10 ng/ml. Recovery was estimated and corrected for by addition of deuterated standards prior to all extractions. Unlabeled PAHs were extracted from sediments using dichloromethane and analyzed by gas chromatography–mass spectrometry (GC-MS) following the methods of Sloan et al. [10].

The lipid content of *A. brasiliensis* was determined by extracting 0.1 to 0.2 g of tissue with 2.5 ml dichloromethane, 5 ml methanol, and 2 ml water overnight, adding more dichloromethane and water, and then centrifuging to separate the lipid phase. After filtering the lipid fraction, it was dried at 60°C overnight and weighed.

Sediment total organic carbon (TOC) was quantified by a Control Equipment 440 Elemental Analyzer (Control Equipment, Lowell, MA, USA) after acid vapor treatment to eliminate inorganic carbon. Grain-size analysis was done by wet sieving.

RESULTS

Sediment characteristics

The six test sediments were all predominantly sands (Table 1). Sediments SC and CO were both from high-energy coastal beaches and contained negligible amounts of silt and clay (0.0 and 0.5%, respectively). Even the muddiest sediment, RR, contained only 15.9% silt and clay. Given the sandy nature of the sediment, organic carbon concentrations were all relatively low, generally <0.5% and exceeding that only in the sediments from the storm sewer outfall (SS = 1.4%).

Sediments AR, SS, and CO were all spiked with approximately equal amounts of [^{3}H]phenanthrene (~200 μg/kg, dry weight basis). Concentrations of unlabeled phenanthrene, however, varied by an order of magnitude and were particularly high in sediment AR containing combustion-derived aluminum refinery sludges, a result that is not surprising considering that the sludge itself contains over 0.5% phenanthrene. The specific activity of the [^{3}H]benzo[*a*]pyrene used in these experiments was much greater than that of the phenanthrene; therefore, it was possible to spike at relatively low concentrations. All sediments contained only 0.40 to 1.07 μg/kg [^{3}H]benzo[*a*]pyrene. Unlabeled benzo[*a*]pyrene varied from 9 to 358 μg/kg, with the highest concentration in SS, presumably derived from urban runoff discharged via the storm sewer.

Tissue lipid content

Total tissue lipid concentrations averaged 6.9% (SD = 2.1%) and varied from 3.8 to 12.8% among 41 individuals tested. There were no obvious differences among animals held in the different sediment types nor was lipid content dependent upon holding time (based on animals held in sediment AR for periods of 1–28 d).

In vitro solubilization

When incubated in digestive fluids, relative solubilization among sediments was expressed as a percentage of the particle-associated PAH originally present based on liquid scintillation counting ([^{3}H]PAH) or GC-MS (unlabeled PAH). There were substantial differences among the sediments in their relative desorption of PAH (Table 2). The proportion of [^{3}H]benzo[*a*]pyrene solubilized ranged from 12.9% in sediment SS to 52.0% in sediment SC. The proportion of unlabeled benzo[*a*]pyrene solubilized from the combustion-derived sludge in the aluminum refinery (AR) sediment was 26%, slightly higher than the 19% of ^{3}H-labeled benzo[*a*]pyrene spike solubilized from the same sediment.

Table 2. Solubilization of [^{3}H]PAH[a] and unlabeled PAH in digestive fluid[b]

PAH/sediment	PAH concn. in digestive fluid (μg/L)	Proportion of total PAH solubilized (%)
[^{3}H]benzo[*a*]pyrene		
SS	0.038 ± 0.001	12.9 ± 0.4
AR	0.045 ± 0.004	19.2 ± 1.7
RR	0.046 ± 0.001	25.4 ± 1.5
CO	0.114 ± 0.004	31.1 ± 0.4
BB	0.087 ± 0.012	42.0 ± 6.6
SC	0.086 ± 0.001	52.0 ± 1.1
Benzo[*a*]pyrene		
AR	10 ± 1	26 ± 2
[^{3}H]phenanthrene		
SS	16.4 ± 6.4	21.5 ± 8.8
AR	23.0 ± 8.0	37.2 ± 12.3
CO	48.3 ± 4.1	49.3 ± 6.4
Phenanthrene		
AR	308 ± 28	70 ± 6
CO	36 ± 10	71 ± 18

[a] PAH = polycyclic aromatic hydrocarbons; see footnote to Table 1 for all abbreviations.

[b] Values shown are means and standard deviations with $n = 5$ in most cases.

[^{3}H]phenanthrene data are only available for three of the sediments (AR, SS, and CO). The relative ranking of [^{3}H]phenanthrene solubilization among these three sediments was identical to that observed for [^{3}H]benzo[*a*]pyrene: sediment SS showed the least desorption of [^{3}H]phenanthrene (21.5%), followed by AR (37.2%) and CO (49.3%). The unlabeled compound was again solubilized to a greater extent than the radiolabeled equivalent: 70% of the phenanthrene in AR and 71% in CO.

Digestive absorption

The large size of *A. brasiliensis* made it possible to collect sediment and quantify [^{3}H]PAH activity throughout the entire length of the digestive tract by collecting sediment samples from the foregut, midgut, hindgut, and rectum. Our initial experiments were conducted with sediments AR, SS, and CO spiked with [^{3}H]phenanthrene, and the gut contents of three worms were composited to ensure sufficient material for analysis. In later experiments with sediments SC, BB, and RR spiked with [^{3}H]benzo[*a*]pyrene, we maintained the gut contents of each individual separate, and nearly all individuals provided sufficient material from each of the four gut segments.

Figure 1 illustrates [^{3}H]PAH concentration in ambient bulk sediments as well as in sediments collected from the foregut, midgut, hindgut, and rectum of *A. brasiliensis*. Data in this figure have arbitrarily been grouped into individuals collected after a short period of exposure (24–72 h; solid symbols) and those that had been in the contaminated sediments for an extended period (240–336 h; open circles). It is evident that substantial variation existed among individuals in the [^{3}H]benzo[*a*]pyrene content of gut sediments. This variation was far greater than the variation inherent in the ambient sediments themselves (i.e., compare broad distribution of foregut data points to that of ambient sediments in any given treatment). The concentration of [^{3}H]benzo[*a*]pyrene in the ambient sediments was uniform, particularly at the beginning of the exposure period. Over time, and perhaps due to the presence of the animals, [^{3}H]benzo[*a*]pyrene concentrations were reduced and became more heterogeneous (i.e., compare tight clustering of solid symbols for ambient sediments with the broader distribution of the open circles for the same ambient sediment).

Among the three sediments labeled with [^{3}H]benzo[*a*]pyrene, there was, to varying degrees, a tendency for contaminant concentrations to be greatest in the foregut and midgut. Contaminant concentrations in these gut segments were often greater than those in the ambient sediments and tended to decrease in sediment samples collected from more posterior gut segments (i.e., hindgut and rectum). These trends were most apparent in sediment SC and to a lesser degree in sediment BB. There was little change in [^{3}H]benzo[*a*]pyrene concentrations throughout the gut in worms feeding on sediment RR.

[^{3}H]benzo[*a*]pyrene concentrations in the ambient sediments and gut segments were compared between short periods of exposure (24–72 h) and longer exposures (240–336 h) by a two-tailed Mann–Whitney *U* test. In sediment BB, there was little effect of length of exposure, with significant differences ($p < 0.05$) present only in the rectal sediment. In sediment SC, and to a lesser extent in sediment RR, there were significant differences depending on exposure time. In these two sediments, the ambient sediment had a lower concentration

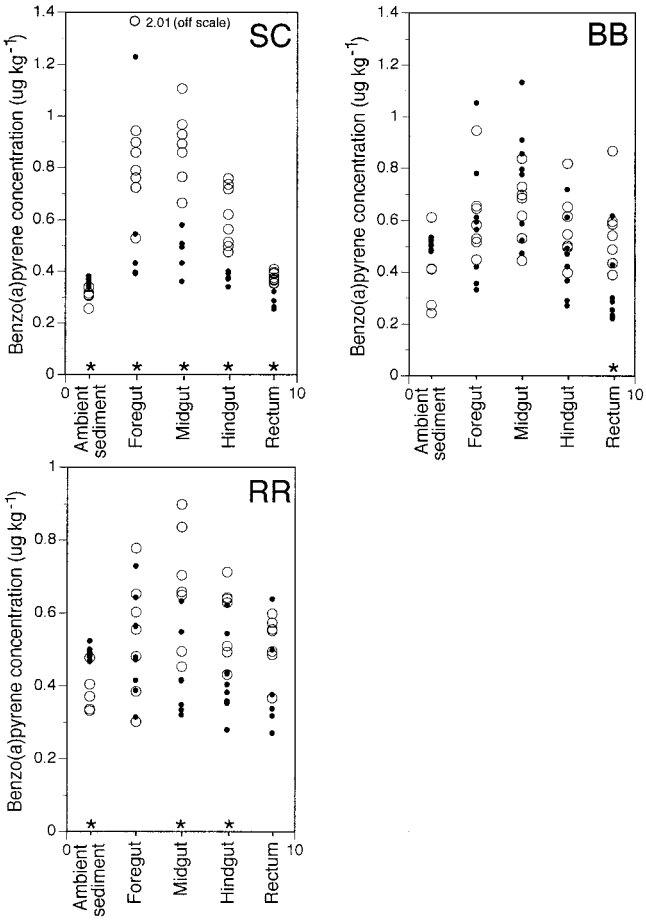

Fig. 1. The concentration of [^{3}H]benzo[*a*]pyrene in the ambient bulk sediments and in sediments dissected from the foregut, midgut, hindgut, and rectum of *Arenicola brasiliensis*. Each point represents data from a single individual. Solid symbols represent data from animals sacrificed after a 24- to 72-h exposure to the contaminated sediments. Open circles represent data from individuals sacrificed after 240 to 336 h. Asterisks above the abscissa indicate sediments for which there was a significant difference between 24- to 72-h and 240- to 336-h data points (Mann–Whitney *U* test, two-tailed, $p < 0.05$).

after 240 to 336 h, yet the gut segments tended to have higher [^{3}H]benzo[*a*]pyrene concentrations after the longer exposure period.

[^{3}H]phenanthrene data (Fig. 2) are not as extensive as those for [^{3}H]benzo[*a*]pyrene. Each data point within the gut represents a three-individual composite sample, and only data from 24 to 72-h exposure periods are available. The ambient sediments were uniformly labeled by [^{3}H]phenanthrene. There were too few data points to establish if foregut [^{3}H]phenanthrene concentrations were greater than those of the ambient sediments as they were for [^{3}H]benzo[*a*]pyrene. There was an appearance of decreased concentrations over the length of the gut in worms from all sediments.

The data of Figures 1 and 2 are also presented in tabular form (Table 3), showing the percentage change of the median [^{3}H]PAH concentrations between ambient sediment and the foregut or between successive gut segments. When foregut sediment [^{3}H]PAH concentrations differed significantly from ambient sediments, the foregut contained higher concentrations (four of the eight cases: sediment SC at both time periods, BB, and RR at 240–336 h). The significant increases were in the range of 18 to 20% during the first few days of

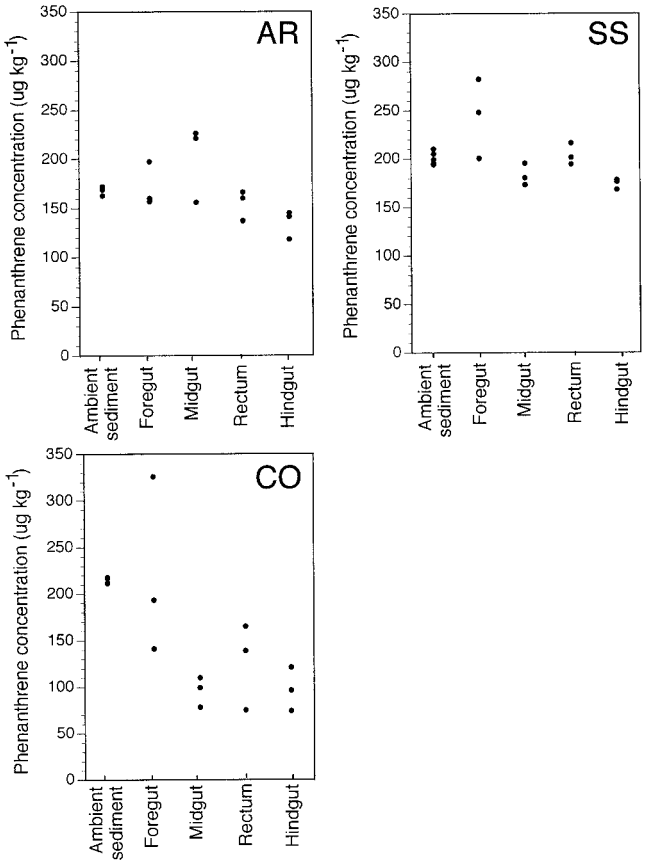

Fig. 2. The concentration of [^{3}H]phenanthrene in the ambient bulk sediments and in sediments dissected from the foregut, midgut, hindgut and rectum of *Arenicola brasiliensis*. Each point represents sediment composited from three individuals sacrificed after a 24- to 72-h exposure to the contaminated sediments.

exposure ([^{3}H]benzo[*a*]pyrene in sediments SC and BB). However, after exposure for 10 d or more, the enrichment of the [^{3}H]benzo[*a*]pyrene in the foregut contents was even more pronounced (167 and 49% in SC and RR, respectively).

With the exception of sediment RR, the five other sediments all showed a significant decrease in [^{3}H]PAH concentrations over the length of the digestive tract when comparing foregut contents to those of the rectum. Overall absorption efficiency (foregut vs rectum) was 27 to 55% for [^{3}H]benzo[*a*]pyrene (sediments SC and BB) and 12 to 50% for [^{3}H]phenanthrene (sediments AR, SS, and CO).

Although determination of overall absorption efficiency is straightforward from the data of Table 3, care should be taken in using these data to establish in which gut segment absorption occurs, since sediment from any given gut segment represents the average concentration over the entire length of that segment. For example, the 28% decrease in [^{3}H]benzo[*a*]pyrene concentrations observed between hindgut and rectum segments in sediment SC (24–28 h) should not be construed to indicate that absorption necessarily occurred in the rectum. Absorption in the posterior portions of the hindgut, anterior portions of the rectum, or a combination of the two would all yield the same result.

Bioaccumulation

To compare digestive solubilization from the various sediments with more commonly accepted measures of bioavailability, *A. brasiliensis* was placed in each of the six test sediment types, and PAH body burden was determined after various periods. Our methods allowed differentiation between radiolabel associated with parent [^{3}H]PAHs and that associated with metabolites. All sediment activity was associated with parent compound, but evidence of biotransformation became increasingly apparent in the tissue data with time. After 3 weeks of exposure to [^{3}H]benzo[*a*]pyrene, about 30% of the total tissue activity was associated with metabolites, whereas about 60% of the [^{3}H]phenanthrene-derived activity was present as metabolites. Therefore, to minimize the effect of biotransformation on apparent bioavailability by the various measures, all body burden data presented for radiolabeled PAHs represent the sum of parent and metabolite-associated activity. Data presented for unlabeled PAHs are for the parent compound only, as metabolite data were not provided by the GC-MS analysis.

The most extensive data set available is for sediment AR (Fig. 3). This figure, data from the other five sediments (not shown), and previous work with the confamilial *Abarenicola pacifica* have all demonstrated linear uptake of radiolabeled benzo[*a*]pyrene during the first 72 h [11]. Therefore, [^{3}H]benzo[*a*]pyrene body burdens measured over the first 3 d of exposure (usually nine data points) were used to derive an

Table 3. Change (as percent) of median [^{3}H]PAH[a] concentrations between ambient sediment and the foregut and between successive gut segments[b]

Sediment/time of exposure (h)	Ambient sediment to foregut	Foregut to midgut	Midgut to hindgut	Hindgut to rectum	Absorption efficiency (foregut to rectum)
[^{3}H]Benzo[*a*]pyrene					
SC: 24–48	+18*	+17	−22*	−28*	−35*
SC: 240–336	+167*	+8	−34*	−37*	−55*
BB: 24–336	+20*	+20	−29*	−14	−27*
RR: 24–72	−3	−20	+6	−16	−29
RR: 240–336	+49*	+19	−4	−12	0
[^{3}H]Phenanthrene					
AR: 24–72	+5	+38	−28	−12	−12*
SS: 24–72	+25	−27*	+12	−12*	−29*
CO: 24–72	−11	−49*	+40	−31	−50*

[a] PAH = polycyclic aromatic hydrocarbons; see footnote to Table 1 for all abbreviations.

[b] Percentage changes are relative to the preceding gut segment, not the initial foregut concentration (i.e., percent change from midgut to hindgut is relative to PAH concentration in the midgut). Percent change from foregut to rectum is considered the overall absorption efficiency. Asterisk indicates a significant change based on a Mann–Whitney U test ($p \leq 0.05$, one-tailed test expecting an increase in concentration from bulk sediment to foregut and a decrease with each successive gut segment thereafter).

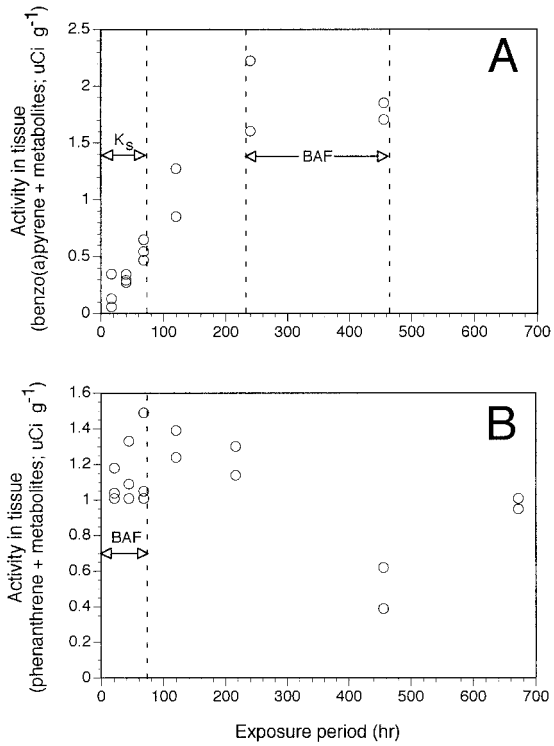

Fig. 3. Body burden of [^{3}H]benzo[*a*]pyrene-derived activity (**A**) or [^{3}H]phenanthrene-derived activity (**B**) as a function of period of exposure to the contaminated sediments. Each point represents a single individual. Data from sediment AR are shown because they provide the greatest temporal coverage, but data from all other sediments tested are comparable. There were no consistent differences among the sediments in the relative phenanthrene body burdens over 24, 48, and 72 h. Derivation of K_s and bioaccumulation factor values from these data are described in the text.

uptake clearance (K_s). The uptake clearance was calculated as the slope of the uptake curve divided by the sediment contaminant concentration, and this value represents the amount of sediment that would have to be completely cleared of PAHs to provide the measured body burden [3]. K_S values ranged over an order of magnitude among the sediments (Table 4). Uptake clearances were lowest for sediments CO and SS (0.0173 and 0.0194 g sediment tissue/h, respectively). [^{3}H]benzo[*a*]pyrene bioavailability, as parameterized as K_s, was greatest for sediments SC and BB (0.0860 and 0.1016 g/g/h, respectively). Sediment AR containing the unlabeled, in situ–contaminated benzo[*a*]pyrene showed a smaller K_s value (0.0214 g/g/h) than for the same sediment spiked with [^{3}H]benzo[*a*]pyrene (0.0498 g/g/h). This was, in part, a function of the inclusion of metabolites in the [^{3}H]PAH data, but, even if they were excluded, the K_s value derived from the parent [^{3}H]benzo[*a*]pyrene data only was greater for the spiked and radiolabeled sediments (0.0389 g g^{-1} h^{-1}).

Steady-state body burdens of [^{3}H]benzo[*a*]pyrene for *A. brasiliensis* were determined by averaging data from approx. seven individuals sampled at days 10 and 14 of the exposure (or 10 and 19 for sediment AR). The observations from these experiments (e.g., Fig. 3) and past work with *Abarenicola pacifica* [11] have all shown relatively constant benzo[*a*]pyrene body burdens after 10 d. Steady-state tissue residues were expressed relative to exposure concentrations by calculation of bioaccumulation factors (BAFs), the ratio of steady-state tissue residue and sediment concentration, both on dry weight basis and without carbon or lipid normalization. No data are available for sediments CO and SS, but the ranking of the remaining four sediments on the basis of BAF values was identical to that obtained by uptake clearance (RR < AR < SC < BB).

Quantifying the bioaccumulation of phenanthrene is more problematic. In work with *A. brasiliensis* in sediment AR (Fig. 3), as well as our past experience with *Abarenicola pacifica*, we have found maximum phenanthrene body burden attained in 24 h or less. This maximum tissue concentration persists for about 1 week, after which body burden drops abruptly to a level about 15 to 30% of the maximum. Because of the rapid rate at which the maximum body burden was reached, it was not possible to calculate a K_s value for phenanthrene. The tissue concentration data and the BAF values derived from those data reflect the maximum body burden attained and were calculated by averaging tissue concentrations over exposure periods from 21 to 72 h or 48 to 72 h (typically six to nine data points), depending on the sediment. Phenanthrene BAF values ranged over less than a factor of two for the ^{3}H-labeled sediments, from 2.96 in sediment CO to 4.48 in sediment AR. Unlabeled phenanthrene was not bioaccumulated to the same extent, relative to its sediment concentration, as the radiolabeled compound. Bioaccumulation factor values for the unlabeled PAH were far smaller than the radiolabeled equivalent in sediments SS and CO but nearly twice as great in sediment AR.

Table 4. Bioaccumulation data derived from exposure of whole worms to the contaminated sediments[a]

Sediment	Benzo[*a*]pyrene K_s (g sediment per g tissue per h)	Benzo[*a*]pyrene BAF[b] (kg sediment per kg tissue)	Phenanthrene BAF (kg sediment per kg tissue)
^{3}H-labeled			
CO	0.0173 ± 0.0023	—	2.96 ± 0.46
SS	0.0194 ± 0.0031	—	4.27 ± 0.54
RR	0.0349 ± 0.0041	10.1 ± 1.8	—
AR	0.0498 ± 0.0041	11.6 ± 1.7	4.48 ± 0.67
SC	0.0860 ± 0.0104	14.7 ± 3.7	—
BB	0.1016 ± 0.0079	20.6 ± 4.9	—
Unlabeled			
SS	—	—	0.64 ± 0.46
CO	—	—	0.93 ± 0.56
AR	0.0214 ± 0.0025	—	8.30 ± 2.91

[a] The uptake clearance (K_s, with standard error) and steady-state bioaccumulation factor (mean BAF, with standard deviation) are shown for benzo[*a*]pyrene. Phenanthrene BAF values reflect the maximum body burden attained (generally over a period of 24–72 h) and do not represent steady-state conditions.

[b] BAF = bioaccumulation factors; see footnote to Table 1 for all abbreviations.

Comparison of bioavailability measurements

Bioavailability of particle-associated PAH to *A. brasiliensis* in the test sediments can be parameterized by four measures using the data presented above: the proportion of particle-associated PAH solubilized by in vitro extraction with digestive fluids (Table 2), the absorption efficiency derived by comparing PAH concentrations in the foregut sediments to those in rectal sediments (Table 3), the uptake clearance (Table 4), and BAF, determined either at steady state (benzo[*a*]pyrene) or at maximum body burdens (phenanthrene) (Table 4). The relative bioavailability of PAHs from the six sediments was compared using these four measures. Although any or all of these measures could be normalized to sediment organic carbon content prior to making these comparisons, no such normalizations were made for two reasons. First, bioavailability measures varied by factors of two to six among the sediments (depending on which of the four bioavailability measure listed above was chosen), whereas sediment organic content varied by a factor of 23 among the sediments. Since variation in organic carbon content was so great, organic carbon would overwhelmingly determine the relative bioavailability ranking of the sediments on an organic carbon-normalized basis, regardless of their relative prenormalization bioavailability values. Second, the purpose of organic carbon normalization is to factor out the influence of organic carbon in determining bioavailability to evaluate the role of residual, non–carbon-related factors. In our comparisons of bioavailability based on the four measures, we expect organic carbon differences among the sediments to play an important role, and we have no a priori justification to exclude their influence.

The relationships among the four bioavailability measurements with respect to [^{3}H]benzo[*a*]pyrene were tested by Pearson product–moment correlations (Fig. 4). Because of the difficulty of establishing significant correlations with few data points (three, four, or six), no allowance was made for the effect of multiple comparisons, and $p < 0.1$ was accepted as a threshold for significance. Correlations between absorption efficiency and any other measure of bioavailability (% solubilization, K_s, or BAF) were all nonsignificant ($p > 0.1$) and failed to show even a suggestion of a monotonic trend. This result, however, is probably due in large part to the availability of only three data points for [^{3}H]benzo[*a*]pyrene absorption efficiency (sediments RR, BB, and SC) and the difficulty of demonstrating significance with such a limited data set.

Those sediments that desorbed proportionately more [^{3}H]benzo[*a*]pyrene during digestive fluid incubation also tended to show the greatest uptake clearance and steady-state bioaccumulation factors. The correlation between digestive fluid solubilization and K_s was statistically significant ($p < 0.1$). The correlation between solubilization and BAF ($p > 0.2$) was not significant, but Figure 4 suggests a direct relationship. Uptake clearance and BAF values were the most highly correlated ($r = 0.942$; $p < 0.1$) of any of the bioavailability measurements tested.

Bioavailability of phenanthrene could be parameterized by three measures (percent solubilized, absorption efficiency, and BAF at maximum body burden). None of the paired correlations among these measures were significant (all $p > 0.1$). This is likely a result of the availability of data for only three sediments (AR, SS, and CO) as well as theoretical reasons discussed below that would make correlations among the various measures less likely for phenanthrene.

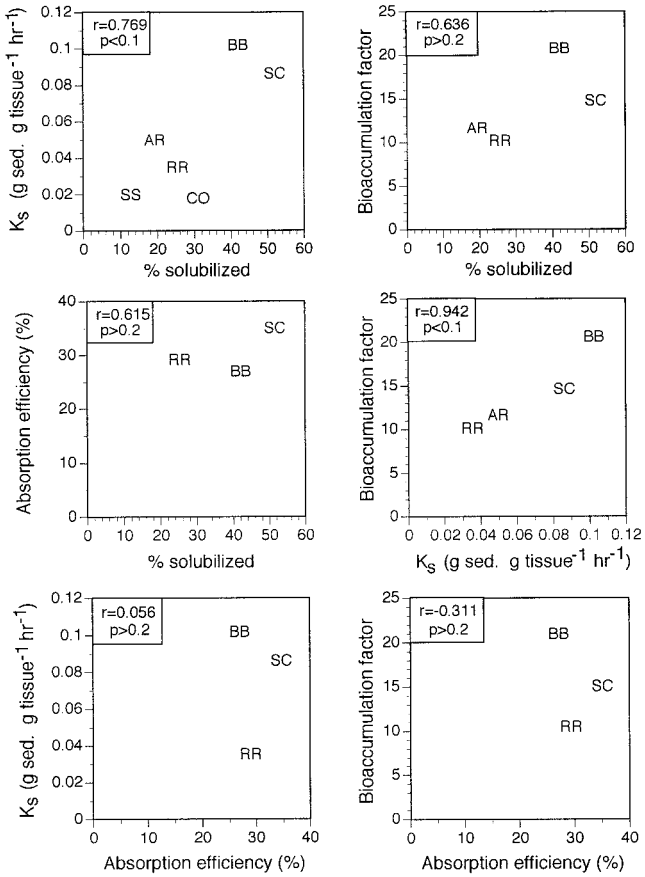

Fig. 4. Relationships between the various measures of bioavailability for [^{3}H]benzo[*a*]pyrene in the test sediments. Data are not available for all measures of bioavailability in all six sediments. R values (Pearson product–moment correlations) and associated probabilities are shown.

DISCUSSION

Particle selective feeding

One of the unique aspects of these experiments was the ability to recover sediment from discrete segments of the digestive tract of *A. brasiliensis* and to quantify PAH concentrations throughout the length of the gut. Such an approach is feasible only because of the large size of the animal (typically about 15 cm in length, 2 cm in width). The advantage of this approach is that it permits direct measurement of both the enrichment of PAHs in ingested sediments due to particle selective feeding and the absorption of PAHs from the sediment during gut passage.

Particle-selective feeding is a common phenomenon among deposit-feeding invertebrates [12] and is known to result in an enrichment of contaminant concentration in ingested material relative to concentrations in the ambient sediment [4,5,13]. In our experiments, we have no direct grain-size evidence of feeding selectivity, but it can be inferred from the enrichment of PAHs in foregut sediments relative to the bulk, ambient sediments. Assuming the foregut sediment represents unaltered ingested material (discussed further below), only slight evidence of particle-selective feeding was seen in worms exposed to the sediments for a few days or less. In two of the six sediments, there was an 18 to 20% increase in PAH concentrations relative to the ambient sediment, whereas the increase was not significant in the remaining four sediments.

These observations are consistent with data from the confamilial species *Abarenicola pacifica,* indicating relatively low selective abilities in comparison to other deposit feeders [14].

After 10 or more days of exposure, the enrichment of [^{3}H]benzo[*a*]pyrene in the foregut sediments relative to bulk sediments was even more dramatic for two of three sediments. This greater enrichment was a consequence of both a decrease over time in concentration in the bulk sediments (sediments SC and RR) and an increase over time in the [^{3}H]benzo[*a*]pyrene concentration of foregut sediments (SC only). The higher [^{3}H]benzo[*a*]pyrene concentrations in the foregut over time in sediment SC are consistent only with a change in the animal's selective ability. Animals feeding in the sediment for 10 to 14 d appeared to select more contaminated particles than those that had fed for less than 3 d. The mechanism for this shift in selectivity is unclear but could be related to changes in feeding rate. It may be significant that the effect was observed only in sediment SC, a high-energy beach sand containing virtually no silt and clay-sized particles or organic carbon (0.06%).

Several other potential explanations for these observations are not consistent with the data. First, in animals held in the contaminated sediments for an extended period, elimination of PAHs via the gut contents may become increasingly important [15]. This explanation, however, is unlikely to be the reason for an increase over time in PAH concentration of SC foregut sediments, given that worms held in sediment SC had only half the body burden of worms in sediment BB, but no comparable enrichment of foregut sediments was seen in the BB animals.

Second, progressive removal of finer particles from sediment due to feeding could explain the reduced PAH concentrations in the bulk sediment over time and could result in an increase in apparent selectivity. This mechanism, however, would not explain the greater PAH concentration of foregut sediments on days 10 to 14, relative to days 1 to 3, in worms from sediment SC. Determination of the degree to which *A. brasiliensis* altered substrate characteristics in the 2 kg of sediment provided each animal is complicated by the fact that, unlike other arenicolid polychaetes (e.g., *Abarenicola pacifica* and *Arenicola marina*), *A. brasiliensis* does not deposit its fecal material in a coil on the sediment surface. Thus, determination of feeding rate is difficult.

Absorption efficiency

Comparison of PAH concentrations in foregut and rectal sediments of *A. brasiliensis* allows direct measurement of absorption efficiency. It is possible that some absorption of PAHs may have occurred in the foregut, and thus our estimates of absorption may be conservative, but it is unlikely that this bias is significant given the short length of the foregut and the resulting brief residence time. In addition, we suspect that little digestion occurs in the foregut, because of (1) the absence of visible quantities of digestive fluid prior to the midgut, (2), the low surfactant and enzymatic activity in the foregut (J. Judd, personal communication; see [8] for similar data on the congener *A. marina*), and (3) the low bacteriolytic activity in the foregut of *A. marina* [16].

Most other deposit-feeding invertebrates are far smaller than *A. brasiliensis,* requiring that absorption efficiency be inferred by more indirect means. The greatest obstacle to absorption efficiency measurements has historically been the complications associated with particle-selective feeding behavior and the fact that measurements of the bulk sediment may not be representative of the material actually ingested. To avoid the complications caused by particle-selective feeding, other approaches used to measure absorption efficiency in deposit feeders have included dual labeling of sediment with both a conservative tracer and the nonconservative contaminant of interest [13] and the use of TOC as a tracer to account for selectivity [4]. Our experiments yielded estimates of digestive absorption efficiency of 27 to 35% (depending on sediment) for [^{3}H]benzo[*a*]pyrene and 12 to 50% for [^{3}H]phenanthrene. These estimates exclude values calculated after exposure periods greater than 72 h, since elimination of previously assimilated PAH or their degradation products via gut contents may become a significant confounding factor with time [15]. Although absorption efficiency is likely to be species and sediment specific, our estimates are comparable to values reported in the literature. Work with another annelid, *Lumbriculus variegatus,* has shown benzo[*a*]pyrene absorption efficiencies to range from 0 to 26% ([17], TOC method) to 11 to 26% ([17], dual-label method). Estimates of benzo[*a*]pyrene absorption efficiency by the amphipod *Diporeia* sp. include 56% ([17], dual-label method), 45 to 57% ([17], TOC method), and 46 to 60% ([5], TOC method).

Dietary absorption or assimilation of PAH from a given quantity of ingested sediment has proven to be highly variable among individuals, even when the animals were feeding on the same sediment under identical conditions [17,18]. Our study provides some insight into this reported variation. Polycyclic aromatic hydrocarbon concentrations in the foregut sediments varied by more than a factor of two among individuals and were far more variable than those found in multiple aliquots of the ambient sediment. As the foregut sediments represent material recently ingested, this variability must reflect differences in particle selectivity among individuals. Moreover, the capacity for digestive fluids to solubilize PAH varies by a factor of three among individuals, even when the animals were collected at the same time within a few meters of one another [7]. Interindividual variation in selectivity and digestive fluid composition may be related to differences in feeding rate between individuals. Nevertheless, with such variation in the characteristics of ingested material and its subsequent fate within the gut, it is not surprising that absorption efficiency measurements exhibit extraordinary inter-individual differences.

The digestive fluid solubilization approach currently does not incorporate any aspects of contaminant absorption across the gut wall, although clearly it is as important to contaminant uptake as the initial solubilization within the gut. Hydrophobic compounds are absorbed by passive diffusion across the gut wall [19], and we suspect that a hydrophobic molecule dissolved within the digestive fluid would rapidly partition into the lipid membranes lining the gut lumen, particularly since *Arenicola* sp. appears to retain the digestive fluid in the gut or reabsorbs it; little leaves the animal via the feces [16]. The ordinal ranking of the sediments in comparisons of solubilization to absorption efficiency was not particularly good, but data were only available for three sediments. It is encouraging that there was general agreement in magnitude between the proportion of PAH solubilized in digestive fluid and the proportion absorbed during gut passage. Absorption efficiencies of PAHs during gut passage were 27 to 35% for benzo[*a*]pyrene and 12 to 50% for phenanthrene, similar to the extent of in vitro solubilization by digestive fluids from the same sediments (13–42% for benzo[*a*]pyrene and 22–49% for

phenanthrene). These data suggest that extent of solubilization of sediment-bound contaminants during gut passage is a critical constraint on uptake and that much of the solubilized PAHs may be absorbed across the gut wall. Although absorption efficiency with respect to the bulk sediment may be less than 50%, with respect to the solubilized fraction it approaches 100%. Further confirmation of these results would be desirable, but if supported they indicate that measurement of solubilization potential alone may serve as a good approximation of bioavailability even in the absence of explicit measurement of the absorptive step, as does the digestive fluid extraction procedure.

Bioavailability

The majority of the data provided by this work pertains to bioavailability of the spiked [^{3}H]PAHs, but limited comparative data are available for the same unlabeled PAHs present in the sediments at the time of sediment collection. It was apparent that the spiked PAHs did not have a bioavailability equal to that of the in situ–contaminated PAHs, presumably because of differences in phase associations between the spiked radiolabeled PAHs, added in a solvent carrier, and the environmental PAHs. Uptake clearance and BAF estimates between the labeled and unlabeled compounds varied by factors of two to seven, depending on the PAH and the sediment. Differences in bioavailability between the labeled and unlabeled compounds were not consistent in direction or between compounds. The labeled PAH were more bioavailable in some instances (benzo[*a*]pyrene uptake clearance in sediment AR and phenanthrene BAF in sediments SS and CO), whereas the in situ–contaminated PAH were more bioavailable in other cases (all digestive fluid solubilizations and phenanthrene BAF in sediment AR).

There have been few studies that have compared bioavailability of spiked contaminants to that of in situ–contaminated equivalent compounds that have had far longer time for adsorption, but the limited work available has generally shown the spiked contaminants to be more bioavailable. Sediment desorption rates have been found to be greater [20], and bioaccumulation in amphipods has been shown to be greater when the PAHs had been recently spiked into the sediment [21]. The reasons for the anomalous results in our study are not clear but may in part be a result of differences in PAH concentration. For example, when the radiolabeled and unlabeled phenanthrene were in relatively comparable concentrations (sediments SS and CO), the radiolabeled compound was more bioavailable based on BAF values. When the concentration of the unlabeled phenanthrene was an order of magnitude higher than that of the labeled compound (sediment AR), then the unlabeled phenanthrene produced a higher BAF. Uptake clearance estimates are conceptually concentration independent, but may not be so in practice [22], and perhaps the same may apply to bioaccumulation factors. These data indicate that bioavailability of a spiked compound is not equivalent to the same compound in environmentally contaminated sediments, but a more quantitative analysis would require careful manipulation of exposure concentrations and a study more focused specifically on this question than was done here.

This study is unique in that it allowed assessment of bioavailability by four measures: (1) percent PAH solubilized by digestive fluid, (2) absorption efficiency during gut passage, (3) uptake clearance (K_s), and (4) BAF. These four measures all share the common trait that they are, to varying degrees, biologically based measures of bioavailability. Chemical approximations, such as the organic carbon-normalized sediment concentrations, have been shown to have some predictive value in estimating BAF [23], uptake clearance rates [24], and solubilization by digestive fluid [7].

Benzo[*a*]pyrene showed good agreement among the various bioavailability measures, excluding absorption efficiency, with which comparisons were difficult because of the few data points. This agreement is noteworthy since each measure of bioavailability used was subject to unique biases and limitations. The uptake clearance and steady-state body burden both are measures of bioaccumulation and reflect the sum of uptake from all possible routes. This would include accumulation through nondietary pathways, such as by absorption of dissolved contaminant across the integuement or gills. For strongly hydrophobic contaminants such as benzo[*a*]pyrene, the route of contaminant uptake in deposit feeders is largely through the diet [15,25]; therefore, one may expect good correlation with bioaccumulation-based measures of bioavailability and measures of exclusively dietary bioavailability (e.g., percent solubilization in digestive fluid and absorption efficiency).

As a measure of bioavailability, uptake clearance is influenced by ingestion rate in deposit feeders, with rapidly feeding individuals accumulating contaminant at a faster rate than more slowly feeding animals [11,18]. Ingestion rate is an important determinant of exposure but is not inherently a component of bioavailability. That uptake clearance correlated so well with other measures of bioavailability in this study (e.g., percent solubilization and steady-state BAFs) suggests either that feeding rates were similar in all sediments or that feeding rate covaried with bioavailability differences among the sediments (e.g., both decreasing with increasing organic carbon). Feeding rates in arenicolids are known to generally decrease with increasing sediment organic carbon content [11,26].

Steady-state body burdens are as much a function of depuration or metabolism as they are of bioavailability; thus, if they have utility as a bioavailability measure, it is only within a taxon, and perhaps with further constraints on population, season, and sex. In this study, steady-state body burdens of benzo[*a*]pyrene were used as a comparative measure of bioavailability and showed good correlation with other measures. Such an approach was feasible only because *A. brasiliensis* individuals from a single population were randomly distributed among the multiple sediment types, thus minimizing differences in biotransformation rates among the treatments. We were able to further minimize potential biotransformation differences among the sediments by summing the tissue activity of the parent compound and degradation products. However, had this not been possible (e.g., in working with unlabeled compounds), then biotransformation would result in steady-state BAFs that underestimated the bioavailability of PAH [27].

For benzo[*a*]pyrene, the proportion of contaminant solubilized by incubation in digestion fluid was significantly correlated with the uptake clearance, and correlation with steady-state body burden was suggestive (but not statistically significant, possibly because of only four data points). For phenanthrene, correlations between digestive fluid solubilization and other bioavailability measures were all nonsignificant. This may be due to the limited phenanthrene data set (three sediments), but there are also a priori reasons to expect poor correlation with body burden-based measures of bioavailability.

Phenanthrene has a much lower K_{ow} than does benzo[*a*]pyrene (log K_{ow}s = 4.5 and 6.0, respectively [28]), and uptake from the dissolved phase may play a more important role for phenanthrene. It has been estimated that the deposit-feeding amphipod *Diporeia* sp. obtains 100% of its benzo[*a*]pyrene body burden from ingested sediments, but only 12% of its phenanthrene in this manner [15]. When uptake from dissolved phases predominates, as it may for phenanthrene, digestive fluid solubilization may have less predictive value as a measure of bioavailability.

Bioavailability, with respect to sediment-bound contaminants, is the fraction of the total contaminant on the sediment particles that is available for bioaccumulation, whereas bioaccumulation is the actual accumulation of the contaminant [15]. To assess the bioavailability of sediment-associated contaminants potentially absorbed through dietary routes, digestive fluid solubilization does not rely on exposure of live animals to the sediment and therefore is not influenced by certain behavioral and physiological factors that are not necessarily inherent components of bioavailability but are determinants of bioaccumulation. An illustration of this distinction is provided by the somewhat anomalous results observed with sediment CO. A relatively high proportion of sediment-associated [^{3}H]benzo[*a*]pyrene (31%) and [^{3}H]phenanthrene (49%) were extracted in vitro by digestive fluid. In addition, we also observed a high (50%) in vivo extraction of [^{3}H]phenanthrene, as measured by comparing concentrations in foregut and rectal sediments. However, this apparent bioavailability did not translate into elevated body burdens since sediment CO had both the lowest uptake clearance of any sediment for [^{3}H]benzo[*a*]pyrene and the lowest peak BAF for [^{3}H]phenanthrene. All data from sediment CO presented in this paper were collected over a 3-d exposure period, but after about 7 d the worms emerged from the sediments, and they died shortly thereafter. Sediment CO was visibly contaminated with crude oil, and we suspect that toxicity may have affected bioaccumulation results, such as by reducing feeding rate. The digestive fluid solubilization and absorption efficiency data suggest the [^{3}H]PAHs of sediment CO were highly bioavailable, but this bioavailability did not translate into high bioaccumulation.

CONCLUSIONS

In vitro digestive fluid extraction is an intuitively attractive technique for assessing the bioavailability of contaminants from ingested sediments. It is likely to be of greatest value for contaminants that are strongly particle associated and for which ingestion is a major route of uptake. For such compounds, as typified by benzo[*a*]pyrene in these studies, there was good agreement between digestive fluid solubilization and the more traditional uptake clearance as measures of relative bioavailability among six sediments. There was a weaker, but suggestive, relationship to BAFs. General numerical agreement between the proportion of contaminant solubilized in digestive fluid and the proportion of contaminant absorbed during gut passage suggests that solubilization is the limiting factor in determining the bioavailability of sediment-bound contaminants. Thus, simple measurement of digestive solubilization alone provides a meaningful measure of bioavailability.

The results presented here are based on the use of digestive fluid from one species (*A. brasiliensis*) to extract PAHs from a variety of sandy sediments. Prior to broad application of the technique, it will be important to establish the utility of in vitro extraction from more organic-rich sediments and to test the technique with digestive fluid of other taxa. Preliminary information in these areas is available [6,7], but further work is needed. If the encouraging results to date can be generalized to other situations, the in vitro extraction approach is potentially a valuable tool in applications such as (1) answering mechanistic questions concerning pollutant bioaccumulation from contaminated sediments, (2) evaluating the effect of handling and storage conditions (e.g., length of storage and effects of sediment freezing) on bioavailability, and (3) quantifying the effect of remedial actions on changing contaminant bioavailability.

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