Commercially Available Chemicals That Mimic a Deposit Feeder's (*Arenicola marina*) Digestive Solubilization of Lipids

IAN M. VOPARIL* AND LAWRENCE M. MAYER Darling Marine Center, University of Maine, Walpole, Maine 04573

To develop a simple and cost-effective bioavailability test for sediment-bound contaminants, the solubilization strengths of mixtures of four commercially available surfactants and four proteins were compared to that of digestive fluids from a deposit-feeding benthic polychaete Arenicola marina. Initial tests indicated that sodium taurocholate, a vertebrate bile salt, was the most accurate mimic of A. marina gut fluids' solubilization of individual polycyclic aromatic hydrocarbons (PAH). Further testing with nutritional lipids and other hydrophobic contaminants confirmed the similarities of these fluids. Bovine serum albumin (BSA) solubilization of PAH was the most efficient of all the proteins tested. A cocktail of sodium taurocholate and BSA was compared to A. marina's solubilization of 12 PAH from four different contaminated sediments (from Boston, Charleston, Jacksonville, and San Diego harbors). The two solutions released most PAH to similar extents; 40 of 48 PAH-sediment combinations were released at amounts within a factor of 2 in cocktail and gut fluid solutions. Therefore, the cocktail may serve as a surrogate for real gut fluids and allow easier adoption of the in vitro incubation approach to bioavailability testing.

Introduction

In vitro incubations of the digestive fluids of deposit feeders with sediments can be used to quantify those contaminants that become digestively available to an animal during gut passage (1). After mixing sediment and digestive fluids under physiologically reasonable conditions, one quantifies the amount of contaminant desorbed into the fluid, based on the presumption that contaminants must first be solubilized by digestive fluids in order to be bioavailable. For hydrophobic compounds in general, solubilization in the gut is thought to be the limiting step during assimilation, as uptake into the cells lining the gut is a passive process and a function of concentration in the digestive fluid (2). For very hydrophobic organic compounds such as polycyclic aromatic hydrocarbons (PAH; 3) and chlorinated hydrocarbons (4), gut fluid solubilization has thus far been shown to be equivalent to bioaccumulation.

The gut fluid extraction approach has a number of advantages over conventional bioaccumulation studies using live animals for sediment risk assessment (5). First, in vitro

incubations can be consistently applied to sediments with a wider range of abiotic parameters (e.g., salinity, sediment grain size, total organic carbon) than could be tolerated by any single animal species for bioaccumulation studies. Second, bioaccumulation experiments often run for 28 days while in vitro incubations last only a few hours, with associated cost savings and faster data availability.

However, the limited quantity of gut fluid available from most animals makes use of the in vitro technique difficult. Even when using large polychaetes such as *Arenicola marina*, usually only 1 mL of fluid is available per individual (6, 7). The small volume of gut fluid available from each animal becomes problematic when attempting to assay numerous samples as well as a threat to communities of animals compelled to support this endeavor.

Therefore, we sought to develop a "cocktail" of commercially available substances that would mimic solubilization by digestive agents in gut fluids. Such a cocktail would offer the advantages of gut fluid incubations without the difficulties of animal collection and dissection. For the bioavailability of hydrophobic chemicals, important constituents of the cocktail are likely surfactants and proteins (7). For the nonselective, deposit-feeding polychaete A. marina (lugworm), surfactant micelles are responsible for 80–90% of the solubilization of the PAH benzo[a]pyrene by gut fluids, with the rest likely due to globular proteins (8). A solution of sodium dodecyl sulfate (SDS) has been used to mimic bioaccumulation of two organic contaminants (hexachlorobenzene and tetrachlorobiphenyl) by two deposit feeders, Nereis succinea and Pectinaria gouldii (4).

We modeled the cocktail after gut fluids from *A. marina* because this animal appears to be a good general model for benthic macroinvertebrates. For example, *A. marina's* digestive mobilization of sedimentary contaminants occurs at concentrations at the median amount solubilized by gut fluids of a 17 other species of benthic invertebrates (7). Although individual animals often show plasticity in digestive strength due to a number of factors including age (6) and diet (10), incorporation of such variability would be confounding at this early stage of cocktail development. Therefore, we compiled gut fluids from many individuals to create a gut fluid "standard".

Our approach was to start simply by testing gut fluid's and commercially available compounds' abilities to mobilize individual PAH. The most successful surfactant mimic was challenged with an expanded set of hydrophobic compounds. Our study ultimately reached practical and environmental relevance with testing of four contaminated sediments collected from the field. In this paper, we refer to the collection of hydrophobic compounds studied as "lipids", in accordance with the operational definition of this group of compounds (11).

Materials and Methods

Arenicola marina Gut Fluids. A. marina (Linnaeus, 1758) (lugworm) individuals were collected from sandflats near Lubec, ME, in July 1999. Animals were stored in seawater for up to 4 h, and mid-gut fluids were removed by dissection. Fluids from the mid-gut have maximal enzyme activities and surfactant concentration (6). Individuals' fluids were pooled, passed through a 0.45 μ m PTFE membrane filter, decanted into plastic containers, and stored at -80 °C until use.

The surfactancy of gut fluid was measured using the contact angle technique (6). The critical micelle dilution (CMD) was 15% for this fluid, which indicates a surfactant concentration of approximately 13.3 mM in the original gut

^{*} Corresponding author phone: (831)459-1533, fax: (831)459-4882; e-mail: ivoparil@es.ucsc.edu.

 $^{^\}dagger$ Present address: Ocean Sciences Dept., University of California Santa Cruz, Santa Cruz, CA 95064.

TABLE 1. Properties of Commercial Surfactants

surfactant	$M_{\rm W}$	CMC in water (mM)	CMC in ASW (mM)
Triton X-100	625	0.2 ^a	1.0 ^c
Brij 35	1200	61.6 ^a	0.1 ^c
sodium dodecyl sulfate	289	7.3 ^a	0.4 ^c
sodium taurocholate	538	1.5-15.0 ^b	2.3

^a Reported in ref 45. ^b Reported in ref 37. ^c Determined using ASW without Ca²⁺.

fluid (9). Gut fluid proteins were isolated using cold ethanol precipitation. A sample of the gut fluid was mixed with ice-cold 100% ethanol (1:9 by vol), vortexed for 30 s, and centrifuged for 15 min at 4 °C at 10 000g. The protein precipitate was washed twice more with ethanol, dried under N_2 gas, and reconstituted in ASW to the same concentration as the original gut fluid (42.0 g L^{-1}).

Commercial Surfactants and Proteins. Sodium dodecyl sulfate (SDS; PanVera Corp., Madison, WI), sodium taurocholate (a vertebrate bile salt; U.S. Biochemical Corp., Cleveland, OH), Triton X-100 (TX-100), and Brij 35 (both from Sigma, www.sigma-aldrich.com) were mixed with artificial seawater (ASW; using the recipe of ref 12) to create solutions with the same CMD as *A. marina* gut fluids (15%). A surfactant's critical micelle concentration (CMC) depends on the ionic strength of the medium, so it was necessary to determine the CMC of these surfactants in ASW instead of relying on reported values measured in distilled water (Table 1). Seawater without calcium was required for SDS, TX-100, and Brij 35 solutions as these surfactants precipitated otherwise.

Four commercially available proteins were tested to mimic PAH solubilization by nonmicellar components of gut fluid. Chicken egg albumin (Fraction V), bovine hemoglobin, casein, and bovine serum albumin (Fraction V) (all from Sigma) were dissolved in ASW at the same concentration as the proteinaceous material isolated from *A. marina* gut fluid by ethanol precipitation. Bovine hemoglobin and casein were difficult to dissolve at the concentration required, and the amount of these proteins in solution is less than the nominal concentrations reported; their solubility behavior in ASW excluded them from further use in cocktail development.

Individual Compound Experiments. The four commercially available surfactants were tested for their abilities to solubilize seven PAH (naphthalene, fluorene, phenanthrene, pyrene, chrysene, benzo[a]pyrene, and dibenzanthracene (all from Sigma)). Purified gut fluid proteins and the four commercial protein solutions were incubated with each of phenanthrene, pyrene, and benzo[a]pyrene. Sodium taurocholate (the best gut fluid mimic) and *Arenicola* surfactant solutions were further scrutinized with cholesterol, palmitic acid, myristic acid, stearic acid, oleic acid, linoleic acid, arachidonic acid, tripalmitin, hexachlorobenzene, and hexachlorobiphenyl—all ¹⁴C-labeled. Radioisotopes were purchased from American Radiolabeled Chemicals (www. arc-inc.com) except for hexachlorobenzene (Amersham, www.apbiotech.com) and hexachlorobiphenyl (Sigma).

Lipids were dissolved in carrier solvent (chloroform for PAH, toluene for all others), added to clean glass test tubes, and dried under $\rm N_2$ gas for 15 min. Triplicate incubations of the fluids of interest (gut fluids or solutions of the commercial compounds) and each solubilizate were placed on a rotary shaker (120 rpm) in the dark (PAH are photosensitive; 13) for 4 h. After incubation, solutions were clarified by filtration (0.45 μm PFTE membrane) and analyzed.

PAHAnalysis. PAH were extracted from the solution phase by chloroform (1:10 by volume) in triplicate and quantified using a Hitachi F-4500 fluorescence spectrophotometer at

TABLE 2. Geochemical Characteristics of the Sediments

	Charleston, SC	Jacksonville, FL	San Diego, CA	Boston, MA
surface area (m² g ⁻¹)	20.6	17.4	18.5	15.4
TOC (mg g^{-1})	47.3	22.8	12.8	71.7
total PAHa	37.1	64.1	852.0	588.0
$(\mu g g^{-1})$				

^a Sum of the 12 PAH measured for bioavailability.

the following (excitation/emission) wavelengths: naphthalene (275/325 nm), fluorene (265/305 nm), phenanthrene (280/360 nm), pyrene (335/380 nm), chrysene (270/380 nm), benzo[a]pyrene (370/430 nm), and dibenzanthracene (300/388 nm). We corrected for background autofluorescence of these fluids—always less than 10% of the measured PAH concentrations. PAH in chloroform were quantitatively diluted until measurements at three different dilutions were linear and proportional, as fluorescence can be quenched above certain concentrations resulting in a nonlinear relationship between fluorescence and concentration. Concentrations were calculated by comparison to external standards of individual PAH in chloroform.

Radioisotope Analysis. Filtered solutions were dispensed directly into 10 mL of ScintiVerse BD cocktail (Fisher Scientific) and counted on a LKB Wallac 1217 RackBeta liquid scintillation counter. Data were quench-corrected by comparison to quench curves generated by adding different concentrations of ¹⁴C or ³H toluene scintillation standard to each of the gut fluid and the sodium taurocholate solutions.

Contaminated Sediment Experiments. Gut fluid and a cocktail formulated to match (15.6 mM sodium taurocholate and 5.0 g L⁻¹ BSA in ASW) were compared in their release of 12 PAH from four contaminated marine sediments from around the United States. Boston, MA (referred to as Little Mystic Channel sediment in ref 8), and Charleston Harbor, SC, sediments were intertidal and collected by hand. San Diego, CA (referred to as Pier 8 sediment in ref 8), and Jacksonville Harbor, FL, sediments were subtidal and collected by grab from a ship. The sediments were characterized for surface area, total organic carbon, and total PAH concentrations (Table 2) using routine geochemical methods.

Sediments and 2 mL of gut fluid or cocktail were incubated in triplicate at a solid-fluid ratio of 0.25 g dry weight sediment (mL solution)⁻¹ as solid-fluid ratio can influence the amount of PAH released (8). Following incubation, the sedimentsolution slurry was centrifuged (1200g for 12 min) and the fluid phase filtered (0.45 μ m PFTE filter). PAH were isolated using liquid-liquid separation with dichloromethane (11, 14), spiked with internal standards (phenanthrene- d_{10} , benzo-[a]anthracene- d_{12} , and benzo[a]pyrene- d_{12}) to correct for recovery efficiencies, purified by silica gel chromatography (EPA 3630C), and quantified via HPLC following a previously described method (8). Using UV absorption (at 254 nm), the detection limit was approximately 0.002 μg (mL initial solution) -1 for individual PAH. Fluorescence detection could also be used with limits of detection approximately an order of magnitude less.

Statistics. All statistics were determined using Systat 9 (www.systat.com). We compared pairs of samples using a paired T-test with separate variances and $P \leq 0.05$. To determine which commercial surfactant solubilized PAH most like *A. marina* gut fluid, molar solubilization ratio (MSR, see below) data were log-transformed (to normalize error variance for data that span more than 3 orders of magnitude) and a simple linear regression was fit to scatter plots for the gut fluid against each commercial surfactant. Gut fluid MSRs were treated as the dependent variable. While a MODEL II

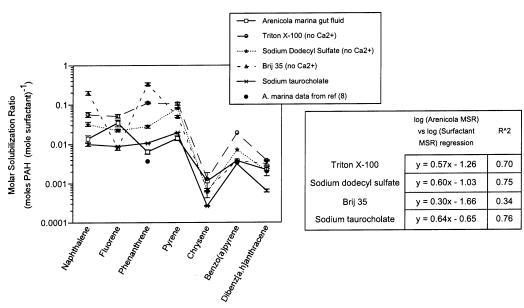


FIGURE 1. Solubilization of individual PAH by surfactants and gut fluid. Abscissa is the specific PAH arranged according to decreasing aqueous solubility in water. Ordinate is the molar solubilization ratio; note the log scale. To determine which surfactant best mimicked A. marina gut fluid, PAH solubilization data for each surfactant were regressed against those for A. marina (see text). SDS and sodium taurocholate behaved most like gut fluids for all of the PAH tested. Error bars are ± 1 SD.

regression would be appropriate for our data (as both variables are subject to error), we followed the suggestions of Sokal and Rohlf (15) for conditions when there is a causal relation between variables and used a simple linear model. A regression of y=x with $R^2=1$ would therefore indicate that the solution was a perfect match of gut fluid. This approach was also used to compare Arenicola gut fluids and sodium taurocholate solubilization of the expanded set of lipids as well as the comparison of gut fluid and cocktail release of PAH from contaminated sediments.

Results

Surfactant Solubilization of Individual Compounds. To compare the relative effectiveness of a particular surfactant in solubilizing a given compound, data are expressed as a molar solubilization ratio (MSR). The MSR is defined as the number of moles of lipid solubilized per mole of surfactant added to solution (16) and normalizes for differences in the molecular weight of each surfactant and PAH. Samples with most of their surfactant aggregated into micelles ought to have higher MSR values than in fluids having only monomers of surfactants in solution, because micelles are particularly able to solubilize lipids.

Ranges among PAH MSRs in any particular surfactant solution (approximately 2 orders of magnitude) were less than the solubility range in water (approximately 5 orders of magnitude) (Figure 1). For an individual PAH, MSRs in different surfactants usually ranged 1 order of magnitude, except for phenanthrene and naphthalene, which spanned 2 orders of magnitude. Triton X-100 was the most efficient solubilizer of all PAH, except naphthalene and phenanthrene. The rank order of solubilization of different PAH by A. marina gut fluid or sodium taurocholate solutions followed aqueous solubility (Spearman rank correlation coefficients $p \leq 0.001$).

Using the linear regression analysis described above, sodium taurocholate best mimicked *A. marina* gut surfactants' solubilization of pure PAH. The calculated regression was

 $log(Arenicola MSR) = 0.64 \times log(taurocholate MSR) - 0.65, R^2 = 0.76$

which had a slope closer to unity, a smaller *y*-intercept, and greater R^2 value than the second best surfactant, SDS.

A few of the PAH—surfactant combinations tested can be corroborated by previous work. Measured MSRs for Brij 35 and TX-100 solubilization of phenanthrene and pyrene are within a factor of 2 of reported values (16-19) as is BaP solubilization into sodium taurocholate (20). However, our TX-100 solubilization of naphthalene was 1 order of magnitude lower than that found previously (16, 18). The differences between our measurements and those previously reported may be due to our use of ASW as the solvent rather than distilled water. Salinity affects the critical micelle concentration of surfactants (21) as well as the aqueous solubility of PAH (22).

We found that MSRs tend to normalize variation caused by plasticity in an animal's digestive physiology (i.e. different surfactant concentrations) as long as micelles are the dominant form of surfactant in the fluid. In Figure 1, phenanthrene, pyrene, and benzo[a]pyrene solubilities in another A. marina gut fluid are included (marked as A. marina data from ref 8). This fluid was collected during a different season the previous year and had different surfactancy (CMD of 30% vs 15%), yet the MSR for benzo[a]pyrene overlaps the value for the gut fluid studied herein (3.74 \times 10⁻³ \pm 0.10 \times 10^{-3} vs $3.48 \times 10^{-3} \pm 0.18 \times 10^{-3}$). Only the MSR for phenanthrene was significantly different, increasing from 3.69×10^{-3} to 6.38×10^{-3} in the fluid with lower CMD. For lipids with an aqueous solubility greater than that of phenanthrene, the MSRs may have been affected by a significant fraction of the solute being solubilized by nonmicellar components of the gut fluid. If so, a fluid with a high CMD (low surfactancy) would display lower MSRs than a surfactant-rich solution. For more hydrophobic compounds, in which solubilization is almost completely micellar, this effect on MSR is not significant. For example, three additional A. marina gut fluids were collected and tested for BaP solubilization. These fluids had CMDs of 12%, 20%, and 70% (this last sample was unusually weak), and one standard deviation of the MSRs for BaP solubilization was $\pm 24\%$ using these three fluids. However, with gut fluid diluted with artificial seawater below the CMD (from Figure 2D of ref 8), the MSR was 5.25×10^{-4} —almost an order of magnitude

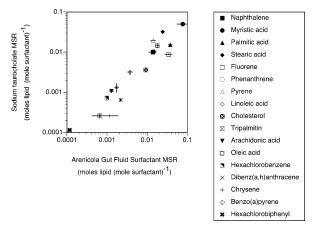


FIGURE 2. Comparison of *A. marina* gut fluid and sodium taurocholate solubilization of lipids. Abscissa is the solute's MSR in Arenicola fluid, and ordinate is MSR in sodium taurocholate solution. Solutes are arranged in the legend according to aqueous solubility with less-soluble compounds at the bottom. Error bars are ± 1 SD.

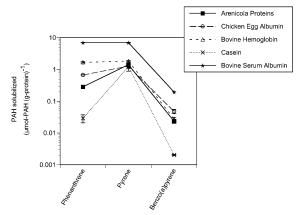


FIGURE 3. Solubilization of individual PAH by proteins. Abscissa is the particular PAH. Ordinate is the amount of PAH dissolved by protein solution, normalized by weight. Bovine serum albumin is the most efficient solubilizer of PAH. Among the PAH, proteins solubilized benzo[a]pyrene the least. Error bars are ±1 SD.

lower than when micelles are present.

For the expanded set of test lipids, sodium taurocholate matched *Arenicola* surfactant solubilization even better than when tested with only PAH—with the regression (Figure 2)

$$\begin{split} \log(Arenicola~{\rm MSR}) = \\ 0.91 \times \log({\rm taurocholate~MSR}) - 0.01,~R^2 = 0.91 \end{split}$$

The intercept indicates that gut fluid tended to solubilize slightly more of a particular compound, on average, than sodium taurocholate. Both *A. marina* gut fluid and sodium taurocholate preferentially solubilized free fatty acids such as myristic, palmitic, and stearic acid over naphthalene and had a strong preference for BaP; thus, solubilization by surfactants does not strictly follow aqueous solubility. However, the rank orders of lipid solubilization into each surfactant solution were strongly related to that of their aqueous solubility (Spearman rank correlation coefficients $p \leq 0.0001$).

Protein Solubilization of Individual Compounds. Bovine serum albumin (BSA) was the most efficient protein for solubilizing PAH, followed by bovine hemoglobin (Figure 3). Though proteins variably enhanced the solubilization of phenanthrene and benzo[a]pyrene, the complexation of pyrene by most proteins was remarkably consistent at \sim 1 μ mol g⁻¹, except for BSA. Lacking molecular characterization

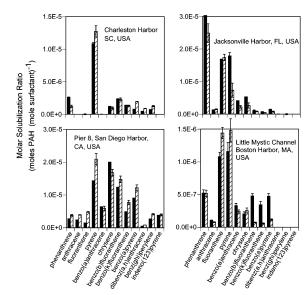


FIGURE 4. Comparison of cocktail and *A. marina* gut fluid release of PAH from four sediments. Each graph is for a different sediment; solid bars are for the cocktail; striped bars are for *A. marina* gut fluid. Abscissa for all graphs is the same and as appears in the bottom graphs. The ordinate for all graphs is the MSR of each individual PAH. For most sediments the cocktail mimics both the absolute amount of individual PAH released by gut fluid and the relative trends among PAH. Error bars are ± 1 SD.

of the proteins in gut fluid that would allow normalization per mole of protein, we normalize the concentration of PAH to the weight of protein in solution (i.e., μ mol-PAH (g-protein) $^{-1}$). Although bovine hemoglobin (BH) and chicken egg albumin (CEA) most closely matched *A. marina* gut fluid proteins, BSA was chosen for use in further cocktail development based upon BSA's greater efficiency in solubilizing PAH and its ready dissolution in seawater. For the cocktail, a less concentrated BSA solution (5.0 g L $^{-1}$) could be used to mimic gut fluid proteins, as BSA $^{-1}$ BaP solubilization is linear over a broad concentration range (δ).

Work with Contaminated Sediments. The patterns of PAH release by *A. marina* gut fluid are complex for the different sediments, yet the cocktail reproduces them well (Figure 4). Cocktail and gut fluid PAH concentrations for 40 of 48 PAH—sediment combinations are within a factor of 2. Release of PAH from Boston sediment was the most difficult to mimic; the cocktail underestimated fluoranthene and pyrene solubilization by $\sim 20\%$ but overestimated benzo[*b*]-fluoranthene, benzo[*k*]fluoranthene, and benzo[*a*]pyrene by severalfold.

Regression analysis of cocktail versus gut fluid solubilization for all sediment—PAH combinations indicates that the cocktail is accurate in reproducing gut fluid PAH concentrations (Figure 5). With all sediment—PAH combinations grouped together, the fitted linear regression is

$$log(Arenicola MSR) = 1.06 \times log(cocktail1 MSR) + 0.30, R^2 = 0.84$$

The 1:1 line is contained in the 95% confidence interval of the regression's slope and intercept (-0.60 < intercept < 1.20; 0.90 < slope < 1.22); thus, we conclude that the cocktail accurately mimics solubilization by *A. marina* gut fluid.

Discussion

The cocktail offers a means to apply a standard measure of bioavailability to disparate sediments, which after an initial investigation with cocktail can then be prioritized for additional investigation. In vitro incubations with cocktail can use any sediment, even though sediments have wider

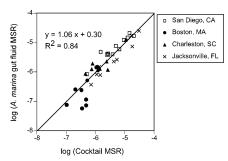


FIGURE 5. Regression analysis shows that the cocktail is both precise and accurate in predicting *A. marina* gut fluid release of PAH from these sediments. MSR data was log-transformed to normalize error variance. The 1:1 line is contained in the 95% confidence interval of the fitted linear regression.

ranges of characteristics, e.g., grain size, total organic carbon, enzymatically hydrolyzable amino acids (food), and salinity, than are acceptable to any single species that could be used in bioaccumulation tests. Though other measures of bioavailability have been proposed, e.g., equilibrium partitioning theory (EqP), we believe that gut fluid extractions are more relevant for deposit feeders—animals that are exposed to the brunt of sedimentary contaminants. Deposit-feeder exposure is via a micellar fluid, while EqP calculates exposure due to contaminants solvated by water (23).

For a deposit feeder, the process of lipid bioaccumulation can be broken down into a series of steps. Ingested sediment and digestive fluids mix in the gut, where lipids are desorbed from the sediment and perhaps attacked by digestive enzymes. Solubilized lipids are transported via diffusion and advection to the gut wall and compounds are absorbed (assimilated) into tissues. Our cocktail development has focused on the solubilization step of digestion. For vertebrates, lipid assimilation into tissues is a passive process, directly controlled by the concentration of lipid solubilized by digestive fluids; lipids must be solubilized by micelles in order to be assimilated (2). We suspect the same importance of solubilization for invertebrates based upon work with organic contaminants. Similarities between gut fluid solubilization and bioaccumulation of hydrophobic compounds such as PAH and chlorinated organic compounds suggest a transfer efficiency approaching 100% in deposit feeder guts (3-5). In vitro incubations do not account for biotransformation and subsequent excretion of contaminants, which is common in benthic invertebrates (24-26). Therefore, gut fluid and cocktail techniques are metrics for a maximum amount of, or upper limit to, digestive exposure to contaminants for deposit feeders.

We reported molar solubilization ratios (MSR) to normalize the amount of lipid solubilized in gut fluid and cocktail solutions to the amount of surfactant. When used for sediment assessment, interest is in the concentration of contaminant to which an animal is exposed, not an MSR. Therefore, in practice, a cocktail must be adjusted to match the digestive surfactancy of A. marina, which is plastic and likely varies with many factors including age (4, 6) and diet (10). We found surfactant concentrations in A. marina to range from no evidence of micelles (<2 mM) to surfactant concentrations of 25 mM (I.M.V., personal observation). Our experience with A. marina shows that individuals usually have surfactant concentrations of 6-13 mM, corresponding to CMDs of 15-30%. To mimic the median of this range, a cocktail should consist of 10 mM sodium taurocholate and 5 g L⁻¹ BSA in ASW.

Other extractants have been used to predict the bioavailability of hydrophobic organic contaminants from soils and sediments. Ethanol, tetrahydrofuran, cyclodextrin, Triton X-100, and supercritical CO_2 extractions have been proposed

to assess bioavailability (27–30). These studies have found correlations between the amounts of contaminants in extractants and other standard measures of bioavailability but are not quantitatively accurate predictors of exposure.

The surfactant SDS has been found to match two other deposit-feeding polychaetes' exposure to either hexachlorobenzene or tetrachlorobiphenyl (4). SDS, when in solution without Ca²⁺, was almost as good a mimic of *A. marina* gut fluids as sodium taurocholate. We tested a SDS cocktail with the Jacksonville Harbor sediment but found low recovery of PAH (data not shown), which was not in good agreement with the gut fluids. We suspect that shell fragments elevated dissolved Ca²⁺ concentrations to levels that caused SDS precipitation, as observed while determining CMD of the surfactant solutions.

Our cocktail formulation is similar to those developed to describe the mobilization of hydrophobic contaminants from soils accidentally ingested by humans. Hand to mouth behavior in children is the primary route of human exposure to soil-bound contaminants (31). In human exposure cocktails, conditions in the small intestine are approximated using mixtures of bile salts, suites of lipids that represent ingested lipids and their digestive products, and often digestive enzymes (20, 32-34). Sodium taurocholate is a bile salt constituent. These formulations use concentrations of bile salts that are similar to that developed here for A. marina digestive exposure (e.g., ref 20 used sodium taurocholate concentrations up to 12 mM and ref 32 used 20 mM of bile salts to mimic fed stages of human digestion). In humans, bile salt micelles play the central role in releasing hydrophobic organic compounds such as PCB and PAH from soils, making the compounds bioaccessible (33, 34). With some fine-tuning of the concentrations of bile salts and the addition of lipids presumably present in the gut during in vivo digestion, a taurocholate cocktail may thus be applicable to both vertebrates' and invertebrates' digestive exposure to organic contaminants.

The functional similarities for lipid solubilization by sodium taurocholate and A. marina gut fluid surfactants are striking. Both micellar systems have evolved to transport ingested nutritional lipids, though the composition of ingested material and associated lipids is presumably quite different for humans and marine deposit feeders. Both vertebrate and invertebrate surfactants contain a lipid moiety conjugated via an amide bond to an amino acid. Amide bonds tend to make surfactants more stable in alkaline solutions (35) and form smaller micelles (36). In vertebrates, the lipid moiety is sterol-based (37), while in invertebrates it is fatty acid-based (9, 38). Lester et al. (39) suggested that this substitution was due to crustaceans' inability to synthesize cholesterol. However, invertebrate polychaetes such as A. marina can synthesize cholesterol (40), suggesting that the use of fatty acid-based surfactants is due to some, as yet undetermined, aspect of their performance in saline solution.

The universal use of amide-linked amino acids as the hydrophilic end of biosurfactants may serve to minimize the loss of these compounds via adsorption or precipitation. For example, all commercial surfactants tested here, except sodium taurocholate, precipitated when dissolved in ASW containing calcium. Biosurfactants are also less likely to denature proteins and cause malfunction of digestive proteins. Commercial surfactants such as SDS are often used to denature proteins (41). At concentrations similar to that in the gut (>millimolar), SDS readily forms complexes with proteins in a very predictable manner—approximately one SDS molecule per two amino acid residues (42).

Our empirical comparison of cocktail and gut fluid suggests that digestive enzymes such as esterases and lipases have little effect on PAH digestive availability from contaminated sediments, though these enzymes are present in gut fluids (6). One could envision a situation where the lack of enzymes in this cocktail would cause problems for sedimentary contaminant risk assessment. For example, fatty alcohols are products of the digestive cleavage of wax esters (43) and may serve as cosurfactants in A. marina gut fluids that increase BaP solubilization and hence digestive bioavailability (44). However, our test sediments are probably as rich in nutritional lipids as are likely to be found in practice, so cosurfactant formation is not likely a concern with contaminated sediments.

We stress that bioavailability depends on a species-specific interaction between a particular organism and the geochemical characteristics of sediment. A priori, our approach of mimicking digestion likely will not predict suspension-feeder bioaccumulation because these animals' exposure is mostly from the freely dissolved phase (23). While this cocktail was developed with focus on a particular deposit-feeding polychaete, A. marina, we realize the potential interest in applying the cocktail to other animals. *A. marina* gut fluids from >30 individuals solubilized BaP at a range of concentrations with a mean equal to the median amount solubilized by gut fluids from 17 of 18 other species of benthic invertebrates across several phyla (7), indicating that our test animal may be a good average model for benthic macroinvertebrates. However, justification of our cocktail's application to other animals requires additional work in identifying and quantifying the digestive surfactants of those animals. Surfactant identification would enable CMC calculations from CMD measurements, which are easily obtainable with contact angle titrations, and hence MSRs to compare the abilities of the different animals' surfactants to solubilize test compounds.

The cocktail provides a simple, cost-effective method to rapidly screen and rank sediments for associated, potentially bioavailable organic contaminants. This approach is a predictive tool for contaminant bioavailability, potentially substituting for longer, more expensive bioaccumulation tests, and can be incorporated into the early stages of a tiered framework for sediment assessment. By mimicking the natural constituents of digestive fluid with commercially available substances, we provide a readily available protocol that can be applied to a wide range of sediments and hydrophobic contaminants to address their potential to cause biological impacts.

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Literature Cited

- (1) Mayer, L. M.; Chen, Z.; Findlay, R. H.; Fang, J.; Sampson, S.; Self, R. F. L.; Jumars, P. A.; Quetel, C.; Donard, O. F. X. Environ. Sci. Technol. 1996, 30, 2641-2645.
- (2) Shiau, Y. F. In *Physiology of the Gastrointestinal Tract*, 2nd ed.; Johnson, L. R., Ed.; Raven Press: New York, 1987; pp 1527-
- (3) Weston, D. P.; Mayer, L. M. Environ. Toxicol. Chem. 1998, 17, 820 - 829.
- (4) Ahrens, M. J.; Hertz, J.; Lamoureux, E. M.; Lopez, G. R.; McElroy, A. E.; Brownawell, B. J. Mar. Ecol. Prog. Ser. 2001, 212, 145–157.
- (5) Weston, D. P.; Millward, R. N.; Mayer, L. M.; Voparil, I. M.; Lotufo, G. R. Sediment extraction using deposit-feeder gut fluids: A potential rapid tool for assessing bioaccumulation potential of sediment-associated contaminants; ERDC/EL TR-02-18, US Army Engineer Research and Development Center: Vicksburg, MS, 2002.

- (6) Mayer, L. M.; Schick, L. L.; Self, R. F. L.; Jumars, P. A.; Findlay, R. H.; Chen, Z.; Sampson, S. J. Mar. Res. 1997, 55, 785-812.
- Mayer, L. M.; Weston, D. P.; Bock, M. J. Environ. Toxicol. Chem. **2001**, 20, 1890-1900.
- (8) Voparil, I. M.; Mayer, L. M. Environ. Sci. Technol. 2000, 34, 1221-
- Smoot, J. C.; Mayer, L. M.; Bock, M. J.; Wood, P. C.; Findlay, R. H. Mar. Ecol. Prog. Ser. 2003, 258, 161-169.
- (10) Bock, M. J.; Mayer, L. M. J. Exp. Mar. Biol. Ecol. 1999, 240, 77-92.
- (11) Bligh, E. G.; Dyer, W. J. Can. J. Biochem. Physiol. 1959, 37, 911-
- (12) Parsons, T. R.; Maita, Y.; Lalli, C. M. A Manual of Chemical and Biological Methods for Seawater Analysis; Pergamon Press: Oxford, U.K., 1984.
- (13) Payne, J. P.; Phillips, C. R. Environ. Sci. Technol. 1985, 19, 569-
- (14) Chen, I. S.; Shen, C.-S.; Sheppard, A. J. J. Am. Oil Chem. Soc. **1981**, 58, 599-601.
- (15) Sokal, R. R.; Rohlf, F. J. Biometry: the principles and practice of statistics in biological research, 3rd ed.; WH Freeman & Co.: New York, 1997.
- (16) Edwards, D. A.; Luthy, R. G.; Liu, Z. Environ. Sci. Technol. 1991, 25, 127-133.
- (17) Grimberg, S. J.; Nagel, J.; Aitken, M. D. Environ. Sci. Technol. **1995**, 29, 1480-1487.
- (18) Guha, S.; Jaffe, P. R.; Peters, C. A. Environ. Sci. Technol. 1998, 32. 930-935.
- (19) Prak, D. J.; Pritchard, P. H. Water Res. 2002, 36, 3463-3472.
- (20) Laher, J. M.; Barrowman, J. A. Lipids 1983, 18, 216-222.
- (21) Rosen, M. J. Surfactants and Interfacial Phenomena: John Wiley & Sons: New York, 1989.
- (22) Whitehouse, B. G. Mar. Chem. 1984, 14, 319-332.
- (23) DiToro, D. M.; Zarba, C. S.; Hansen, D. J.; Berry, W. J.; Swartz, R. C.; Cowan, C. E.; Pavlou, S. P.; Allen, H. E.; Thomas, N. A.; Paquin, P. R. Environ. Toxicol. Chem. 1991, 10, 1541-1583.
- (24) DiĜiulio, R. T.; Benson, W. H.; Sanders, B. M.; Van Veld, P. A. In Fundamentals of Aquatic Toxicology; Rand, G. M., Ed.; Taylor & Frances: New York, 1995; pp 523-561.
- (25) Stegeman, J. J. Mar. Environ. Res. 2000, 50, 61-62.
- (26) McElroy, A.; Leitch, K.; Fay, A. Mar. Environ. Res. 2000, 50, 33-38.
- (27) Tang, J.; Liste, H.-H.; Alexander, M. Chemosphere 2002, 48, 35-
- (28) Reid, B. J.; Stokes, J. D.; Jones, K. C.; Semple, K. T. Environ. Sci. Technol. 2000, 34, 3174-3179.
- Volkering, F.; Breure, A. M.; Rulkens, W. H. Biodegradation 1998, 8. 401-417.
- Loibner, A. P.; Gartner, M.; Schlegl, M.; Hautzenberger, I.; Braun, R. In In Situ and On Site Bioremediation; Leeson, A., Alleman, B. C., Eds.; Battelle Press: Columbus, OH, 1997; Vol. 4, no. 5, pp 617-622.
- (31) Stanek, E. J.; Calabrese, E. J.; Pekow, P. J.; Yeatts, K. B. J. Soil Contam. 1998, 7, 227-242.
- (32) Holman, H. N.; Goth-Goldstein, R.; Aston, D.; Yun, M.; Kengsoontra, J. Environ. Sci. Technol. 2002, 36, 1281–1286.
- (33) Oomen, A. G.; Sips, A. J.; Groten, J. P.; Sijm, D. T. H.; Tolls, J. Environ. Sci. Technol. 2000, 34, 297-303.
- (34) Hack, A.; Selenka, F. Toxicol. Lett. 1996, 88, 199-210.
- (35) Mizushima, H.; Matsuo, T.; Satoh, N.; Hoffmann, H.; Graebner, D. Langmuir 1999, 15, 6664-6670.
- (36) Folmer, B. M.; Nyden, M.; Holmberg, K. J. Colloid Interface Sci. **2001**, 242, 404-410.
- Small, D. M. In The Bile Acids; Nair, P. P., Kritchevsky, D., Eds.; Plenum Press: New York, 1971; Vol. 1, pp 249–356.
- (38) Vonk, H. J. Comp. Biochem. Physiol. 1969, 29, 361-371.
- (39) Lester, R.; Carey, M. C.; Little, J. M.; Dowd, S. R. Science 1975, 189, 1098-1100.
- (40) Goad, L. J. In Marine Natural Products: Chemical and Biological Perspectives; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol. 2, pp 75-172.
- (41) Jones, M. N. In Surfactant Activity of Proteins' Chemical and Physiochemical Modifications; Magdassi, G., Ed.; Marcel Dekker: New York, 1996; pp237–284. (42) Reynolds, J. A.; Tanford, C. *Proc. Natl. Acad. Sci. U.S.A.* **1970**,
- 66, 1002.
- (43) Place, A. R. Am. J. Physiol. 1992, 263 (Regulatory Integrative Comp. Physiol. 32), R464-R471.
- Voparil, I. M.; Mayer, L. M.; Place, A. R. Environ. Sci. Technol. **2003**. 37. 3117-3122.
- (45) Kile, D. E.; Chiou, C. T. Environ. Sci. Technol. 1989, 23, 832-838.

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