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IN VITRO DIGESTIVE FLUID EXTRACTION AS A MEASURE OF THE BIOAVAILABILITY OF SEDIMENT-ASSOCIATED POLYCYCLIC AROMATIC HYDROCARBONS: SOURCES OF VARIATION AND IMPLICATIONS FOR PARTITIONING MODELS

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Abstract—In vitro extraction of contaminated sediments using the digestive fluid of a deposit-feeding polychaete has recently been proposed to study contaminant bioaccumulation mechanisms and perhaps to better quantify the bioavailable contaminant fraction. This approach was evaluated using digestive fluid from the polychaete *Arenicola brasiliensis* and six marine sediments containing both spiked radiolabeled polycyclic aromatic hydrocarbons (PAHs) and in situ–contaminated unlabeled PAHs. The proportion of total contaminant extracted by digestive fluid from each sediment varied from 22 to 71% and 13 to 52%, for phenanthrene and benzo[*a*]pyrene, respectively. The proportions of contaminant solubilized were inversely correlated with the sediments' organic carbon content. The extent of PAH solubilization among sediments by *A. brasiliensis* digestive fluid was highly correlated with that of digestive fluid from the echiuran *Urechis caupo* and appears to be a consequence of surfactant properties of the fluids rather than of their enzymatic activity. The proportion of PAHs solubilized in vitro was similar to in vivo measurements of solubilization for contaminant exposures lasting about 24 h. However, with continued exposure, in vivo PAH concentrations in the digestive fluid increased fivefold, suggesting that digestive fluid is retained in the gut longer than sediment and thus accumulates PAHs through sequential digestion of many gut volumes. This phenomenon may enhance contaminant fugacity in the gut and increase the potential for bioaccumulation or toxicity.

Keywords—Bioavailability Polycyclic aromatic hydrocarbons Digestion *Arenicola brasiliensis* Partitioning

INTRODUCTION

Deposit-feeding organisms may ingest several times their own body weight in sediments every day; thus, ingestion is potentially an important route for the bioaccumulation of particle-associated contaminants. For organic contaminants with a high octanol–water partitioning coefficent, bioaccumulation through the diet may be of equal or greater importance than uptake from the dissolved phase in interstitial or overlying water [1–3]. However, assessment of the potential contribution of ingested contaminant to a deposit-feeder's body burden is complicated by the fact that a substantial fraction of particle-bound contaminants is not bioavailable. Studies with several organic compounds have shown that often over half of the sediment contaminant remains in the sediment after passage through an animal's digestive tract [4–6].

Standard methods of contaminant analysis rely on a strong solvent (organic compounds) or acid (metals) to extract all or nearly all of the contaminant from sediments. These methods are not intended to quantify the bioavailable fraction, and the total amount of contaminant extracted often has little relationship to the amount of contaminant that is actually bioaccumulated [7]. A new approach to assessment of the bioavailability of particle-associated contaminants has recently been proposed that employs the digestive fluid of deposit feeders to solubilize contaminants [8]. Digestive fluid of a deposit-feeding organism is removed from the gut lumen, and the sediments of concern are then incubated with that fluid in vitro. The fraction of the total contaminant that is solubilized in those fluids is then quantified on the presumption that sediment-associated contaminants must first be solubilized in the gut to be bioavailable. The approach has the simplicity of a chemical extraction, but, by using digestive fluid rather than an exotic solvent, the approach provides more environmental realism than is achieved by conventional chemical methods.

Because of the novelty of in vitro digestive fluid extraction, much information is needed on the potential and the limitations of the procedure. The present study is intended to expand upon the limited data provided in Mayer et al. [8] and in particular to (1) examine contaminant solubilization over a greater number of sediments, (2) identify some of the factors that control solubilization of contaminants in digestive fluid, (3) compare the contaminant solubilization potential of digestive fluid from two species, and (4) establish if in vitro extraction mimics in vivo processes. In a separate paper [9], we examine the bioavailability of contaminants by digestive fluid extraction in comparison to other more traditional measures of bioavailability. Although the digestive fluid extraction technique is applicable to both metals and organic contaminants, this research examines polycyclic aromatic hydrocarbons (PAHs) because of the high surfactant activity of digestive fluid [8] and its obvious implications for solubilization of other hydrophobic contaminants.

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MATERIALS AND METHODS

Sediment collection and preparation

Six sediments from estuaries and the open coast of California, USA, were collected for use in these experiments. All sediments selected were predominantly sand, since the species from which the digestive fluid was obtained (*Arenicola brasiliensis*) is naturally found in a coarse-grained substrate, and it was necessary to maintain the animals in sediment during some of these experiments. The sediments used and their designations were AR—home sediment of *A. brasiliensis* amended with wet scrubber sludge from an aluminum refinery (0.04% sludge on wet weight basis); wet scrubbers are used to remove particles from refinery exhaust gases, and the resulting sludges contain high levels of PAHs; SS—sediments collected from Colorado Lagoon adjacent to a storm sewer outfall serving Long Beach, California, USA; CO—well-sorted beach sand from the southern California coast, visibly contaminated with crude oil as a result of a past spill; SC—well-sorted beach sand from the relatively pristine coastline of Sonoma County, California, USA; BB—sediments from an intertidal area of Bodega Bay, California, USA; and RR—a mix of sandy and muddy sediments from subtidal areas in central San Francisco Bay, California, USA.

All sediments were wet sieved on a 1.0-mm screen, and the coarser material was discarded. The sediments were used in three independent exposure series: [^{3}H]benzo[*a*]pyrene (all six sediments), [^{3}H]phenanthrene (AR, SS, and CO sediments only), and unlabeled phenanthrene and benzo[*a*]pyrene (AR, SS, and CO sediments only). For the first two experimental series, the sediments were spiked with tritium-labeled phenanthrene (267 mCi/mmol, National Cancer Institute, Bethesda, MD, USA) or benzo[*a*]pyrene (57–78 Ci/mmol, Amersham Life Science, Arlington Heights, IL, USA). The PAHs had a radiochemical purity of 97 to 98% as assayed by the supplier either immediately before ([^{3}H]phenanthrene) or 1 to 2 months prior ([^{3}H]benzo[*a*]pyrene) to shipment. The [^{3}H]PAHs, dissolved in acetone, were added to the sediments, and the mixtures were stirred for 3 min with a concrete-mixing paddle mounted in an electric drill. Sediment was spiked in batches of 20 kg wet weight (much of this sediment was used in a companion study [9]). The labeled sediments were then held at 4°C for 13 to 15 days prior to use. After completion of the aging period, the sediments were mixed with the paddle for an additional 2 min prior to use in any experiments.

Three of the sediments (AR, SS, and CO) contained 3 to 5 ppm of unlabeled PAH, either present in situ at the time of sediment collection (SS and CO) or by laboratory addition of the wet scrubber sludges (AR). Results based on spiked radiolabeled PAH are denoted by [^{3}H] (e.g., [^{3}H]benzo[*a*]pyrene), whereas the [^{3}H] prefix is omitted when discussing data from the unlabeled compounds.

Animal collection and exposure

Arenicola brasiliensis were collected from the intertidal zone on the Pacific coast near San Francisco, California, USA. Individuals to be used for recovery of digestive fluid were held in seawater without sediment for 23 to 36 h to allow evacuation of the gut and to increase ease of recovery of digestive fluid. After the holding period, the animals were dissected to expose the digestive tract, which consists of a foregut, midgut, hindgut, and rectum. Digestive fluid was withdrawn with a pipette through the wall of the midgut. The fluid was centrifuged at 500 *g* for 10 min to remove sediment particles. For the [^{3}H]benzo[*a*]pyrene experiments, fluid from 30 individuals was composited. Digestive fluid from each *A. brasiliensis* individual was kept segregated for the [^{3}H]phenanthrene tests. Digestive fluids were frozen at −80°C and stored approximately 4 d prior to use.

One component of these experiments utilized digestive fluid from the echiuran *Urechis caupo*. These organisms were collected intertidally from Bodega Bay, California, USA, and held in seawater for 6 h, after which the gut was removed by dissection. The posteriormost portion of the gut containing seawater (which the animal brings in through the anus for respiratory purposes) was discarded. The digestive tract of *U. caupo* is extremely long (approximately 100 cm in a 15-cm-long animal), with no inflated reservoir of digestive fluid (i.e., midgut) as there is in *A. brasiliensis*. Therefore, the digestive tract was cut into short lengths and placed in a centrifuge tube. The need to cut the digestive tract resulted in some contamination of the digestive fluid with hemal fluid, but the brown color of the mixture, as opposed to red of the hemal fluid, indicated the fluid obtained was largely digestive fluid as intended. The fluid was centrifuged at 500 *g* for 10 min to remove large particles and was then frozen at −80°C until use.

Some *A. brasiliensis* were used for exposure studies rather than sacrificed immediately for recovery of digestive fluids. These individuals were placed in stainless-steel trays containing [^{3}H]benzo[*a*]pyrene-labeled sediment with approximately 2 kg (dry weight) of sediment per *A. brasiliensis* individual. Any individuals that did not burrow promptly were replaced. The animals were held at 15°C and 35‰, with overlying seawater continuously aerated and replaced approximately every 5 d. After exposure periods of 1, 2, 3, 10, and 14 d, some of the animals were sacrificed and digestive fluid was removed from the midgut. Digestive fluid was collected from these individuals without allowing time for gut evacuation, although the presence of sediment in the gut makes recovery of fluid more difficult and reduces fluid yield. Approximately 40% of these worms contained 0.2 to 0.8 ml of recoverable digestive fluid in the midgut, and fluid from these individuals was used for [^{3}H]PAH quantification.

Digestive fluid extraction

The sediment to be extracted (0.5 g wet weight, approx. 0.4 g dry weight) and the digestive fluid (0.8 ml) were placed in glass centrifuge tubes. The liquid:solid ratio used was intended to approximate the ratio observed in the gut of actively feeding *A. brasiliensis*, although that proportion is quite variable among individuals. Preliminary data have also shown that extraction efficiency is relatively independent of the fluid:sediment ratio used anywhere within the range of 0.2 to 0.8 g dry sediment per 0.8 ml fluid. The digestive fluid and sediment mixture was vortexed to ensure complete mixing and then continual agitation was provided for 4 h on an orbital shaker at 400 rpm. Preliminary data indicated that the proportion of [^{3}H]phenanthrene and [^{3}H]benzo[*a*]pyrene extracted was essentially constant for any time period from 20 min to 4 h. After completion of the extraction, the samples were centrifuged at 2,100 *g* for 10 min, and the supernatant was recovered for PAH quantification.

With each series of digestive fluid extractions with any given sediment, a single replicate seawater extraction was done for comparative purposes using the same liquid:solids ratio

Table 1. Physical and chemical characteristics of sediments used in these experiments[a]

	AR[b]	SS	CO	SC	BB	RR
Total solids (%)	74	77	81	82	79	76
Total organic carbon (%)	0.51	1.39	0.23	0.06	0.23	0.21
Grain size (%)						
>500 μm (coarse sand)	0.5	20.5	32.2	19.9	0.8	1.6
250–500 μm (medium sand)	24.9	40.0	60.2	63.8	48.7	51.7
125–250 μm (fine sand)	50.5	27.4	5.4	16.2	42.8	28.3
63–125 μm (very fine sand)	22.4	7.8	1.7	0.0	1.5	2.5
<63 μm (silt and clay)	1.7	4.3	0.5	0.0	6.3	15.9
PAH (μg/kg)						
Total unlabeled PAH	4,736	4,320	2,834			
Unlabeled phenanthrene	1,160	213	123			
Unlabeled benzo[*a*]pyrene	99	358	9			
[^{3}H]phenanthrene	169	199	217			
[^{3}H]benzo[*a*]pyrene	0.73	0.97	1.07	0.36	0.51	0.49

[a] No data are available on unlabeled polycyclic aromatic hydrocarbons (PAH) in sediments SC, BB, and RR, but these sites are not near known major PAH point sources.
[b] See Materials and Methods for definitions of sediments.

(0.8 ml liquid to 0.5 g wet sediment). The sample was processed, and PAH concentration in the seawater was determined by the same procedures used for the digestive fluid.

Sample analysis

Radioactivities of ambient sediment and digestive fluids containing [^{3}H]PAH were both determined by placing the material in Hionic-Fluor scintillation cocktail (Packard Instrument, Meriden, CT, USA). Concentrations of [^{3}H]PAHs were determined from activity using either a Packard Tricarb 4640 or Beckman LS6500 liquid scintillation counter. Background activity was subtracted, and quench correction was done by the external standards method.

To insure that sediment activity was associated with the parent [^{3}H]PAHs and not with degradation products, sediment samples were extracted by a hexane:sodium hydroxide partitioning method [9]. The proportion of activity partitioning into the hexane was comparable to that of PAH standards, so the contribution of degradation products to total activity was assumed to be minimal.

Nonradiolabeled PAHs were extracted from digestive fluids using a single-phase dichloromethane:methanol extraction [8], consisting of 7.5 ml dichloromethane, 15.0 ml methanol, and 5.0 ml 50-mM phosphate buffer (pH 7.4). After partitioning into aqueous and organic phases by addition of dichloromethane and water, the dichloromethane fractions were cleaned by passing over $NaSO_4$ and silicic acid columns. Analyses of cleaned extracts were conducted by reverse-phase high-performance liquid chromatography using a Hitachi 7000 system with photodiode array (PDA) identification of peaks. If concentrations were sufficient, PDA was used for quantification; at lower concentrations, an in-line, programmable fluorescence detector was used. Deuterated standards were added prior to all extractions and analyses were corrected for their recoveries. Nonradiolabeled PAHs were extracted from sediments using dichloromethane and analyzed by gas chromatography-mass spectrometry following the methods of Sloan et al. [10].

Total organic carbon in each sediment was quantified by a Control Equipment 440 Elemental Analyzer after acid vapor treatment to eliminate inorganic carbon. Grain-size analysis was done by wet sieving, with silts and clays combined as pan weight.

One component of this investigation involved analysis of enzyme activity in digestive fluid. Activities of lipase, protease, esterase, amylase, and cellulase enzymes in *A. brasiliensis* digestive fluid were assayed using the methods described in Mayer et al. [11]. Briefly, after dilution of digestive fluid in 0.1 M sodium phosphate buffer (pH 7.5), fluorescent probes consisting of methylumbelliferyl palmitate, alanine methylcoumarinylamide, methylumbelliferyl butyrate, methylumbelliferyl-α-D-glucoside, and methylumbelliferyl-β-D-glucoside were added, and the release of fluorescent tags was monitored over time. Fluorescence was measured on an F4500 Hitachi spectrofluorometer, and fluorescent signals were converted to concentration of tag by spiking the tags alone into separate aliquots of digestive fluid.

RESULTS

Sediment characteristics

All sediments used in these experiments were primarily sand (Table 1). Sediment AR, the home sediment of *A. brasiliensis*, was predominantly fine sand (50% by weight between 125 and 250 μm). The other sediments were slightly coarser and dominated by medium sand. All sediments contained relatively little silt and clay. The home sediment (AR) contained only 1.7% silt and clay, and the muddiest sediment, RR, contained only 15.9%. All sediments also contained relatively little organic carbon. Sediment SC from a high-energy beach contained <0.1%, and most other sediments contained 0.2 to 0.5%. The highest organic content (1.39%) was found in sediment SS, collected from a storm sewer outfall site.

Data on unlabeled PAH are available only for sediments AR, SS, and CO. In these sediments total unlabeled PAH concentrations ranged from 2,834 to 4,736 μg/kg. Sediment AR, containing combustion-derived wet scrubber sludges from the aluminum refinery, contained 1,160 μg/kg unlabeled phenanthrene, the dominant PAH in the sludges. Unlabeled benzo[*a*]pyrene concentrations were very low in sediments contaminated with crude oil (9 μg/kg) but highest in sediments contaminated by urban runoff (358 μg/kg). The concentrations of labeled PAHs were far more uniform among the sediments. [^{3}H]phenanthrene concentrations ranged from 169 to 217 μg/kg, and [^{3}H]benzo[*a*]pyrene concentrations ranged from 0.36 to 1.07 μg/kg.

Table 2. Interreplicate variability among polycyclic aromatic hydrocarbons (PAH) analysis of sediments and digestive fluids[a]

Sample type	Sediment	PAH	Mean concn.	Standard deviation	*n*	Coefficient of variation (%)
Sediment concentrations (μg/kg dry wt.)	SC	[3H]BaP	0.358	0.016	8	4.4
	BB	[3H]BaP	0.505	0.022	8	4.3
	RR	[3H]BaP	0.489	0.018	8	3.7
Digestive fluid with each replicate extraction done using fluid from a separate individual (μg/L)	SS	[3H]BaP	0.0658	0.0218	18	33.1
	CO	[3H]BaP	0.163	0.047	5	28.9
	AR	[3H]Phen	23.0	8.0	5	34.6
Digestive fluid with each replicate extraction done using a single fluid composited from 10–30 individuals (μg/L)	AR	Unlabeled BaP	9.8	0.8	5	8.5
	AR	Unlabeled Phen	308	28	5	9.1
	BB	[3H]BaP	0.0759	0.0128	5	16.8
	RR	[3H]BaP	0.0450	0.0011	5	2.5

[a] The data sets shown are those with the greatest number of replicates (*n*). BaP = benzo[*a*]pyrene; Phen = phenanthrene.

Interreplicate variability

The use of 0.5-g aliquots of sediment for the digestive fluid extractions required that the [3H]PAH spike be distributed uniformly throughout the 20 kg of sediment within each spiking batch. Analysis of 0.5-g sediment aliquots for PAH homogeneity indicated this objective had been met (Table 2). The standard deviation of multiple subsamples was within about 3 to 4% of the mean value. This level of uncertainty was therefore inherent in all subsequent digestive fluid extractions.

Arenicola brasiliensis yields an average of 1 ml digestive fluid per individual, and occasionally 2 ml, thus, it is possible to do an in vitro extraction (0.8 ml required) with the digestive fluid of a single individual. In extractions of [3H]benzo[*a*]pyrene from sediment SS, 18 separate extractions, each done with digestive fluid from a unique individual, solubilized 9 to 28% of the PAH, depending on the individual. This test and others with multiple extractions of a single sediment type using fluid from discrete individuals produced a coefficient of variation of about 30% in PAH solubilization (Table 2). This degree of variation was far beyond the 3 to 4% coefficient of variation inherent in the level of sediment contamination and indicates substantial differences in digestive fluid composition, and hence PAH solubilization potential, among individuals. Using a fluid composited from many individuals, the coefficient of variation among many replicate extractions of the same sediment type was reduced substantially and ranged from 2.5 to 16.8%. Except for our first experiments with phenanthrene, most benzo[*a*]pyrene results presented herein are based on experiments using a composite digestive fluid.

Digestive fluid solubilization

The concentrations of PAH attained in the digestive fluid after completion of the extractions spanned several orders of magnitude; thus, it is unlikely that saturation of the digestive fluid was a significant constraint in these experiments. The concentrations of [3H]benzo[*a*]pyrene in digestive fluid ranged from 0.04 to 0.10 μg/L, and the unlabeled compound reached a maximum of 10 μg/L in extractions of the AR sediments. For [3H]phenanthrene, digestive fluid concentrations ranged from 16 to 48 μg/l, with unlabeled phenanthrene present at 36 and 308 μg/L in extractions of sediments CO and AR, respectively.

Data are presented as a proportional amount of PAH solubilized relative to the total particle-bound contaminant initially present (Fig. 1). The total amount of particle-associated PAH in each sediment was quantified either by counting in a pseudocumene-based scintillation cocktail ([3H]PAH) or by following dichloromethane extraction (unlabeled PAH). In no case was digestive fluid capable of solubilizing as much PAH

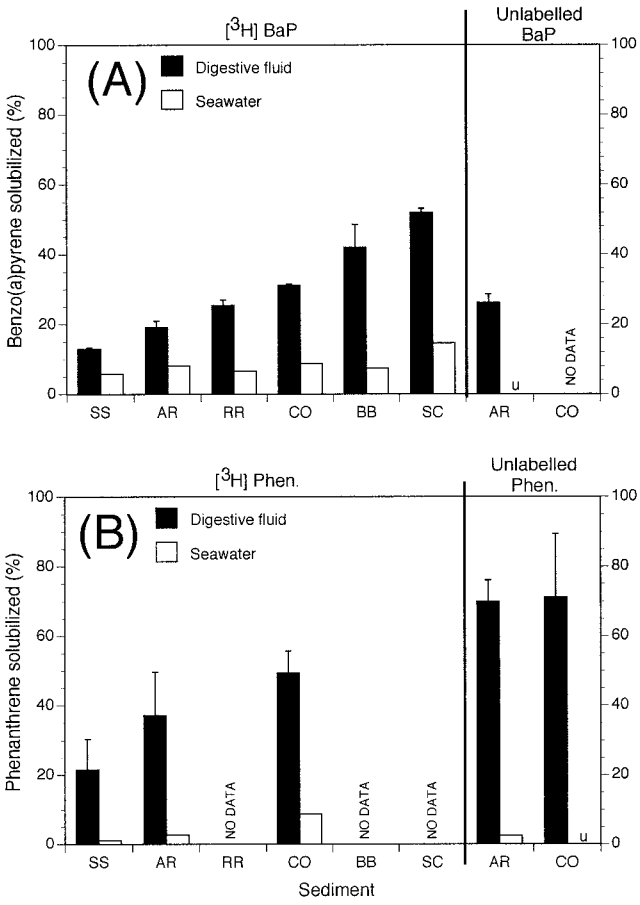

Fig. 1. The proportion of particle-associated benzo[***a***]pyrene (**A**) and phenanthrene (**B**) solubilized upon incubation of sediments in *Arenicola brasiliensis* digestive fluid and seawater. Mean and standard deviation of the five replicate digestive fluid extractions with each sediment are shown. U indicates polynuclear aromatic hydrocarbons were undetected with a concentration <5 ng/ml digestive fluid, corresponding to a maximum possible solubilization in seawater of 13.5% for benzo[*a*]pyrene from home sediment AR (amended with wet scrubber sludge from aluminum refinery) and 10.1% for phenanthrene from sediment CO (well-sorted beach sand from southern California coast, visibly contaminated with crude oil).

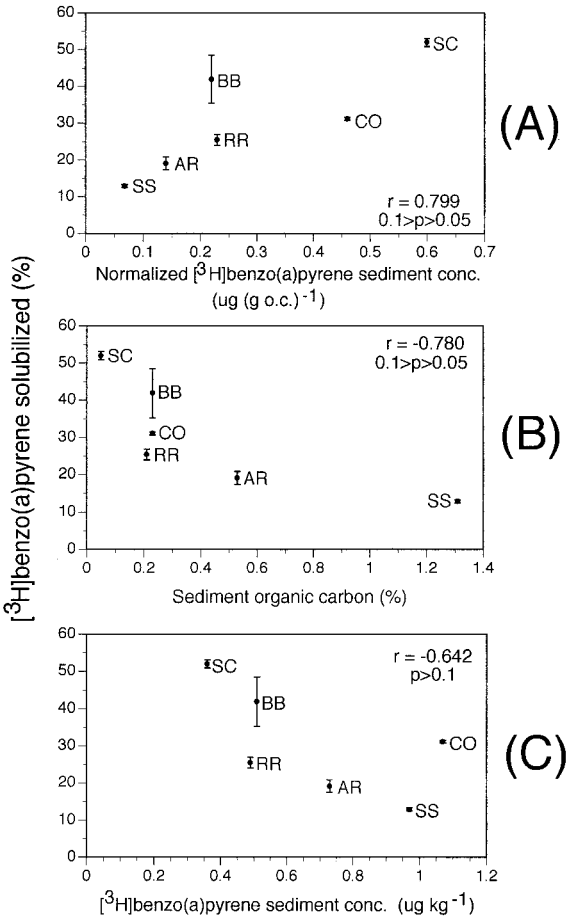

Fig. 2. The proportion of [^{3}H]benzo[*a*]pyrene solubilized from the six sediments by *Arenicola brasiliensis* digestive fluid (means and standard deviations) as a function of the contaminant's organic carbon-normalized sediment concentrations (**A**), sediment organic carbon content (**B**), and the contaminant's dry weight-based sediment concentrations (**C**).

as these strong organic solvents, and in most instances over half the sediment-bound PAH remained particle associated after incubation in digestive fluid.

There were dramatic differences among the sediments in the proportions of PAH that were solubilized during digestive fluid extraction. Of the sediments spiked with [^{3}H]benzo[*a*]pyrene, sediment SS desorbed the smallest proportion of the total contaminant (mean = 12.9%; standard deviation ± 0.4%). The greatest proportion (52.0% ± 1.1) was extracted from sediment SC, a sand from a high-energy beach. The proportion of [^{3}H]benzo[*a*]pyrene solubilized from all other sediments was between these two extremes: AR = 19.2% ± 1.7, RR = 25.4% ± 1.5, CO = 31.1% ± 0.4, and BB = 42.0% ± 6.6. The proportion of unlabeled benzo[*a*]pyrene solubilized from the combustion-derived sludge in the aluminum refinery (AR) sediment was 26.1% ± 2.4, slightly higher than the proportion of the ^{3}H-labeled benzo[*a*]pyrene spiked in the same sediment.

The proportions of [^{3}H]benzo[*a*]pyrene solubilized from the six sediments were directly correlated with the organic carbon-normalized PAH concentrations within the sediments (Fig. 2A). This relationship appeared to be largely a function of the organic carbon content of the sediment rather than of differences in dry weight-based concentrations to which the sediments had been spiked. Examining these two alternatives independently demonstrated that the proportions of

Table 3. Organic carbon-normalized partitioning coefficients between sediment and digestive fluid or sediment and seawater[a]

	Phenanthrene log K_{oc}		Benzo[*a*]pyrene log K_{oc}	
Sediment	Digestive fluid	Seawater	Digestive fluid	Seawater
[^{3}H]-labeled PAH[b]				
AR	3.16	4.31	3.44	3.81
SS	2.96	4.26	3.16	3.50
CO	3.27	4.05	3.50	4.04
SC	—	—	3.85	4.40
BB	—	—	3.40	4.18
RR	—	—	3.69	4.28
Unlabeled PAH				
AR	2.86	4.32	3.29	—
CO	3.17	—	—	—

[a] Digestive fluid K_{oc} values are the mean of three to five replicates; seawater K_{oc} values are based on a single sample.
[b] PAH = polycyclic aromatic hydrocarbons; AR = home sediment 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 within central San Francisco Bay, CA, USA.

[^{3}H]benzo[*a*]pyrene solubilized were inversely correlated with sediment organic content (Fig. 2B). Spiking concentrations, on the other hand, showed no relationship with PAH desorption (Fig. 2C). These results are not suprising given that the dry weight [^{3}H]benzo[*a*]pyrene concentration differences among the sediments were small (threefold variation) relative to the variation in organic content (23-fold).

Data for phenanthrene are not as extensive as for benzo[*a*]pyrene, and the variability around mean values are greater (Fig. 1) because of the use of digestive fluid from single individuals of *A. brasiliensis*, rather than from a composite sample, for each replicate extraction. Nevertheless, the relative ranking of the sediments observed for benzo[*a*]pyrene persisted for phenanthrene, with SS showing the least desorption of [^{3}H]phenanthrene (21.5% ± 8.8), followed by AR (37.2% ± 12.3) and CO (49.3% ± 6.4). This ranking was inversely related to their organic content, as noted for [^{3}H]benzo[*a*]pyrene. The unlabeled compounds were again solubilized to a greater extent than the radiolabeled equivalents: 69.8% ± 6.2 in AR and 71.1% ± 18.4 in CO.

Phenanthrene was consistently solubilized to a greater extent than benzo[*a*]pyrene from any given sediment. The only other PAH for which data are available is unlabeled benz[*a*]anthracene in sediment AR (initially present in AR sediment at 195 μg/kg). Solubilization of benz[*a*]anthracene in digestive fluid was 53.2% ± 3.9 relative to 26.1 for benzo[*a*]pyrene and 69.8 for phenanthrene.

Digestive fluid was far more effective at solubilizing PAHs than seawater. Seawater extractions, done in a manner identical to the digestive fluid extractions, solubilized 1.0 to 8.7% of the particle-associated [^{3}H]phenanthrene and 5.9 to 14.6% of the [^{3}H]benzo[*a*]pyrene. The greater solubilization potential of digestive fluid was also apparent on the basis of partitioning coefficients (Table 3). The organic carbon-normalized partition coefficients derived from sediment and digestive fluid were consistently smaller than those derived from partitioning be-

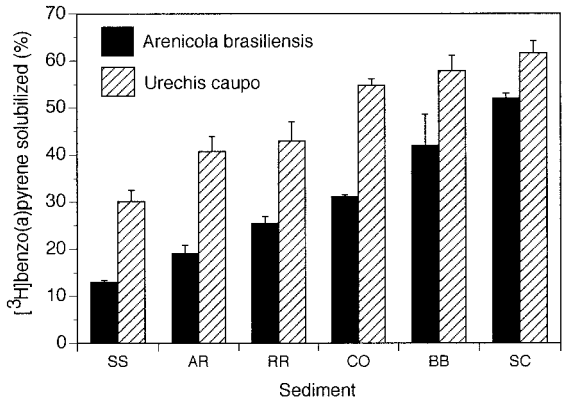

Fig. 3. The proportion of [^{3}H]benzo[*a*]pyrene solubilized from the six sediments by in vitro digestive fluid extraction using fluid from the polychaete *Arenicola brasiliensis* and the echiuran *Urechis caupo*. The means and standard deviations are shown for five replicates (*A. brasiliensis*) or two or three replicates (*U. caupo*).

tween sediment and seawater, reflecting greater PAH solubility in digestive fluid. Sediment:seawater log K_{OC} values for [^{3}H]phenanthrene (4.05–4.32) were in close agreement with values reported in the literature (4.08–4.36 [12–14]). Sediment:water log K_{OC} measurements for [^{3}H]benzo[*a*]pyrene (3.50–4.40) were more than two orders of magnitude below literature values (5.81–7.45 [15–17]). However, the concentrations of [^{3}H]benzo[*a*]pyrene in the aqueous phase of the seawater extractions were consistently well below reported water solubility (0.01–0.03 μg/L vs reported solubility of 3.8 μg/L [18].

Interphyletic comparisons

The polychaete *A. brasiliensis* was the principal test species for most experiments reported here, but limited work was done using digestive fluid from the echiuran *U. caupo* to determine if results could be generalized to other taxa. The same six sediments spiked with [^{3}H]benzo[*a*]pyrene and extracted with *A. brasiliensis* digestive fluid were also extracted with digestive fluid from *U. caupo*. The relative ranking of the six sediments in terms of the proportion of solubilized [^{3}H]PAHs was identical regardless of the animal species used (Fig. 3). The proportions of [^{3}H]benzo[*a*]pyrene solubilized by *U. caupo* digestive fluid were SS = 30.1% ± 2.4, AR = 40.7% ± 3.1, RR = 42.9% ± 4.1, CO = 54.8% ± 1.4, BB = 57.9% ± 3.2, and SC = 61.6% ± 2.6. Digestive fluid of *U. caupo* was a better solubilizer of PAH than that of *A. brasiliensis*, with about 10 to 24% more of the total [^{3}H]benzo[*a*]pyrene solubilized from any given sediment by *U. caupo*.

Relationships with enzymatic activity

As noted earlier, the digestive fluid collected from individual *A. brasiliensis* showed substantial variability in their capacity to solubilize PAHs. To determine if PAH solubilization was dependent upon enzymatic degradation of organic matter and whether the variation was a consequence of varying enzyme activities among individuals, the digestive fluid of 18 individuals was collected. An aliquot of each fluid was used for assays of enzymatic activity, and the remainder was used for a PAH solubilization assay by standard procedures. The proportion of [^{3}H]benzo[*a*]pyrene solubilized from sediment SS by each of the 18 digestive fluid samples varied form 9.4 to 27.8%, with a mean value of 15.7% ± 5.2 (Table 3). This mean is comparable to the value of 12.9% ± 0.4 shown in Figure 1 as derived from a separate extraction using a digestive fluid composited from 30 individuals.

The enzyme activities quantified included lipase, protease, esterase, amylase, and cellulase (Table 4). The individual variations in enzyme activities were as great as the variations in PAH solubilization potential, with the standard deviation among multiple individuals often about one-third of the mean value. However, correlations between the proportion of [^{3}H]benzo[*a*]pyrene solubilized by the digestive fluid of each individual and the activity of each enzyme class in that same fluid were all statistically nonsignificant (Pearson product–moment correlation, $p > 0.1$).

In vitro and in vivo comparisons

The relevance of in vitro digestive fluid extraction to in vivo processes was evaluated by comparing the in vitro data to PAH concentrations in the digestive fluid from actively feeding worms. *Arenicola brasiliensis* were placed in [^{3}H]benzo[*a*]pyrene-contaminated SC, BB, and RR sediments and allowed to feed for time periods ranging from 24 to 336 h (1–14 d). The animals were then sacrificed, ^{3}H activity in the digestive fluids was quantified, and the in vivo data were compared against the activity achieved in the in vitro extractions of the same sediments.

While more data from in vivo extractions would have been desirable, the limited data available suggest good agreement between in vitro and in vivo extractions (Fig. 4). Dissolved ^{3}H activity achieved in 4-h in vitro extractions was similar to the activity in the digestive fluids found in worms feeding on the sediments after the shortest (24-h) observation period. This similarity was apparent for all three sediments.

Another result apparent in Figure 4 is a tendency for the ^{3}H activity of the in vivo digestive fluid to increase with the time the animal has spent feeding in the contaminated sedi-

Table 4. Correlations between polycyclic aromatic hydrocarbons (PAH) solubilization potential and enzyme activity in digestive fluid[a]

	Maximum	Minimum	Mean	Standard deviation	Correlation coefficient (*r*)
% PAH solubilized	27.8	9.4	15.7	5.2	—
Lipase activity	12.14	4.00	7.52	2.87	−0.101
Protease activity	74.24	14.07	37.90	16.64	0.259
Esterase activity	379.49	125.78	246.19	74.17	0.188
Amylase activity	3.89	0.00	1.70	0.93	−0.164
Cellulase activity	7.96	3.71	5.78	1.46	−0.047

[a] Digestive fluid extraction data based on solubilization of [^{3}H]benzo[*a*]pyrene from sediment SS (see footnote to Table 3). All enzyme data given as micromolar product per milliliter of digestive fluid per minute. All correlations are nonsignificant ($p > 0.1$, Pearson product–moment correlation, $n = 18$).

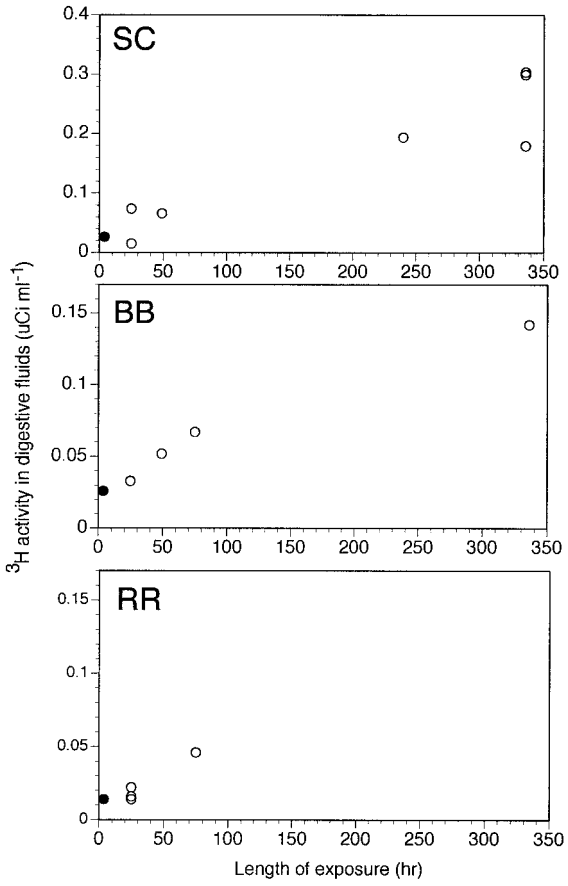

Fig. 4. [^{3}H]benzo[*a*]pyrene-derived activity of digestive fluids used in the in vitro extractions of sediments SC (beach sand from Sonoma County, CA, USA), BB (from intertidal area of Bodega Bay, CA, USA), and RR (mix of sandy and muddy sediments from subtidal areas within central San Francisco Bay, CA, USA (**solid circles**) or collected from the midgut of worms feeding on these same contaminated sediments (**open circles**). The solid circles are means of five replicate in vitro extractions; the standard deviation is obscured by the mean symbol. The in vitro extraction is shown as a 4-h exposure, the length of the extraction period. **Open circles** from the in vivo extractions represent digestive fluid from single individuals sacrificed after various periods of exposure.

ment. This trend was most apparent in sediments SC and BB because of the availability of data from worms that had fed in those sediments for 10 to 14 d but is suggested even by the 72-h data of sediment RR. The digestive fluid extracted from those individuals that had been exposed to the contaminated sediments for 10 to 14 d contained about five times as much ^{3}H activity as those individuals that had been in the sediment only 1 d or as attained in the 4-h in vitro extractions.

DISCUSSION

For most sediments and contaminants investigated in these studies, the majority of the particle-associated PAHs remained adsorbed to the sediment following extraction by digestive fluid. Given the similarity of the in vitro and in vivo extraction results, we conclude that the same result would apply during ingestion and digestion of sediments by deposit-feeding organisms; that is, much of the chemically quantifiable PAH is not absorbed by the animal during gut passage. That the proportion of extractable PAH is quite variable among different sediments means that risk characterizations and sediment management decisions based on total sediment-associated contaminant are likely to be misleading. It is generally recognized that standard methods of chemical analysis fail to quantify the bioavailable fraction [2,7]. Extractability by digestive fluid may be a far better measure of bioavailability, at least as it pertains to dietary uptake from particle-associated phases [9].

The extent of PAH solubilization by digestive fluids from any given sediment was a function of that sediment's organic carbon content in all cases examined (benzo[*a*]pyrene and phenanthrene extraction in *A. brasiliensis* digestive fluid and benzo[*a*]pyrene extraction in *U. caupo* digestive fluid). This result is consistent with general equilibrium partitioning theory [19] and the strong role sediment organic carbon is known to play in controlling partitioning of a hydrophobic contaminant. There is a strong organic carbon dependency in the partitioning of hydrophobic organics between sediment and seawater [12] and between sediment and biota [20]; thus, it is not suprising that sediment organic carbon concentrations were correlated with the extent of the partitioning between sediment and digestive fluid.

More data are needed from sediments containing higher organic carbon concentrations than those used herein. The highest organic carbon sediment tested (1.39% organic carbon in sediment SS) would still be considered relatively low in comparison to the range of organic carbon concentrations normally encountered in marine sediments. The only data available for more organic-rich sediments are those of Mayer et al. [8], in which 3 to 8% of phenanthrene and benzo[*a*]pyrene were extracted by *Arenicola marina* digestive fluid from two sediments containing 4 to 4.6% organic carbon. It is tempting to ascribe this very low extractability (relative to the 13–70% we observed) to the greater organic carbon content of the Mayer et al. sediment, but further validation is necessary using identical digestive fluid over a range of organic carbon contents.

Further evidence that digestive fluid extraction is demonstrating fugacity-driven partitioning comes from the observation that the proportion of PAHs solubilized by digestive fluid was higher for PAHs with lower octanol–water partitioning coefficients (K_{ow}). In any given sediment, proportionately more phenanthrene (log K_{ow} = 4.57 [21]) was extracted than was benzo[*a*]pyrene (log K_{ow} = 5.98 [21]). Using sediment AR only, there was a progressive decrease in the proportion of unlabeled PAH solubilized as the compounds' K_{ow} increased (phenanthrene: 69.8%, log K_{ow} = 4.57; benz[*a*]anthracene: 53.2%, log K_{ow} = 5.90; benzo[*a*]pyrene: 26.1%, log K_{ow} = 5.98). This K_{ow} dependence of digestive fluid extraction was also observed for one of two sediments tested by Mayer et al. [8].

For a few of the sediments, comparative data are available on digestive fluid solubilization of both the ^{3}H-labeled compounds and the equivalent unlabeled PAH (phenanthrene in sediments AR and CO; benzo[*a*]pyrene in sediment AR). In all cases, the digestive fluid extracted more of the unlabeled compound than of the ^{3}H-labeled compound. We had expected the opposite to be the case because sediment CO had been contaminated by PAH in situ decades prior to collection, and thus the PAH had had far longer for adsorption than the 2 weeks provided for the spiked ^{3}H-labeled compounds. Aging generally serves to reduce the extractability and/or bioavailability of hydrophobic contaminants [7,22–24]. Furthermore, the PAHs of sediment AR had been derived from the high-temperature vaporization of petroleum coke and pitch during aluminum refining and thus had been expected to be poorly

extractable because of their pyrogenic origin [15,25]. It is not clear why the unlabeled PAHs were more extractable in digestive fluid, although the greater extractability of phenanthrene from sediment AR is consistent with a greater bioaccumulation factor for the unlabeled compound relative to the radiolabeled compound for *A. brasilienis* exposed to the sediment [9].

Most importantly, it should be recognized that adsorption and desorption behaviors of spiked PAHs are not equivalent to the identical compounds that may have had greater opportunity for aging or are associated with different sedimentary phases. The labeled compounds provide a means to study mechanisms and test hypotheses but cannot be used as surrogates to predict environmental fate of in situ contaminants [26,27].

Digestive fluid was capable of solubilizing at least twice as much (and often far more) of the particle-bound PAHs than was seawater, indicating that sediment:water partitioning predictions (e.g., [19]) are not applicable to the gut environment of deposit feeders. Nevertheless, the extent of benzo[*a*]pyrene solubilization in seawater was suprisingly high, and, although well below aqueous solubility, it was greater than would have been predicted based on values of K_{oc} reported in the literature. Phenanthrene solubilization, on the other hand, was in good agreement with literature K_{oc} measurements. The high benzo[*a*]pyrene solubilization could be related to the presence of nonsettling particles or colloidal matter resulting in an overestimate of truly dissolved phase concentrations [17,28], association of a few percent of the total activity with degradation products rather than the parent [^{3}H]benzo[*a*]pyrene, or incomplete adsorption of the spiked [^{3}H]benzo[*a*]pyrene to the sediment after the 2-week aging period [29].

We suspect that the mechanism behind PAH solubilization in digestive fluid is the fluid's high surfactant activity, as has been speculated for *A. marina* [8]. Casual observation of physical properties (e.g., frothiness) as well as contact angle measurements [11] indicate that the digestive fluids of deposit-feeding animals have high surfactant activity. Surfactants are well-known solubilizing agents for PAHs [30]. The lack of correlation we observed between enzyme activities in the digestive fluid (lipase, protease, esterase, amylase, and cellulase) and the extent of PAH solubilization make it unlikely that enzyme hydrolysis played a role in releasing PAH from these sediments.

There was considerable variation among individuals in the ability of their digestive fluids to solubilize PAH. Extraction of PAH from a single sediment using digestive fluid from different individuals yielded a threefold difference in PAH extractability. These differences are probably attributable to interindividual variation in surfactant properties of digestive fluid. In work with many invertebrates, including the congeneric *A. marina,* surfactancy varied dramatically among individuals [11]. We suspect these differences are ephemeral within any individual and are related to factors such as feeding rate, composition of recently ingested material, or the degree of retention of gut fluids. Nevertheless, when sampling many individuals at any one time, one encounters a broad range of surfactancy in their digestive fluids and, hence, in their potential ability to solubilize PAHs. Thus, to compare contaminant extractability among sediments, or for any in vitro extractions in which constancy of solubilization potential is desirable, it is necessary to composite the digestive fluid from multiple individuals to insure homogeneity.

In all three sediments tested, solubilization of sediment-bound PAHs by in vitro digestive fluid extraction mimicked the extent of solubilization achieved in vivo over a short-term exposure period (i.e., animals exposed 24 h). This encouraging result suggests that in vitro extraction can be a powerful tool in studying the mechanisms of bioaccumulation from ingested sediments. The in vitro approach allows manipulations of sediment or digestive fluid characteristics that would not be possible in vivo, yet the results appear generalizable to contaminant desorption processes occurring in the gut.

With continued exposure of *A. brasiliensis* to [^{3}H]benzo[*a*]pyrene-contaminated sediment over 14 d, the concentration of radiolabel in the digestive fluid increased to about five times the concentration observed after only 1 d of exposure. The increase in concentration of PAHs in gut fluids during extended feeding is evidence for lack of saturation. This buildup is conceivably due to secretion of labeled parent PAHs or radiolabeled metabolites back into the gut. However, we tentatively reject this explanation based on bioaccumulation data from these same individuals, presented elsewhere [9]. *Arenicola brasiliensis* attained steady-state body burdens after about 10 d of exposure, at which time the worms held in sediment BB reached an [^{3}H]benzo[*a*]pyrene tissue concentration approximately twice that of worms held in sediment SC (data derivable from Tables 1 and 4 in Weston and Mayer [9]). Were the increase over time of PAH concentrations in the digestive fluid due to elimination via the gut contents, one would expect the digestive fluid of BB worms to be greater than that of SC worms, reflecting the greater body burden attained in sediment BB. There was no indication that this was the case, since after 14 d of exposure, digestive fluid from a sole BB worm actually contained less radiolabel than did that of any of three SC worms. Definitive conclusions, however, are hampered by the availability of data from only one individual after 14 d of exposure to sediment BB.

As an alternative explanation for the increase in PAHs within the digestive fluid, we note that the increase occurred over several days, equivalent to the passage of many gut volumes of ingested sediment. In the congeneric *A. marina,* we have also observed high concentrations of low molecular weight amino acids, representing food hydrolyzed from ingested sediment, present in the digestive fluid in excess of the amount that could be obtained from a single gut volume of ingested sediment [11]. Antiperistaltic pumping of the gut of this species has been observed [31], suggesting that the residence time of gut fluid is greater than that of ingested solids. Increased residence time of gut fluid allows buildup of both digestive agents and their products, enhancing digestive and absorptive kinetics [11]. One consequence of such behavior would be concomitant buildup of contaminants in the digestive fluid.

An important implication of these accumulations of high-contaminant concentrations is enhanced fugacity within the gut. Fugacity-based models (e.g., [32]) have had difficulties accounting for bioaccumulation in various animals, which show apparent bioaccumulation in excess of that predicted by the organic carbon-normalized sedimentary concentrations. One explanation for this discrepancy has been the volumetric reduction of food during gut passage, leading to higher concentrations and, hence, to fugacity in hindgut regions [33,34]. However, this explanation is not likely to be applicable to deposit-feeding species in which food volume remains constant during gut passage [35]. The buildup observed here provides an alternative explanation in that digestive solubilization

into a relatively stationary gut fluid over many gut volumes of sediment will also increase fugacity beyond that predicted by a single sediment passage.

The high degree of similarity between in vitro extraction results using *A. brasiliensis* and *U. caupo* digestive fluids is encouraging. The two species are phylogenetically diverse (*A. brasiliensis* in phylum Annelida and *U. caupo* in phylum Echiura) and also differ in feeding strategy (*A. brasiliensis* is a subsurface deposit-feeder; *U. caupo* is a filter feeder). However, in spite of these differences, the relative rankings of the six sediments tested in terms of the extent of [^{3}H]benzo[*a*]pyrene desorption in digestive fluid were identical regardless of whether the digestive fluid was derived from *A. brasiliensis* or *U. caupo*. That approximately 20% more of the total particle-associated [^{3}H]benzo[*a*]pyrene was extracted by *U. caupo* digestive fluid than by *A. brasiliensis* digestive fluid suggests that PAH bioavailability is species specific and that *U. caupo* may have a greater dietary accumulation of PAHs than *A. brasiliensis* at comparable levels of exposure. It has previously been noted that copper solubilization from sediment by digestive fluids of *A. marina* and those of *Parastichopus californicus* differed in extent but was also significantly correlated [8]. Thus, although much of our data is arenicolid based, conclusions may be generalizable to other invertebrates.

CONCLUSIONS

Digestive fluid is a far more effective solute than seawater in solubilizing hydrophobic contaminants, yet it is not nearly as efficient as strong organic solvents such as dichloromethane under in vitro incubation conditions. This approach may thus provide a better means to estimate the bioavailable fraction of sediment-bound contaminant than does a total extraction. The similarity between our in vitro results and solubilization measured in vivo provides some support for this hypothesis. The strong dependence of contaminant desorption on sediment organic carbon content, the relationship between extent of solubilization and K_{ow}, and the lack of any apparent correlation between solubilization and digestive fluid enzyme activity all suggest that in vitro digestive fluid extraction is simply a result of fugacity-driven partitioning between particulate and dissolved phases. The presumed importance of fluid surfactancy and the absence of a major enzymatic role in solubilization suggest that it may be possible to develop a synthetic equivalent to digestive fluid based on surfactant properties. Such a substitute would be necessary for routine and widespread use of the approach in the future, but at present in vitro extraction with a deposit feeder's digestive fluid provides a unique tool to study the process of contaminant bioaccumulation via dietary routes.

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