Environ. Sci. Technol. **2004**, *38,* 4549−4556

Effects of Particulate Carbonaceous Matter on the Bioavailability of Benzo[*a*]pyrene and 2,2′,5,5′-Tetrachlorobiphenyl to the Clam, *Macoma balthica*

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We investigated the bioavailability via diet of spiked benzo[*a*]pyrene (BaP) and 2,2′,5,5′-tetrachlorobiphenyl (PCB-52) from different carbonaceous (non-carbonate, carbon containing) particle types to clams (*Macoma balthica*) collected from San Francisco Bay. Our results reveal significant differences in absorption efficiency between compounds and among carbonaceous particle types. Absorption efficiency for PCB-52 was always greater than that for BaP bound to a given particle type. Among particles, absorption efficiency was highest from wood and diatoms and lowest from activated carbon. Large differences in absorption efficiency could not be simply explained by comparatively small differences in the particles' total organic carbon content. BaP and PCB-52 bound to activated carbon exhibited less than 2% absorption efficiency and were up to 60 times less available to clams than the same contaminants associated with other types of carbonaceous matter. These results suggest that variations in the amount and type of sediment particulate carbonaceous matter, whether naturally occurring or added as an amendment, will have a strong influence on the bioavailability of hydrophobic organic contaminants. This has important implications for environmental risk assessment, sediment management, and development of novel remediation techniques.

Introduction

For decades, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) have been prioritized as contaminants of concern in sediment. A 2001 U.S. EPA survey found that among Superfund sites, PCBs and metals were the most common contaminants driving sediment environmental risk, followed by PAHs and pesticides (*1*). Despite years of research, experts still disagree on the best remediation strategies for PCB- and PAH-contaminated sediments. This disagreement stems from the complex interactions of PCBs and PAHs with sediment and the poorly understood effects of these interactions on contaminant bioavailability to benthic organisms at the base of the food web. Limited understanding of the ways in which sediment biogeochemistry governs PCB and PAH bioavailability and fate hinders the development of protective sediment quality guidelines and innovative remedial measures for contaminated sites.

PCBs and PAHs preferentially associate with carbonaceous (non-carbonate carbon containing) matter in sediment, from which they can (re)partition into overlying water and enter the food web. Sediment quality guidelines attempt to address bioavailability by considering a generic organic matter (OM) pool that sorbs the contaminants to an equal degree, based on equilibrium partitioning (*2*, *3*). Output from this organic matter partitioning (OMP) model is used to judge the risk to water quality and animal exposure. Many researchers have documented limitations of the OMP model in accurately predicting the solid−water distribution of nonionic hydrophobic organic contaminants (e.g., refs *4*−*7*). In practice, the OMP model overpredicts aqueous PAH and PCB concentrations in equilibrium with a variety of field sediment samples (*4*−*7*). McGroddy et al. (*5*) found that the OMP model worked well for PCBs but overestimated concentrations of phenanthrene and pyrene by as much as 250 and 30 times, respectively. To the extent that pore water governs bioavailability, the OMP model cannot dependably predict risk to the food web from a given PCB or PAH sediment concentration.

Sediment is heterogeneous at grain and subgrain scales (*8*). The limitations to the OMP model partly arise from that heterogeneity. Specifically, different particle types within carbonaceous matter can exhibit vastly different sorptive affinities for PAHs and PCBs. For example, existing data for phenanthrene reveal orders of magnitude differences in observed partition coefficients for a suite of particle types (ref *9* and references therein). It is increasingly accepted that the OMP model should be replaced by a combination of linear and nonlinear models to better describe contaminant sorption isotherms in sediment (*10*). Although researchers use different nomenclature, most of the devised schemes consider linear absorption and nonlinear adsorption as the dominant processes (ref *10* and references therein). Some researchers propose that the new modeling paradigm consider amorphous organic carbon and soot carbon as separate sorbing matrixes in sediment (*11*−*13*). This idea is supported by research that documents the "supersorbent" capacity of the soot fraction and the common occurrence of soot and soot-like particles in sediment samples (*7*). Accardi-Dey and Gschwend (*13*) showed that a two-compartment model comprising soot carbon and amorphous organic matter could predict sediment−porewater distribution coefficients previously observed for Boston Harbor sediment (*4*). In contrast, Jonker and Koelmans (*14*) recently found that normalization to organic and soot carbon fractions did not accurately describe PAH or PCB sorption to a suite of soot and soot-like materials. This suggests that even a two-compartment equilibrium model may oversimplify contaminant sorption in some sediment.

It is imperative to understand how sorption to different particle types might influence the overall bioavailability of PAHs and PCBs in sediment; but only limited work exists. Ghosh et al. (*9*) showed that PCBs and PAHs in three

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10.1021/es049893b CCC: $27.50
Published on Web 07/24/2004

contaminated harbor sediments were predominantly associated with certain types of carbonaceous matter in sediment, including coal, tar, and cenophores. They found that PAHs were more bioavailable for aerobic bioslurry degradation when sorbed to semi-soft tar pitch than when associated with coal-derived particles. Talley et al. (*15*) employed bioslurry studies, earthworm uptake, and microscale particle characterization in tandem to conclude that the bioavailability of PAHs in sediment is directly related to differences in the form of carbonaceous matter to which PAHs are bound.

Especially little is known about the influence of differential sorption on dietary exposure of PAHs and PCBs, although particle ingestion can be an important exposure route for both chemicals (*16*). It is logical to hypothesize that enhanced contaminant sorption to certain types of carbonaceous particles will result in lower absorption efficiencies in the gut, but this has not been directly investigated. It is possible, for example, that the gut fluid in organisms is a strong extractor of PCBs and PAHs (e.g., refs *17* and *18*); that benthos select against the contaminant-laden particles; or that some of the carbonaceous particles themselves can be digested by benthic organisms, leading to increased contaminant availability.

The objective of this research is to increase our understanding of PCB and PAH sediment biogeochemistry by determining the effect of contaminant association with different forms of particulate carbonaceous matter on bioavailability via diet. The particles tested include forms of carbonaceous matter with widely varying composition and structure (such as wood, coke, char, and anthracite) and are commonly found in sediments. We conducted feeding experiments that measured the absorption efficiency for spiked benzo[*a*]pyrene (BaP) and 2,2′,5,5′-tetrachlorobiphenyl (PCB-52) to clams (*Macoma balthica*) from six different particle types and diatoms, a usual form of food for these clams. Results from our experiments suggest that the bioavailability of PCBs and PAHs is highly dependent upon the type of carbonaceous matter to which the contaminants are bound.

Experimental Section

Test Organism. The clam *Macoma balthica* was chosen because of its uniform presence in the intertidal mudflats of San Francisco Bay, the amount of existing data describing the feeding behavior of *M. balthica*, and the relative ease of handling and maintaining *M. balthica* under laboratory conditions. It is usually a deposit feeder, but is also capable of suspension feeding, and typically ingests sediment particles and algae smaller than 100 μm in diameter (*19*). Gut residence time and other aspects of feeding behavior influential in absorption are also well-known (*19*, *20*). Two days prior to each experiment, 60−80 *M. balthica* were collected by hand from an estuarine, intertidal mudflat at the Palo Alto Baylands Nature Reserve in south San Francisco Bay (station 6 in ref *20*). Total PAH and PCB concentrations at the site were measured as 1.59 ± 0.15 ppm and 14.7 ± 1.5 ppb, respectively (unpublished data), both representing background concentrations for San Francisco Bay. Pereira et al. found PAH concentrations in relatively noncontaminated parts of the Bay ranged from 0.04 to 6.3 ppm (*21*), and cores taken in the same areas by Venkatesan et al. revealed PCB concentrations <1−34 ppb (*22*).

Clams were rinsed in the field, transported to the laboratory in Bay water, and maintained without feeding in aerated 17 ppt seawater at 13 °C. This salinity and temperature were chosen to mimic field conditions and minimize stress to the organisms. The clam shells were brushed 1 d before the start of an experiment to remove loose periostracum and particles adhering to the shell. All clams used in experiments were similar in size (21−24 mm shell length) to minimize effects of size and age on contaminant uptake. Seawater was obtained from Long Marine Facility (University of California at Santa Cruz), where the water is collected via pipeline, sand-filtered, and pumped into storage tanks. Prior to use, the seawater was additionally filtered (0.45 μm), diluted to 17 ppt with Milli-Q water, and stored at 13 °C.

Particle Preparation. The test particles were coke, anthracite, wood, char, peat, and activated carbon. Coke was obtained as 1−2 mm coke breeze from Ispat Inland Inc. (East Chicago, IL). Anthracite (1−4 mm) was obtained from George L. Throop Co. (Pasadena, CA). Coke and anthracite were ground in a ball mill to produce smaller particles. Wood particles were prepared by sanding white pine (Palo Alto Hardware, Palo Alto, CA). Char was produced at the Johns Hopkins University (Baltimore, MD) in a retort furnace under a nitrogen atmosphere at 650 °C/h to 525 °C. It was then crushed using a mortar and pestle. Peat was purchased as Pahokee Peat Soil from the International Humic Substances Society (St. Paul, MN). Fine-mesh activated carbon (TOG 50 × 200) was purchased from Calgon Carbon Corporation (Pittsburgh, PA) and crushed using a mortar and pestle.

Each particle type was wet sieved to obtain particles with 20−25 μm diameters. This size fraction was chosen to provide the test organisms with a relatively uniform particle size within the range clams typically ingest (*19*). The particles were dried overnight (100−150 °C), transferred into 20-mL vials, and stored at room temperature until use. Specific surface area (SSA) was determined by Micromeritics, Inc. (Norcross, GA) by adsorbing N_2 at liquid nitrogen temperatures using the BET method. SSA for char was measured at the Johns Hopkins University. Total organic carbon (TOC) was measured at Huffman Laboratories (Denver, CO) by combustion of acidified samples. Particle characteristics are summarized in Table 1.

Diatom Preparation. The diatom *Phaeodactylum tricornutum* was obtained from SUNY Stonybrook (Stonybrook, NY) and served as food supply for *M. balthica*. *P. tricornutum* cultures were kept in Guillard's nutrient medium (*23*) in 0.45 μm-filtered 35 ppt seawater and grown with 10:14 light:dark for 10 d under artificial light. The cultures were suspended by hand, shaking twice daily. Cell density was measured using a hemocytometer.

Labeling of Particles and Diatoms with [^{3}H]-BaP and [^{14}C]-PCB-52. [^{3}H]-BaP (sp. act. = 76.0 Ci/mmol, Amersham, Piscataway, NJ) and 2,2′,5,5′-[^{14}C]-PCB (sp. act. = 14.4 mCi/mmol, Sigma Chemical, St. Louis, MO) were transferred into amber vials with hexane upon receipt and stored at 4 °C until use. The specific activity of BaP was reduced to 76.0 mCi/mmol by adding nonradioactive BaP (Aldrich, St. Louis, MO) to stock solutions.

Particles were simultaneously spiked with [^{3}H]-BaP and [^{14}C]-PCB-52. Both compounds were added in hexane to glass vials and evaporated to dryness by gentle hand swirling, leaving the compounds adhered to the vial walls. Particles were added to individual vials as suspensions (25 mg of char; 50 mg for all other particles) in 10 mL deionized water. Spike vials were sealed with Teflon septa, covered in aluminum foil to prevent photodegradation, and mixed on a rotator for 21 d. Ten-day-old diatom cultures were spiked similarly but were added as suspensions in 35 ppt seawater. Diatom cultures were further grown at 10:14 light:dark for 7 d after spiking and were suspended by hand-shaking twice daily. Some photodegradation of BaP could have occurred during this step. Unlabeled diatoms for use as cold feed were maintained under the same conditions. Table 1 reports the initial contaminant concentration on the particles and diatoms as well as the initial activity present at the start of the pulse−chase experiments.

TABLE 1. Fraction Organic Carbon (f_{OC}), Specific Surface Area (SSA), and Initial BaP and PCB-52 Concentrations for Particles in Experiments I−III

	f_{OC}	SSA, m^2/g	initial concn, $\mu g/g$		initial activity $\times 10^{-6}$, dpm[a]	
			BaP	PCB-52	^3H	^{14}C
activated C	0.87	1032	564 ± 128	801 ± 216	17.4 ± 4.0	4.12 ± 1.11
coke	0.901	3.2	615 ± 45	727 ± 42	16.2 ± 1.2	3.18 ± 0.19
peat	0.435	0.8	864 ± 16	1013 ± 18	26.7 ± 0.5	5.21 ± 0.09
anthracite	0.797	4.2	628 ± 39	659 ± 51	12.4 ± 0.7	2.16 ± 0.17
char	0.792	84	701 ± 69	492 ± 55	10.6 ± 1.0	1.24 ± 0.14
wood 1	0.464	1.5	561 ± 69	785 ± 46	14.8 ± 1.8	3.44 ± 0.20
wood 2	0.464	1.5	561 ± 40	870 ± 39	17.4 ± 1.3	4.47 ± 0.20
diatoms 1	0.25[b]		1.7 ± 0.4	3.5 ± 0.6	1.02 ± 0.21	0.35 ± 0.06
diatoms 2	0.25[b]		3.0 ± 0.6	9.7 ± 0.9	1.85 ± 0.39	1.00 ± 0.09
diatoms 3	0.25[b]		4.9 ± 0.9	15 ± 0.5	3.22 ± 0.53	1.48 ± 0.05

[a] dpm = disintegrations per minute; initial activity on particles in feeding beaker. [b] *Phaeodactylum* f_{OC} as reported in ref *30*.

Pulse−Chase Experiments. We adapted the pulse−chase method used in previous studies of metal uptake by *M. balthica* (*24*, *25*) to determine clam absorption efficiencies of [^{3}H]-BaP and [^{14}C]-PCB-52 from ingested particulate materials and water. Glass beakers were used to minimize sorption of organic compounds to the feeding apparatus. The absorption efficiencies were tested with groups of clams in three separate experiments: experiment I (diatoms, wood, anthracite, coke), experiment II (diatoms, wood, char, peat), and experiment III (diatoms, activated carbon). All experiments were performed in a controlled-temperature room at 13 °C.

Clams were randomly divided into groups of 13 per treatment. Each clam was attached with Krazy Glue (Columbus, OH) to a glass rod, such that clams were at the bottom of the rod when held vertically, and siphons were oriented upward. Rods were pre-roughened with sandpaper to promote strong attachment. After drying in air for 15 min, each group of 13 rod-mounted clams were suspended from a stainless steel mesh designed to sit atop a 1-L feeding beaker. Rods were secured with O-rings to allow vertical adjustment in the water column. Clams suspended from the mesh were allowed to acclimate for 1 h in a 1-L beaker with 500 mL of 17 ppt seawater immediately prior to the hot feed.

Spiked particles or diatoms were collected on a Nuclepore filter to separate particulate from dissolved compounds in the spiking water. The particles were introduced into the feeding water by agitating the filter in the beaker, containing 500 mL of 17 ppt seawater. A total of 25 mL of unlabeled diatom culture was added to each feeding beaker to stimulate clam feeding. The treatment with spiked diatoms did not receive unlabeled diatoms. Particles and diatoms were kept in suspension using a magnetic stirrer.

Clams were introduced to the feeding beakers and allowed to feed for 8 h. Decho and Luoma (*19*) determined the minimum gut passage time, defined by the first appearance of radiolabel in feces, for *M. balthica* to be 9.6 ± 1.8 h, with a range of 3−16 h. Thus, some clams may have produced feces during our exposure period. However, we chose this feeding time to ensure all clams fed, which was shown in preliminary tests to decrease variability in clam absorption efficiency as compared to hot feeds of shorter duration.

After the hot feed, clams were removed and rinsed. Ten of the clams from each treatment were placed in unlabeled 17 ppt seawater in individual 50-mL depuration beakers with unlabeled diatoms as feed. This allowed for separate feces collection for each clam, which greatly reduced the variability in calculated absorption efficiency as compared to tests in which feces from several clams were pooled and analyzed together. Clams were depurated for 88 h. In studies with ^{51}Cr inert beads, *M. balthica* egested >95% of unabsorbed radioactivity in 72−96 h (*19*). In our experiments, contaminants in the clam tissue after 88 h of depuration were considered absorbed. Feces were collected on glass fiber filters at 14, 26, 40, 64, and 88 h and analyzed for [^{3}H]-BaP and [^{14}C]-PCB-52. An individual clam's feces from different sampling times were sometimes combined to ensure measurable radioactivity. In preliminary tests, negligible contaminant concentrations were detected in water samples taken from the depuration beakers. After each feces collection, the depuration water was refreshed, and more diatoms were added to remove metabolic waste and encourage digestion and depuration.

Following depuration, the soft tissues were separated from the shells, and shell length and wet weights were recorded. Glass fiber filters placed under the clams during dissection to capture internal fluids were also analyzed. Compounds were found to adsorb to clamshells in preliminary tests, but shells were not analyzed in these experiments because shell-sorbed contaminants do not directly contribute to soft tissue absorption efficiency.

Estimating Partition Coefficients and Mass Balances. In experiment III, additional samples were removed from the feeding beaker before and after feeding to characterize particle−water partitioning in our systems. Some samples were filtered on a glass fiber filter, and the filter and filtrate were analyzed. The sum of the radioactivity from the filter and filtrate was operationally defined as the system total. Other samples were centrifuged (Beckman L8-6000 Ultracentrifuge), and the supernatant was analyzed to determine particle-free aqueous concentration. These data were used to estimate potential aqueous uptake and particle−water partition coefficients (K_d) for the activated carbon and diatom systems.

Mass balances were performed by comparing the activity on particles in the feeding beaker at the start of the hot feed to the sum of the activity in particles remaining in the beaker at the end of the hot feed, clam tissues, clam feces, and methanol and hexane rinses of the feeding beaker. This approach assumes negligible dissolved contaminant concentrations and is therefore most appropriate for the activated carbon system. In the activated carbon test, 106.2% of the BaP and 105.5% of the PCB-52 were recovered.

Sample Digestion and Extraction. Feces samples were digested in 2.8 mL of ScintiGest (Fisher Scientific, Pittsburgh, PA), 370 μL of concentrated HCl, and 12 mL of Scinti-Safe Plus 50% Cocktail (Fisher Scientific, Pittsburgh, PA). Samples were vortexed once daily for 5 d. Whole soft tissue of each clam was chopped and digested in 2.8 mL of ScintiGest overnight in a 52 °C oven. Samples were periodically vortexed (totaling 1 min) to aid digestion. A 0.6-mL subsample of digested tissue was transferred to a 20-mL scintillation vial

with 80 μL of HCl and 14 mL of scintillation cocktail. Subsamples were analyzed instead of whole tissue to minimize tissue matrix effects and attain high counting efficiency. To ensure representative sampling, we performed a tissue homogeneity test in which we fed clams following the pulse−chase protocol and counted subsamples from pooled, digested tissue. Coefficients of variation among samples were less than or equal to 0.01 for both compounds, verifying the homogeneity of tissue subsamples.

The digestion and extraction technique described above was chosen after comparing the ability of several methods to recover [^{3}H]-BaP and [^{14}C]-PCB-52 from spiked coke particles prepared using the above methods and equilibrated for 21 d. The extraction methods tested were different combinations of ScintiGest tissue solubilizer, scintillation cocktail, and HCl. Particles were combusted at 750 °C in a carbon combustion oven, and gases were captured in three 1 N NaOH traps in series. Samples from each trap were analyzed and added. Combustion was assumed to recover 100% of the compounds from the particles. Measured combustion recoveries for [^{3}H]-BaP and [^{14}C]-PCB-52 standards in hexane were 96−101%. The extraction method used in our experiments recovered 95% ± 2% of [^{3}H]-BaP and 101% ± 3% [^{14}C]-PCB-52 as compared to combustion.

Radiolabel Analysis. Radioactivity was determined by liquid scintillation counting (model 6500, Beckman Instruments, Fullerton, CA) using a dual-label (^{3}H and ^{14}C) program. Tissue and feces samples produced a yellow-amber color in scintillation cocktail that interfered with counting efficiency. Thus, a quench curve for colored samples was established using solubilized clam tissue as quenching agent. Recoveries for [^{3}H]-BaP and [^{14}C]-PCB-52 in colored samples were 100.02% ± 0.96 and 100.57% ± 0.96, respectively. All tissue and feces samples were corrected for quench using this curve. Noncolored samples were corrected for quench using the manufacturer's quench curve. Background was subtracted from all samples. Since the ^{3}H and ^{14}C counting windows overlap, we also performed a ^{3}H:^{14}C ratio test in colored (tissue) and noncolored matrixes. Varying the ratio had no significant effects on isotope recovery.

Absorption Efficiency. Absorption efficiency (AE) was calculated for each clam using measured tissue and cumulative feces activities and a mass balance approach:

$$\% \text{ AE} = \frac{a_{\text{tissue}}}{a_{\text{tissue}} + \sum a_{\text{feces}}} \times 100 \qquad (1)$$

where a_{tissue} is the activity in *M. balthica* soft tissue after 88 h of depuration and Σa_{feces} is the sum of all activity in feces collected during depuration. This method assumes negligible losses of BaP and PCB-52 to depuration water, which was confirmed in preliminary studies. No attempt was made to correct for absorption of contaminants by the clams from the water during the feeding period.

Researchers have used different terms to describe contaminant uptake efficiency in organisms from sediment (*26*−*28*). In this paper, we use the term "absorption efficiency" to describe the proportion of organic contaminant physiologically incorporated into soft tissues after ingestion and depuration (*19*, *24*, *25*). In some treatments, the absorption efficiency values included uptake from the aqueous phase. As discussed later, the weak sorption of BaP and PCB-52 to diatoms and wood resulted in a fraction of the spiked compound partitioning into water during the feeding experiment. We used a model calculation to evaluate how such a bias might have affected interpretations.

Results and Discussion

Absorption Efficiency. Absorption efficiency results for BaP and PCB-52 from experiments I−III are presented in Figure 1A−C. We observed clear differences in absorption efficiency between compounds and among particle types, even though the sorbents were equilibrated with spiked chemicals for short times. Clam absorption efficiency for PCB-52 was always higher than that for BaP for a given particle type, from all sorbents tested. The inter-compound difference is significant for all particles except activated carbon. Diatoms were tested in all three experiments to assess the replicability and seasonality of the data. The absorption efficiency for both compounds was always highest from diatoms. Mean absorption efficiencies for BaP from diatoms in experiments I−III were 35.2%, 50.7%, and 37.4%. Mean absorption efficiencies for PCB-52 were 89.9%, 86.3%, and 83.3%. The small inter-experiment range of absorption efficiency for both compounds supports the replicable nature of the experiment and allows us to directly compare results from experiments I−III. Some photodegration of BaP during diatom spiking

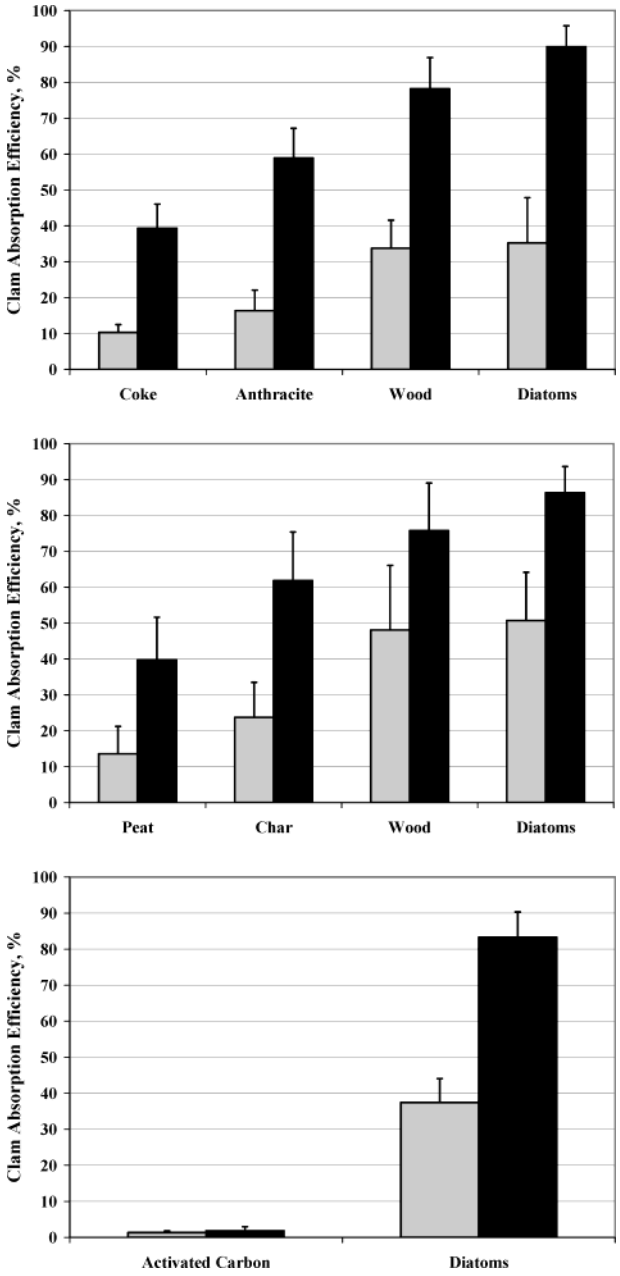

FIGURE 1. Absorption efficiency of BaP and PCB-52 varies significantly among different particle types, as shown here for experiments I−III. Light columns represent BaP; dark columns represent PCB-52. Error bars represent 95% confidence intervals.

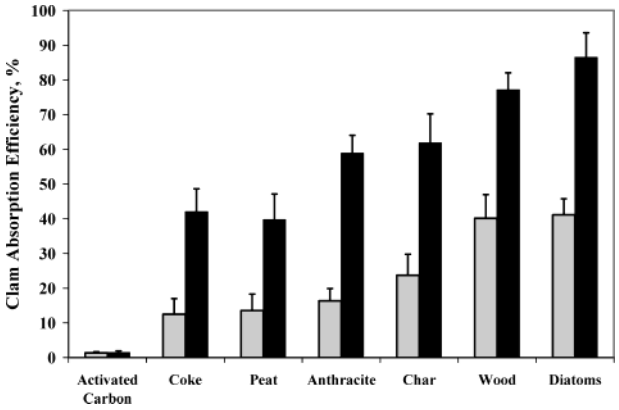

FIGURE 2. Absorption efficiency results for all particles in experiments I−III. Light columns represent BaP; dark columns represent PCB-52. Error bars show 95% confidence intervals. For the particles tested, absorption efficiency is lowest from activated carbon and greatest from wood and diatoms.

could have contributed to the greater variability in the BaP absorption efficiency from diatoms versus PCB-52.

Figure 2 presents the entire data set from experiments I−III. For diatoms and wood, which were tested in more than one experiment, the mean absorption efficiency was determined as the average of all available data points. Significant differences in absorption efficiency are seen between particle types, with 30- and 60-fold differences between activated carbon and diatoms for BaP and PCB-52, respectively. Statistical analyses using ANOVA suggest the particles may be divided into four groups based on differences in absorption efficiency ($p < 0.05$), listed in order of increasing associated contaminant bioavailability: (i) activated carbon; (ii) coke and peat; (iii) anthracite and char; (iv) wood and diatoms. Combining the data reiterates that contaminant bioavailability is strongly dependent upon the type of particle to which the contaminant is bound.

The differences in absorption efficiency we observed among particles may stem from different contaminant−particle sorption interactions, although this was not explicitly tested. Wood and diatoms would be classified as identifiable material of biogenic origin following the scheme presented by Allen-King et al. (*10*). Activated carbon, coke, anthracite, and char represent thermally altered carbonaceous matter. Sorption to wood is expected to be weaker than to particles representing thermally altered carbonaceous matter (*10*), so the high absorption efficiency from wood is not surprising. Karapanagioti et al. (*29*) observed that the Pahokee peat used in these experiments contains a number of "opaque particles" (charcoal and other oxidized woody tissues identified through petrographic analysis) that impart higher sorptive character than would be expected from a peat composed exclusively of humic materials. This may explain the low absorption efficiencies from peat measured in our experiment. Additionally, it is not surprising that activated carbon showed the lowest observed absorption efficiency, given its highly sorptive tendency, a quality that has been exploited in the water treatment sector for decades. Further investigations of the sorptive differences among coke, anthracite, and char may be required for a more complete mechanistic understanding of the relationship between sorption to particles and absorption efficiencies from these particles, although this does not appear to be the only mechanism involved, as discussed later.

Dietary absorption efficiencies of BaP and PCB-52 are reported elsewhere. Wang and Chow (*30*) used a mass balance approach to determine the absorption efficiency for BaP from *P. tricornutum* to green mussels (*Perna viridis*) as 37.2 ± 4.3%. The range of BaP absorption from *P. tricornutum* by *M. balthica* reported herein are consistent with their (*30*) prior findings. Kukkonen and Landrum (*28*) used a dual tracer technique and found that the absorption efficiencies for BaP from sediment to an amphipod (*Diporeia* spp.) and oligochaete (*Lumbriculus variegates*) were 45% and 23−26%, respectively. Our results are within this general range as well.

Higher absorption efficiency for PCB-52 than BaP is observed in some but not all studies elsewhere. In tests with *Diporeia*, for example, Harkey et al. (*31*) found that BaP was bioaccumulated less than 2,2′,4,4′,5,5′-hexachlorobiphenyl, even though the two have comparable octanol−water partition coefficients. Bott and Standley (*32*) report absorption efficiency of BaP to the oligochaete *L. variegates*, ranging from 23 to 75% for bacteria, algae, and sediment, when absorption efficiency values could be calculated. For PCB-52, they measured absorption efficiencies ranging from 21 to 87%. In specific comparisons, BaP uptake surpassed PCB-52 uptake by the oligochaete. In chironomid larvae, by contrast, the trend was reversed. Researchers studying HOC sorption to natural materials have observed the tendency of PAHs to partition more than other relatively nonpolar solutes of similar hydrophobicity, including PCBs (*5*). It is logical that stronger sorption might be related to lower bioavailability, but apparently this is not true for all species. Differences among species in gut fluids, digestive processing, and microscale food choices could confound such simple relationships.

Other researchers suggest that food quality influences organic contaminant bioavailability. For example, Gunnarsson et al. (*33*) report uptake of 3,3′,4,4′-tetrachlorobiphenyl to brittle stars varied 5-fold depending on the nutritional value of the sorbent. It was most bioavailable when associated with labile TOC-like green macroalgae and least when associated with lignin of terrestrial origin. It is possible that nutritional value may influence the consistently high absorption efficiency of PCB-52 and BaP from diatoms observed in our experiments. In contrast, there were large differences in absorption efficiency among wood, coke, and coal despite their undoubtedly low nutritional value.

Assessing Aqueous Uptake during Feeding. As previously mentioned, a portion of BaP and PCB-52 repartitioned into the aqueous phase in the wood and diatom systems. In experiment III, we measured aqueous contaminant concentrations at the beginning and end of the feeding period to assess the degree of repartitioning in the two extreme cases: activated carbon and diatoms. In the activated carbon system, virtually all (>99%) of the BaP and PCB-52 remained sorbed to the carbon particles throughout the feeding experiment. Calculated logarithmic distribution coefficients (K_d, $L/kg_{sorbent}$) ranged from 6.3 to 6.7 for both compounds. These values are 2−3 orders of magnitude lower than those reported by Jonker and Koelmans (*14*). These authors used solid-phase extraction with polyoxymethylene (POM-SPE) to assess very low aqueous concentrations and observed some of the highest partition coefficients to activated carbon ever reported in the literature, by up to 2.5 log units. As these authors suggest, phase separations using centrifugation, as in our experiment, could leave suspended colloidal activated carbon particles in the aqueous phase, leading to an underestimation of partitioning coefficients. Our purpose here is not to precisely determine K_d but to confirm that partitioning in the activated carbon system led to very low aqueous concentrations.

In the diatom system, a significant fraction of both compounds desorbed and was associated with the aqueous phase. At the beginning of the feeding period, 81% of BaP and 55% of PCB-52 in the system were bound to the diatoms. By the end of the feeding period, the percentage of sorbed contaminants declined to 70% for BaP and 45% for PCB-52. Calculated log K_d values at the beginning and end of the

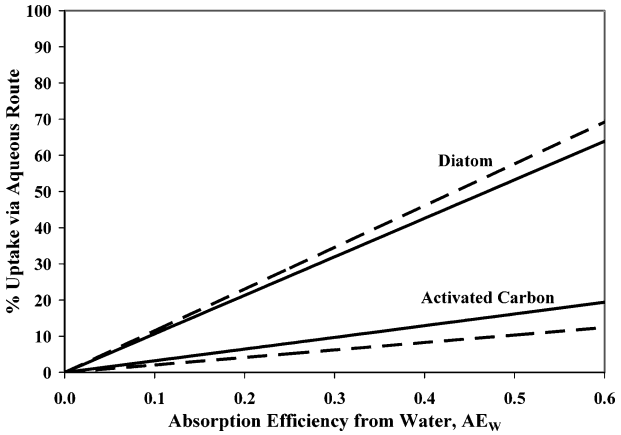

FIGURE 3. Estimated importance of aqueous uptake in activated carbon and diatom feeding tests for a range of assumed absorption efficiencies from water, AE_W. Solid lines represent BaP; dashed lines represent PCB-52.

experiment were approximately 3.4 for BaP and 3.0 for PCB-52, several orders of magnitude below the octanol−water partitioning coefficients for these compounds. The clams would have filtered all the water in the exposure vessel once over the 8-h exposure period, at an average filtration rate of 1.0 L/(g of dry tissue/d) (*24*). Given the partitioning to the aqueous phase and the ventilation rate, it is probable that the clams received a portion of their contaminant exposure through the aqueous route.

We estimated potential uptake of BaP and PCB-52 from the activated carbon and diatom systems using the aqueous contaminant concentrations measured in experiment III, ventilation parameter values from Luoma et al. (*24*), and a range of absorption efficiency from the aqueous phase (AE_w = 0−0.6). Wang and Chow (*30*) calculated AE_W for BaP to the green mussel *Pe. viridis* as 6.6−8.8%, and Bjork and Gilek (*34*) found that PCB AE_W to *Mytilus edilus* varied between 10 and 60%, with that for PCB-49 (a tetrachlorobiphenyl similar to PCB-52) in the range 10−40%. On the basis of these studies and because the clams clearly did not remove all of the radiolabeled contaminant from solution, a maximum AE_w of 0.6 was chosen. This still conservatively constrains the assumption that all uptake was the result of absorption from food. Equation 2, derived from the physiologically based bioaccumulation model in Landrum et al. (*35*), was used to estimate contaminant concentration in soft tissues after depuration (C_{clam-w}, μg/g) that would result from aqueous phase uptake:

$$C_{clam-w} = C_w AE_w Vt \qquad (2)$$

where C_w (μg/L) is the aqueous contaminant concentration in the feeding beakers and V is the ventilation (filtration) rate. The time of exposure (t) was 8 h. Calculated C_{clam-w} values are compared to clam tissue concentrations measured in the laboratory. Estimates were made using the average of initial and final feeding water contaminant concentrations. The relative importance of aqueous uptake was calculated as the ratio of estimated contaminant concentration from aqueous uptake to the measured concentration of BaP or PCB-52 in clam tissue.

The estimated contribution of the aqueous route to overall contaminant uptake is depicted in Figure 3 for the range of AE_W. In the activated carbon test, particle ingestion would dominate even under the most conservative of assumptions. If AE_W were 1%, 99% of uptake would be from food; if it were 60%, then ~90% of uptake would be from food. In the diatom system the aqueous route could account for a portion of overall BaP and PCB-52 uptake. If AE_W exceeds about 0.5,

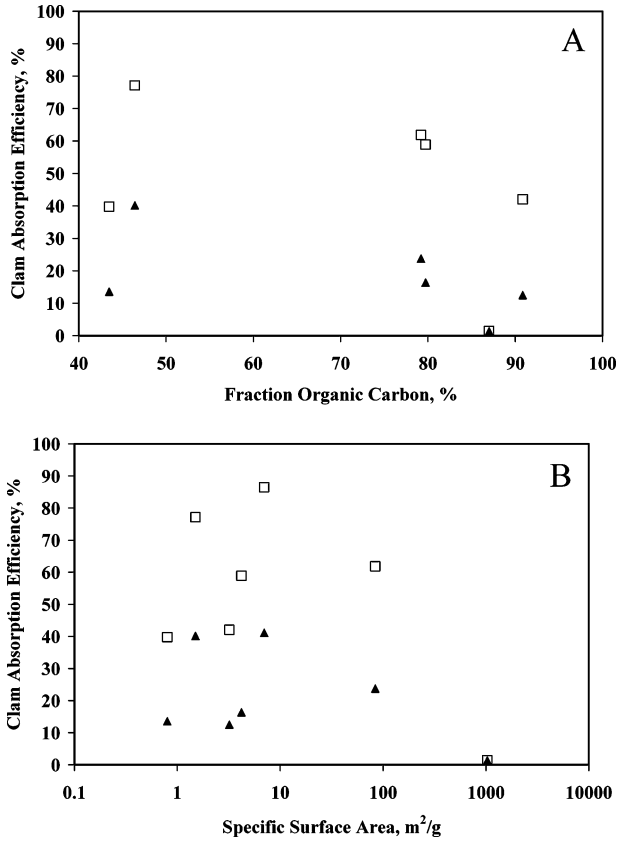

FIGURE 4. Clam absorption efficiency is independent of (A) total organic carbon and (B) surface area. Open squares represent data for BaP, and triangles represent data for PCB-52.

more than 50% of the measured contaminant mass within the clam tissue is estimated to be from the water phase. Such complexities could rearrange the order of purely dietary absorption efficiencies from some of the particle types but would not affect conclusions about the largest differences (e.g., diatoms and other particle types vs activated carbon).

Sorbent Organic Carbon and Specific Surface Area. Figure 4A,B shows that neither total organic carbon nor specific surface area is a sufficient predictor of clam absorption efficiency for BaP or PCB-52 in these experiments. The 2-fold difference in organic carbon content among our particle types cannot explain the 60-fold difference in observed absorption efficiency. Additionally, particle specific surface area did not correlate well with clam absorption efficiency. Activated carbon resulted in the lowest absorption efficiency and has the highest surface area; its SSA is about 10 times greater than char and 100−1000 times greater than the other particles tested. Within the lower SSA range (coke, anthracite, peat, wood, and char), however, there is no clear relationship between SSA and absorption efficiency.

The OMP model assumes that amorphous, humic-like organic carbon is the sediment's sorbing matrix and, as such, oversimplifies the bioavailability of organic contaminants from sediment. According to this model, TOC and bioavailability of organic contaminants are inversely related, yet some researchers have observed the lowest accumulation factors at low-TOC sites (*36*). Others showed increased nutritional quality or "labile organic carbon" increases the bioavailability of sediment-sorbed contaminants (*33, 37*). Researchers examining PAH and PCB sorption in field sediments documented significantly higher partitioning to the soot(-like) carbon fraction than to the normal organic carbon pool (*38, 7*). Our results quantify the extremely low bioavailability of PAH and PCB from soot-like particles. To our knowledge

these are also the first results that directly quantify the extremely broad range of PAH and PCB bioavailability from the individual particle assemblages representative of the carbonaceous fractions of sediment.

Sorptive properties of sediment carbonaceous matter appear to play some role in determining PAH and PCB bioavailability, but it is not one that is simple to extrapolate from all particle types or to all species. Further investigation of how such properties interact with the biological characteristics affecting organic contaminant uptake by different species might lead to generalizations of broader applicability than the OMP model. Even if one assumes that sorption controls bioavailability, a 1:1 relationship between absorption efficiency and K_d is unlikely. We hypothesize that a quantity K_{pc} (partition coefficient between particle and clam) is linearly correlated with K_d. Considering the particles ingested by the clams with associated contaminants ($M_{c,p}$) and assuming that the mass of particles ingested by a clam (M_p) equals the mass of particles in its feces, at equilibrium:

$$K_{pc} = \frac{K_d}{K_{cw}} = \frac{M_{c,p}M_t}{M_{c,t}M_p} \quad (3)$$

where K_{cw} is the partition coefficient between clam and water, M_t is the mass of clam tissue, and $M_{c,t}$ is the mass of contaminant in clam tissue. Inverting the equation for absorption efficiency and substituting mass for activity:

$$\frac{1}{AE} = 1 + \frac{M_{c,p}}{M_{c,t}} \quad (4)$$

Substituting eq 4 into eq 3 and assuming that the ratio of tissue mass to ingested particle mass is constant among particle types:

$$K_{pc} = \left(\frac{1}{AE} - 1\right)\frac{M_t}{M_p} \propto \left(\frac{1}{AE} - 1\right) \quad (5)$$

For the 60-fold variation in absorption efficiency observed in our data, we would estimate a nearly 600-fold variation in K_{pc}. Thus, a 1:1 correlation between absorption efficiency and K_d is probably not a reasonable assumption.

Implications. While there is more to learn about factors controlling contaminant bioavailability from sediments, these findings have potentially significant implications for sediment regulation and remediation. The above results raise questions about the applicability of TOC-based models to marine and freshwater food webs and their usefulness in making policy decisions (e.g., refs *7*, *31*, and *36*). They support researchers' previous claims that additional sediment compartments should be included in equilibrium partitioning models (*11*−*13*). Instead of relying on traditional OMP framework to predict risk at contaminated sites, engineers and scientists should continue to develop models that incorporate both absorption to natural organic matter and adsorption onto particulate carbonaceous materials such as coke and coal. The results of our experiments underscore the importance of considering how the inherent grain and subgrain heterogeneity of sediment could significantly impact contaminant bioavailability.

Our results also provide a strong basis for suggesting activated carbon addition as a novel remediation technique for PAH- and PCB-contaminated sediments. As shown in these tests, BaP and PCB-52 bound to activated carbon are up to 60 times less available to clams than the same contaminants associated with other types of particulate carbonaceous matter frequently found in sediment. West et al. (*39*) found that the addition of Ambersorb (a synthetic resin) to contaminated field sediments decreased porewater concentrations and bioavailability of eight PAHs, lending credence to sorbent addition as a viable option for in situ sediment remediation. Lebo et al. (*40*) compared the reductions in bioavailability of several hydrophobic organic compounds from laboratory-spiked sediment by coarse and fine versions of two different carbons using low-density polyethylene membranes as mock organisms and found fine Ambersorb 1500 to be highly effective. Further investigations are currently underway to assess the efficacy and practicality of activated carbon addition for in situ remediation of field sediments.

Acknowledgments

This work was supported under Stanford University's Bio-X Initiative. The authors thank Sarah Rubinfeld for her assistance in the laboratory and field; Dr. William Ball and Roberta Brown for supplying the char; and Dr. Upal Ghosh for assistance with data analyses. At the time of the experiments, M.J.v.d.H.-G. was affiliated with and supported by Stanford University.

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Received for review January 21, 2004. Revised manuscript received June 2, 2004. Accepted June 10, 2004.

ES049893B