Title

Enhancing the utility of in vitro digestive fluid extraction as a management tool for contaminated aquatic sediments

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ABSTRACT

A technique has recently been proposed to assess the bioavailability of sediment-associated contaminants by in vitro incubation of the sediments in digestive fluids of a deposit-feeding organism. This procedure mimics the chemical environment to which a contaminated particle would be exposed as it passes through the gut, and is based on the presumption that the bioavailable contaminant fraction is that which is desorbable under these conditions. This study was intended to further explore some key assumptions and limitations of this procedure. With regards to use of the technique to measure trace metal bioavailability, it was found that the procedure does allow oxygenation of the gut fluid in comparison to the in vivo gut environment which is near anoxic. This oxygenation did influence solubilization of about half the trace metals tested, though the effect was usually too small to have an appreciable impact on risk assessment decisions. With regards to assessment of bioavailability of organic contaminants, there was an excellent correlation between the proportion of contaminant solubilized in vitro, and that judged to be bioavailable by other in vivo techniques using two deposit-feeding polychaetes. This relationship held not only for polycyclic aromatic hydrocarbons which had been previously studied, but for several other pesticides and chlorinated organic compounds. Moreover, one traditional measure of bioavailability (absorption efficiency as measured by a dual label technique) was shown to underestimate bioavailability due to complications caused by selective feeding. The digestive fluid technique showed considerable promise across a broader range of contaminants and substrate types than had been previously tested, and appears to be a simple and rapid means to assess bioavailability, and obtain information otherwise available only by using live animals and more lengthy and logistically difficult procedures.

INTRODUCTION AND PROBLEM STATEMENT

Sediments serve as a reservoir for many trace metals and organic pollutants of potential ecological concern. These same sediments also serve as a habitat and food source for deposit-feeding invertebrates including annelids, molluscs, crustaceans, and many other taxa. Deposit-feeding organisms may ingest several times their own body weight in sediments every day; thus ingestion is potentially a very important route for the bioaccumulation of particle-associated contaminants. However, the fact that a large fraction of sediment-associated contaminants are not bioavailable complicates efforts to assess potential bioaccumulation from sediments, and hence evaluate the ecological risks they pose. 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, and for trace metals the non-bioavailable fraction can be much greater. Standard chemical methods of sediment analysis are designed to extract all of the contaminant, and thus the resulting quantification is likely to drastically over-estimate the bioavailable contaminant fraction. Resulting management decisions, therefore, have a high potential for error.

Standard methods of chemical analysis involve an extraction step that is intended to extract all of the targeted contaminant from sediments using 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, digestive fluids), and thus are likely to overestimate the fraction of the contaminant that is bioavailable.

A new approach to assessment of the bioavailability of particle-associated contaminants employs the digestive fluid of deposit feeders to solubilize contaminants. 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 in order to be bioavailable. While 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 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.

OBJECTIVES

The research supported by WRC was intended to further develop the digestive fluid extraction procedure, and specifically to address some key assumptions of the technique that had not yet been carefully evaluated. The research was done and will be discussed here under three tasks, each with specific objectives:

Task 1 – To determine if the conditions under which the in vitro extraction is done, and particularly permitting oxygenation of the sample, adequately reflect in vivo gut conditions and contaminant solubilization.

Task 2 – To evaluate a novel method of measuring digestive desorption of contaminants and explore its utility and extent of agreement with traditional measures of absorption efficiency.

Task 3 - To test the digestive fluid extractions across a wider variety of contaminants and substrate types than had been previously utilized.

PROCEDURE

For this study and our past efforts, we have relied on the polychaete Arenicola brasiliensis as a source of digestive fluid, although conceptually, any large deposit-feeder would suffice. A. brasiliensis was collected intertidally, and then held in the lab for 24 hr to evacuate gut contents, thereby increasing recovery of digestive fluid. The animals were dissected to expose the digestive tract and then fluid withdrawn with a pipette through the wall of the midgut. We typically recovered an average of 1 ml per individual.

The test sediment was incubated in digestive fluid (0.5 g wet weight sediment to 0.8 ml fluid for most experiments, roughly approximating the in vivo ratio) with constant agitation for 1-2 hr. After completion of the extraction, the sample was centrifuged at 4000 g for 10 minutes, and the supernatant recovered for quantification of solubilized contaminant. Extraction efficiency (i.e. bioavailability) was expressed as the proportion of the initially sediment-associated contaminant that has been solubilized in the digestive fluid.

Task 1-

Typically, extraction of gut fluid from A. brasiliensis and the subsequent in vitro extraction of sediment has been done under a normal atmosphere simply for the sake of simplifying the procedure, though this does allow oxygenation of the fluid. In order to determine if this procedure affects trace metal solubilization by the fluid, half of the gut fluid was recovered under a nitrogen atmosphere, and the sediment incubations were also done under nitrogen. The relative extraction efficiencies of 11 trace metals from five sediments were evaluated under both conditions (oxic and anoxic). In addition, microelectrodes were used to measure pH, dissolved oxygen and redox potential within the gut of A. brasiliensis in order to determine whether handling of the gut fluid under a normal atmosphere altered its characteristics relative to those found in vivo.

Task 2 –

The polychaete A. brasiliensis was used as a source of digestive fluid for these experiments because of its large size (25 g in weight, 15 cm in length). This size also allows determination of in vivo contaminant desorption from sediments by dissection of the worm and recovery of gut contents along the length of the digestive tract. Comparison of contaminant concentration in foregut sediments (material recently ingested) to that in the rectum (material digested and soon to be defecated) allows us to determine what fraction of the contaminant was solubilized in vivo and available for absorption, and compare this value to the bioavailable fraction determined by in vitro gut fluid extraction. A. brasiliensis was allowed to feed on sediment that had been spiked with radiolabelled benzo(a)pyrene and zinc. In vitro solubilization of these contaminants was compared to in vivo solubilization (difference in contaminant concentration between foregut and rectum). In addition, absorption efficiency of contaminants in both species was measured by a widely-used dual label procedure using C-14 and Chromium-51, thus allowing us to both evaluate the traditional procedure.

Task 3 –

Digestive fluid extraction work has primarily been done with polycyclic aromatic hydrocarbons (PAH). This task included a PAH (benzo(a)pyrene (BaP)), but also expanded the technique to chlorinated organic compounds (DDT, hexachlorobiphenyl (HCBP)) an organophosphate pesticide (chlorpyrifos) and a pyrethroid pesticide (permethrin). In addition, the task explored extraction of a broader range of sediment types than done previously. Eleven sediments spanning a broad range of grain size and organic carbon content were spiked with the above compounds, using C-14 radiolabels. In vitro extractions of these sediments using A. brasiliensis gut fluid were done. In order to compare the in vitro bioavailability to in

vivo bioavailability, sediment desorption during digestion (foregut/rectum comparison method) was measured in A. brasiliensis feeding on the same sediments. In order to evaluate the effectiveness of the in vitro technique in predicting bioavailability to other taxa, the polychaete Nereis succinea was allowed to feed on these sediments, and dietary assimilation efficiency measured by pulse-chase procedures. In these procedures, absorption efficiency is parameterized as a ratio between assimilated compound and the total amount that passed through the gut (assimilated plus defecated).

RESULTS

Task 1-

In vivo microelectrode measurements showed that the gut fluid of A. brasiliensis had a near neutral pH, a redox potential of about +180 mV, and nearly no dissolved oxygen (<1% saturation). Dissection of the gut and recovery of gut fluid under a nitrogen atmosphere maintained these conditions, however, dissection under a normal atmosphere as is normally done, did elevate the dissolved oxygen concentration to 5-20% of saturation.

Introduction of oxygen into the gut fluid typically had no influence on the ability of the fluid to solubilize Cr, Hg, Se, Cd, Fe and Zn as the treatments under nitrogen and under a normal atmosphere yielded comparable metal concentrations in gut fluid after sediment extraction. Three metals (Pb, Ni, Cu) tended to be more efficiently extracted from sediment when the extraction was done under a normal atmosphere, while Mn and As were more efficiently extracted anaerobically. Even in those instances when differences existed, they were generally small (less than a factor of 2).

The extent of metal desorption from sediment during an in vitro digestive fluid extraction is an indicator of bioavailability and could be used as one element in considering ecological risk of that contaminated sediment. We considered the influence of oxygenation of gut fluid from a risk assessment perspective by asking whether the designation of a metal or a sediment as being of concern could be influenced by whether oxygenation of the gut fluid had been allowed during dissection and sediment extraction. In 87% of the cases tested, the differences between the aerobic and anaerobic treatments were either non-existent or too small to influence characterization of risk.

Task 2-

A common traditional measure of absorption efficiency relies upon a comparison of the ratio of the contaminant (in this case, BaP) to a conservative tracer (Cr) in the ambient sediment to the same ratio in fecal material. This technique produced estimates of contaminant absorption from 5 sediments ranging from 21-42% (Table 1). The large size of A. brasiliensis permits collection of recently ingested sediment from the foregut, providing a better estimate of material actually ingested, and avoiding complications created by selective feeding. Use of foregut sediments, rather than ambient sediments, to estimate absorption efficiency yielded estimates that were about 50% higher than the traditional technique, and ranged from

Table 1. Estimates of BaP absorption efficiency by A. brasiliensis calculated by the traditional ambient sediment to feces comparison, and by the more novel foregut to feces comparison. The proportion of contaminant solubilized by in vitro extraction also shown.

Sediment	Sediment to feces absorp. effic. (%)	Foregut to feces absorp. effic (%)	In vitro extraction (%)
BBF	21	38	59
BBC	35	44	67
HMB	25	35	27
DTM	23	38	56
RIC	42	--	49

35-44%. The underestimate of the traditional technique is due to the fact that ingested foregut material was more enriched in BaP, relative to the ambient sediment, than was Cr. A critical assumption of the technique is that the relative degree of BaP and Cr adsorption on any given particle is identical; i.e., the two labels are tracking the same particles. In this case, and we suspect most other past applications of the technique, the animal was ingesting particles more enriched in BaP but deficient in Cr than the sediment as a whole, violating the inherent assumption of the traditional technique.

In vitro solubilization estimates of bioavailability ranged from 27-67%, and were usually slightly higher than estimates of in vivo desorption made by foregut to rectal comparison.

Task 3-

Measurements of in vitro and vivo desorption (Arenicola) or assimilation (Nereis) showed that across the broad range of sediments used in these experiments, usually one-third to two-thirds of the sediment-associated contaminant can be solubilized under digestive conditions, and would be bioavailable (Table 2). The remainder of the contaminant remains adsorbed to the particle, and passes out of the animal via the feces without any toxicological consequence. There were three-fold differences among the contaminants in the extent of their desorption from particles. In vitro desorption was in the order of permethrin>chlorpyrifos>HCBP>DDT>BaP. In general, sediments with higher organic contents showed less in vitro desorption, as would be expected and as we have found in previous work. However, it was interesting that this relationship was not evident for in vivo assimilation suggesting other factors (e.g. type of carbon, feeding rate) may be important. Between the two species, there was no consistent difference in bioavailability across all sediments and contaminants.

The in vivo measurements used in these experiments are considerable more difficult than assessment of bioavailability by in vitro digestive fluid extraction. Moreover they are limited to particular species. For example, arenicolid polychaetes are among the few deposit feeders that are large enough to measure contaminant desorption by comparison of foregut and rectal contents, and pulse-chase procedures, as used for Nereis, only work for those species for which feces can be recovered

Table 2. In vitro gut fluid solubilization and in vivo measures of contaminant bioavailability for all sediments and contaminants tested.

Sediment	TOC (%)	Contaminant	In vitro solubilization (%)	In vivo A. brasiliensis desorption (%)	In vivo N. succinea assimilation (%)
DB	0.06	chlorpyrifos	61	35	76
		DDT	61	56	43
		HCBP	58	71	41
		BaP	19		17
		permethrin	87	67	
BB	0.08	chlorpyrifos	65	51	62
		DDT	58	65	53
		HCBP	61	83	26
		BaP	24		7
		permethrin	67	80	
RF	0.42	chlorpyrifos	53		
		DDT	35		46
		HCBP	42		30
		BaP	20		8
PP	0.43	chlorpyrifos	59	37	43
		DDT	35	8	32
		HCBP	37	31	16
		BaP	15		7
		permethrin	42	41	
TB	0.95	chlorpyrifos	66		
		DDT	42		35
		HCBP	63		24
		BaP	29		26
DM	1.3	chlorpyrifos	46		
		DDT	25		44
		HCBP	40		32
		BaP	20		18
PR	1.3	chlorpyrifos	42		
		DDT	19		39
		HCBP	28		41
		BaP	13		15
BL	1.72	chlorpyrifos	43		
		DDT	22		28
		HCBP	21		29
		BaP	23		16
U	2.08	chlorpyrifos	58		64
		DDT	31		60
		HCBP	41		52
		BaP			31
GG	2.12	chlorpyrifos	43		
		DDT	15		12
		HCBP	23		21
		BaP	33		45
LI	3.1	chlorpyrifos	30		46
		DDT	23		38
		HCBP	29		15
		BaP	25		10

quantitatively. Therefore it is encouraging that a brief in vitro extraction provides an estimate of the bioavailable contaminant that is very similar to the more difficult in vivo methods. In vitro solubilization in A. brasiliensis gut fluid was highly correlated with in vivo A. brasiliensis desorption measurements (Figure 1). This correlation was robust across the broad range of contaminants used in this work (benzo(a)pyrene, chlorpyrifos, permethrin, DDT, hexachlorobiphenyl), demonstrating the efficacy of the technique for many hydrophobic organic toxicants.

In addition, in vitro solubilization in A. brasiliensis gut fluid appeared to be a good predictor of bioavailability to another polychaete, N. succinea (Figure 2), in that the in vitro results showed excellent correlation with in vivo assimilation efficiency measurements.

CONCLUSIONS

Task 1, which examined the influence of gut fluid dissection and handling on trace metal solubilization, demonstrated that the typical procedures used for extraction create a far more oxygenated fluid than would be found in vivo in actively-feeding organisms. While this oxygenation can have some influence on the ability of the fluid to solubilize some trace metals (Pb, Ni, Cu, Mn, As), the effect is generally small and rarely would change characterization of sediment risk. Obtaining anoxic gut fluid to accurately represent in vivo conditions, while possible, is logistically difficult, and would severely limit adoption of the gut fluid solbuilization technique for assessment of contaminant bioavailability. This work has shown that the extra effort is seldom worthwhile, and extraction under a normal atmosphere as has been practiced in the past is an acceptable approach.

Task 2 illustrated the short-comings of the traditional dual label technique to measure absorption efficiency in deposit feeders. Selective feeding by Arenicola violated a key assumption of the technique, and since A. brasiliensis is relatively non-selective in comparison to other deposit-feeding invertebrates, we suspect the problems with the approach are even more significant for other species. Assessment of contaminant availability by comparison of foregut to rectal contents is a preferable procedure for those species large enough to provide sufficient gut contents.

Task 3 was particularly important in demonstrating the applicability of the in vitro digestive fluid technique to a diversity of hydrophobic organic contaminants and across a broad range of sediment organic contents. It was encouraging that the in vitro technique successfully predicted bioavailability not only to A. brasiliensis, the species from which the gut fluid was obtained, but to another deposit-feeding polychaete.

As a result of these studies, many key questions about the in vitro technique have been answered, and data are now available to address its utility and limitations. It appears to be useful as a tool for simple and rapid assessment of contaminant bioavailability from sediments, and provides results applicable to deposit feeders in general. As a result of these studies, the U.S. Army Corps of Engineers has shown interest in using the technique for the assessment of dredged material. We have received $430,000 in funding to pursue this application, and particularly to develop a

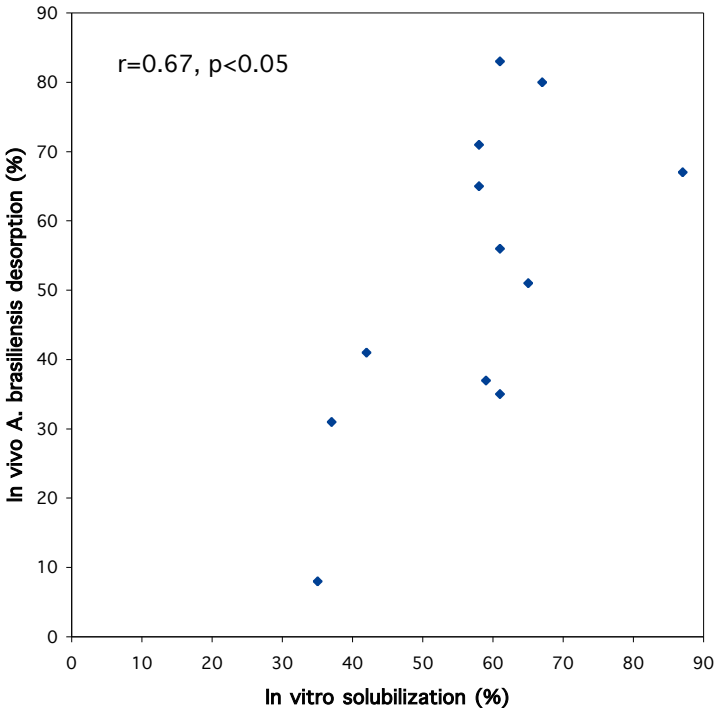

Figure 1. In vitro solubilization of chlorpyrifos, DDT, HCBP and permethrin in comparison to in vivo desorption of the same compounds from the same sediments by A. brasiliensis.

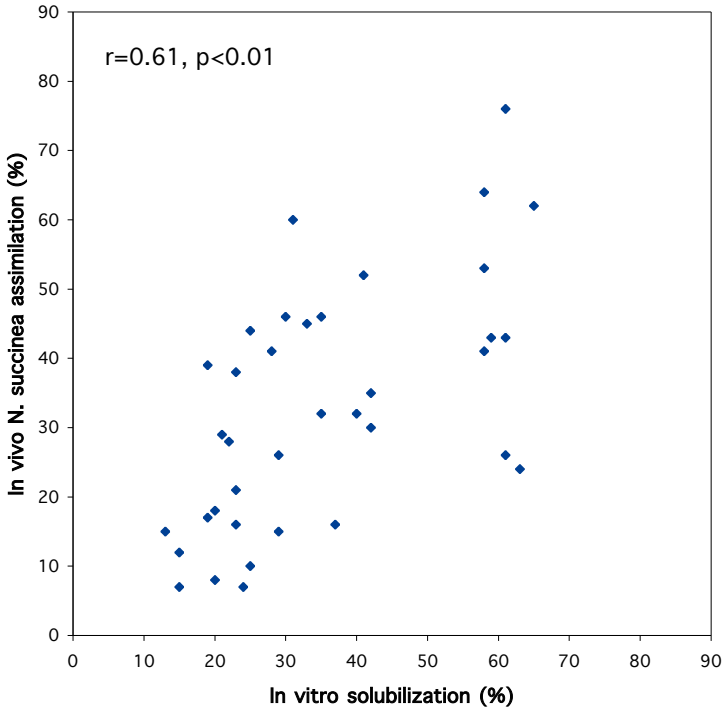

Figure 2. In vitro solubilization of chlorpyrifos, DDT, HCBP and BaP in comparison to in vivo assimilation of the same compounds from the same sediments by N. succinea.

synthetic fluid that mechanistically extracts contaminants as does gut fluid, but would avoid the difficulty of collecting actual polychaete digestive fluid. This work is now underway, and synthetic fluids for both organic and trace metal contaminants are in testing.

LIST OF PUBLICATIONS

A publication based on Task 1 entitled "The effects of extraction conditions on trace metal solubilization in deposit feeder digestive fluid" is in review in Environmental Toxicology and Chemistry. Two additional manuscripts (one on each of Tasks 2 and 3) are in preparation and should be submitted by the end of summer, 2003.