

## Bioaccumulation of Benzo(a)pyrene from Sediment by Fathead Minnows: Effects of Organic Content, Resuspension and Metabolism

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**Abstract.** The accumulation of <sup>14</sup>C-benzo(a)pyrene (BaP) sorbed to sediment was examined in fathead minnows (*Pimephales promelas*) to compare uptake from sediment with a high organic carbon (OC) content (7.7%), to that with a low OC content (2%). Ingestion of sediments was quantified by co-labeling the sediment with <sup>141</sup>Cerium, which was not assimilated by the fish. Results of this study indicated that (1) significantly greater quantities of BaP were dissolved in water over low-OC sediment, compared to water over high-OC sediment, (2) fish disturbed the sediment and increased the concentration of BaP in centrifuged (particle-free) water, (3) fish ingested significantly more of the low-OC sediment than high-OC sediment, perhaps in response to the lower food quality of the low-OC sediment, and (4) uptake of BaP from sediment ingestion contributed <3% of the total flux of BaP into the fish. Primarily as a result of the greater concentration of BaP in water, fish from the low-OC exposures had significantly higher rates of BaP accumulation. However, after 48 h the body burdens in these fish declined by 50%, likely due to the induction of MFO enzymes in response to accumulation of BaP. A smaller effect was apparent in the fish from the high-OC exposures, consistent with the lower dose of BaP they experienced. These results illustrate the complex, and sometime counter-intuitive, interactions that affect the uptake and bioaccumulation of sediment-associated contaminants.

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The accumulation of hydrophobic organic contaminants (HOC) by aquatic organisms can be influenced by a number of environmental and physiological processes. The environmental processes can affect the concentration and physico-chemical form (speciation) of the contaminant. HOCs dissolved in water are more readily bioavailable than the same compound sorbed either to particles (McCarthy 1983; Ridal *et al.* 2001) or to dissolved organic matter, such as humic

substances (McCarthy *et al.* 1985; Kukkonen *et al.* 1991). Partitioning of compounds between water and sediment depends, in turn, on the organic carbon (OC) concentration of the sediment (Karickhoff 1984; Chiou *et al.* 1998; Chu and Chan 2000), and the extent of mixing between the sediment and water (Alkhatib and Castor 2000). Physiological processes, such as respiration rates (Black *et al.* 1991) or feeding (Jimenez *et al.* 1987; Jimenez and Burtis 1988; Kaag *et al.* 1997), can also affect bioaccumulation. For compounds that are rapidly metabolized to more readily excreted polar forms, changes in the activity of biotransformation enzymes can also have a significant effect on the accumulated body burden of the toxicant (Jimenez *et al.* 1987). For example, polycyclic aromatic hydrocarbons, such as benzo(a)pyrene (BaP), are metabolized by the cytochrome P450 mixed function oxidase (MFO) system (and subsequent Phase II reactions) to more polar metabolites (Buhler and Wang-Buhler 1998). Furthermore, the MFO system is induced as a result of exposure to BaP, so that levels of exposure sufficient to induce the MFO system can result in a decrease in the steady-state body burden of the contaminant, due to increased rates of metabolism and metabolite elimination. Fish challenged with MFO inducers, either in water or by intraperitoneal injection, respond with increased MFO activity within two to five days (Jimenez *et al.* 1988; Jimenez and Burtis 1988; Smolowitz *et al.* 1992; Hodson *et al.* 1996).

The objective of this research was to examine the interaction of chemical and biological variables that influence the uptake and accumulation of a model contaminant, BaP, by the fathead minnow, *Pimephales promelas*. Specifically, we examined (1) the effect of the sediment OC content on the partitioning of BaP into the water and on the rate of ingestion of contaminated sediment, (2) the effect of bioturbation of sediment by fish on the aqueous concentration of BaP in the water column, and (3) the apparent increase in the rate of BaP elimination in fish receiving higher levels of exposure to BaP from sediment and water. The contribution of the sediment to bioaccumulation, and the direct and indirect mechanisms by which sediment organic content affects bioaccumulation, are discussed.

## Materials and Methods

### Experimental Design

The uptake and elimination of BaP was conceptualized as an exchange of contaminants between water, fish, and sediment. Exchange of contaminant between fish and water were determined in water-only uptake and elimination experiments using  $^{14}\text{C}$ -BaP. Sediment exposures compared uptake from sediments with a high (7.7%) and low (2.0%) OC content. Sediments were labeled with  $^{14}\text{C}$ -BaP and  $^{141}\text{Ce}$  ( $^{141}\text{Ce}$ ). The  $^{141}\text{Ce}$  binds strongly to particles and is not assimilated by the fish ingesting the sediment. Thus, the  $^{141}\text{Ce}$  can be used as a tracer of sediment particles ingested by the fish. Based on the known  $^{141}\text{Ce}:^{14}\text{C}$  ratio, the rate of BaP ingestion by the fish can also be estimated. Both BaP and Ce share a strong affinity for binding to organic components of the sediment (Takahashi *et al.* 1999), and should have a similar distribution on sediment particles, at least on the macroscopic scale of relevance to sediment ingestion.

For each of the two sediments, partitioning of  $^{14}\text{C}$ -BaP between sediment and particle-free (centrifuged) water was measured both in two control aquaria, containing no fish, and in three exposure aquaria, containing fish. Fish in the exposure aquaria were sampled to measure the uptake and elimination of  $^{14}\text{C}$ -BaP. The rate of sediment ingestion and the sediment content in fish guts were measured by nondestructive gamma-spectroscopy of  $^{141}\text{Ce}$  concentrations in the fish.

### Experimental Materials

Fathead minnows, *P. promelas*, were obtained from Newtown Hatchery (Newtown, OH, USA). Fish were held in large tanks with flowing water at 23°C, and all experiments were conducted at a similar temperature. Fish were fed *ad libitum* daily with Purina Trout Chow until experiments began. Fish used in these experiments were  $0.48 \pm 0.03$  g wet weight. Radiolabeled [ $7\text{-}^{14}\text{C}$ ]-BaP ( $15$  mCi  $\text{mmol}^{-1}$ ) and  $^{141}\text{Ce}$  ( $0.97$  mCi  $\text{mg}^{-1}$  in  $0.5$  M HCl) were obtained from New England Nuclear (Boston, MA, USA). Sediments were obtained from Target Range Pond, a small pond on the Oak Ridge Reservation with high sediment OC content (Oak Ridge, TN, USA), and from Melton Hill Reservoir, a short distance above the Melton Lake dam on the Clinch River (Oak Ridge, TN, USA). Sediments were used without further treatment. The organic content of the Target Range Pond sediments was approximately fourfold greater than the Melton Lake dam sediments.

### Water Exposures

The fish were exposed to  $^{14}\text{C}$ -BaP (with no sediments present) in a flow-through system. The water passed through fine-particle and carbon filters (Sears) and a  $0.45\text{-}\mu\text{m}$  glass fiber filter cartridge (Balston) before entering a mixing bottle at a rate of 200 mL/min. BaP dissolved in dioxane carrier was injected continuously from a gas-tight syringe, using a Sage syringe injector, into the mixing bottle; carrier concentrations were 0.1 mL carrier per L of water. The aqueous solution of BaP flowed into a 100-L glass aquarium where the BaP concentration was maintained at a nominal  $2\text{ }\mu\text{g}$  BaP/L. The volume of water in the tank was maintained at 65 L. Approximately 100 fish were put into the aquaria at the beginning of the experiment. Fish were fed daily with Trout Chow, but excess food and feces were vacuumed from the tank within an hour after feeding in order to reduce opportunities for ingestion of BaP-contaminated particles. Fish were exposed to flowing, contaminated water for 48 h in order to measure BaP uptake. The tank was then drained, rinsed, and refilled with noncontaminated

water. Elimination of BaP was monitored over 4 d in flowing, non-contaminated water (200 mL/min).

### Sediment Exposures

Each of the sediments, with either high or low OC content, were labeled with  $^{14}\text{C}$ -BaP and  $^{141}\text{CeCl}_3$  by slow addition of stock solutions of the radioactive tracers and vigorous stirring of sediment slurries in five replicate 6-L aquaria, and the sediments allowed to settle. Fish were first acclimated to the sediment by maintaining them in aquaria with noncontaminated high- or low-OC sediment for 72 h prior to transferring them to the exposure aquaria containing contaminated sediment of the same type. In a static exposure experiment, groups of 10–12 fish were added to each of the three replicate exposure aquaria for each sediment type. Although this initial mass of fish in 6 L of water exceeds the EPA's guidelines of  $<0.65$  g/L for static tests, the higher frequency of sampling at the beginning of the experiment reduced the load to recommended levels after 8 h of the 240-h experiment. Two additional aquaria were set up without fish. Fish were not fed during the exposure, which lasted 10 d at 23°C. At 2, 4, 8, 16, 32, 48, 72, 96, and 240 h, one fish from each aquaria was first analyzed for the quantity of sediment in the gut by nondestructive gamma-counting of whole-body levels of  $^{141}\text{Ce}$ ; the  $^{14}\text{C}$  radioactivity in the fish was then analyzed by combustion of the entire fish, followed by liquid scintillation counting. Data are reported as the mean and standard error for the three replicate fish at each time point. Triplicate water samples were also analyzed for  $^{14}\text{C}$  and  $^{141}\text{Ce}$  activity. At the end of the exposure, the hindguts of the remaining fish were removed, weighed, and analyzed for  $^{141}\text{Ce}$  and  $^{14}\text{C}$  activity.

### Analytical Methods

Water samples were centrifuged at  $10,000 \times g$  for 30 min in order to remove suspended particles. The OC content of the sediments was measured by wet oxidation with persulfate, using a Total Organic Carbon Analyzer (IO Corp.), and by incineration, using a Leico Carbon Analyzer. Both methods gave similar results.

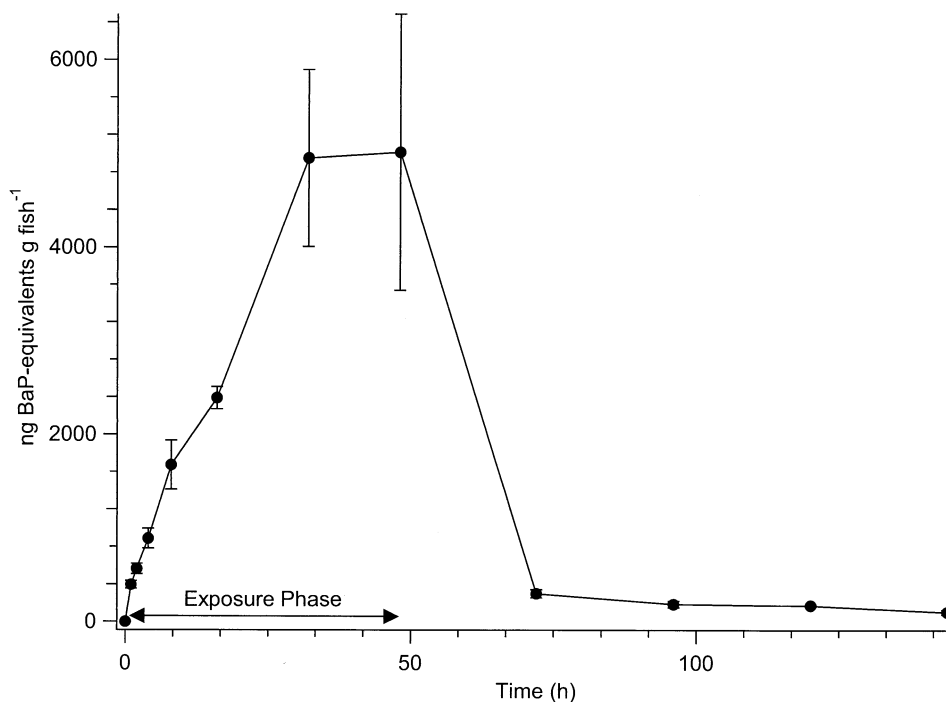
$^{14}\text{C}$ -radioactivity was analyzed by combustion of the whole fish, or the fish hindguts and the sediments, in a Packard Model 306 Oxidizer. The  $^{14}\text{C}$  activity of the combusted sediments or fish and of the water samples were determined using a Packard CDS 460 Liquid Scintillation Counter with automatic quench correction. The measured  $^{14}\text{C}$  activity includes both the parent compound as well as any polar metabolites of  $^{14}\text{C}$ -BaP. Thus, contaminant concentrations are expressed as BaP-equivalents based on the specific activity of the  $^{14}\text{C}$ -BaP.

For the water-only exposures, water samples were also extracted with ethyl acetate in order to determine the proportion of the  $^{14}\text{C}$  activity composed of the parent compound (in the ethyl acetate phase) and that composed of the BaP polar metabolites (in the aqueous phase; Jimenez *et al.* 1987).

$^{141}\text{Ce}$  activity in sediments, water samples, and whole fish or fish hindguts were measured nondestructively using a Packard Auto-Gamma Scintillation Spectrometer. Results were corrected for the decay of the  $^{141}\text{Ce}$  ( $t_{1/2} = 33$  d) during the exposure.

### Data Analysis

The body burdens of contaminant in the fish were corrected for the contribution of sediment-bound  $^{14}\text{C}$  activity in the gut, which was calculated based on the measured  $^{141}\text{Ce}$  levels in an individual fish and the  $^{141}\text{Ce}:^{14}\text{C}$  ratio for that sediment.



**Fig. 1.** Bioconcentration of  $^{14}\text{C}$ -BaP by fish. Plotted values are means  $\pm$  standard error ( $n = 6$ ). Data are shown in terms of BaP equivalents based on  $^{14}\text{C}$  activity

In some cases, the total mass of contaminant accumulated or eliminated by a whole fish or fish gut over a given time interval was calculated from the change in the contaminant level from one sampling time to the next and the total grams of fish present in the aquarium during that time interval. Likewise, in cases where there was an increase in the level of  $^{14}\text{C}$  activity in the water overlying the sediment, the total mass of contaminant represented by that increase was calculated from the change in concentration and the volume of the aquarium.

Statistical differences between contaminant concentrations in the exposure water were determined using a *t*-test (Zar 1996).

## Results and Discussion

### Water Exposure

Following an initial decline during the first two hours of the experiment, the exposure system maintained a stable concentration of parent  $^{14}\text{C}$ -BaP. The  $^{14}\text{C}$  activity in the ethyl acetate extracts of the exposure water averaged  $2.3 \pm 0.1 \mu\text{g/L}$  from 4–48 h. The total radioactivity in the water increased over the course of the exposure due to release of polar metabolites that partitioned into the aqueous fraction of the ethyl acetate extraction. By the end of the experiment,  $^{14}\text{C}$ -polar metabolites constituted half of the total  $^{14}\text{C}$  activity in the water.

In fish,  $^{14}\text{C}$  accumulated rapidly during the 48-h exposure and then declined rapidly when the fish were transferred to contaminant-free water (Fig. 1). The shape of the uptake and elimination curve is similar to that observed by Jimenez *et al.* (1987) for bluegill sunfish that were fed during the experiment.

This Jimenez study also found that a large fraction of the  $^{14}\text{C}$  activity in the fish was due to the polar metabolites, rather than the parent compound, and this is likely true for the fathead

minnows used in our study. This would be consistent with the higher levels of polar metabolites detected in the aqueous phase, based on the ethyl acetate extraction of the water.

### Sediment Exposures

The fractional OC content ( $f_{\text{OC}}$ ) of the sediments used in this study were 7.7% and 2% OC for the Target Range Pond and Melton Hill Reservoir sediments, respectively, and are referred to as high-OC and low-OC sediments, respectively (Table 1). The initial  $^{14}\text{C}$ : $^{141}\text{Ce}$  ratios of the sediments were 3.6 and 3.8 for the low- and high-OC sediments, respectively (Table 1). Centrifugation successfully removed 97–99% of the  $^{141}\text{Ce}$  in water samples. We attribute this small amount of suspended radioactivity to the binding of Ce to small particles and/or dissolved organic matter. Data reported for the aqueous concentrations of  $^{14}\text{C}$ -BaP were corrected for the contribution of suspended particles.

### Accumulation in Fish

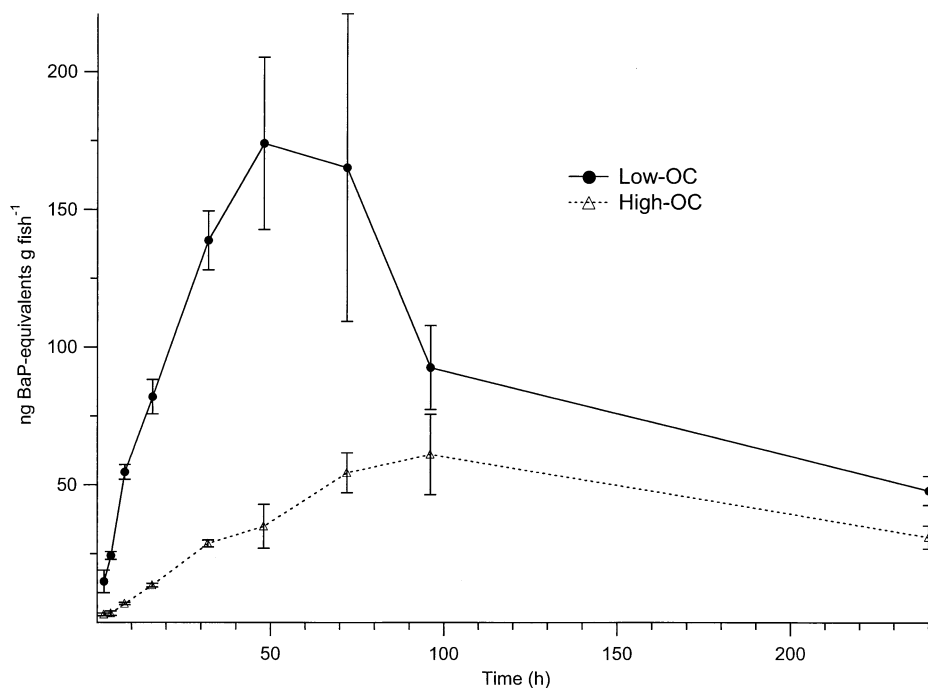
The uptake of  $^{14}\text{C}$  activity in the fish from the high-OC sediment exposure increased slowly for the first 96 h, but declined by the end of the experiment (Fig. 2). In contrast, the dynamics of contaminant uptake were quite different for the fish exposed to the low-OC sediment. For the first 48 h, the levels of  $^{14}\text{C}$ -BaP in low-OC fish increased much more rapidly than for fish in the high-OC exposures, but levels of  $^{14}\text{C}$ -activity then decreased sharply between 72 and 96 h and continued to decline at a slower rate over the remainder of the experiment.

The higher body burdens in fish exposed to the low-OC

**Table 1.** Organic carbon content and radiotracer concentrations for sediments used in this study

Sediment	$f_{OC}$ (%)	$^{141}Ce$ (ng/g sediment)	$^{14}C$ -BaP (ng/g sediment)	$^{14}C:^{141}Ce$ ratio
High-OC Sediment (Target Range Pond)	7.7	28	100	3.8
Low-OC Sediment (Melton Hill Reservoir)	2.0	37	140	3.6

The data are the results of analysis from composite several sediment samples from each exposure condition.



**Fig. 2.** Bioaccumulation of  $^{14}C$ -BaP by fish exposed to low-OC sediments (solid symbols and solid line) or high-OC sediments (open symbols and dotted line). Plotted values are means  $\pm$  standard error ( $n = 3$ ). The data represent the concentration of BaP equivalents in the fish body, after subtracting any sediment-bound  $^{14}C$ -BaP in the gut

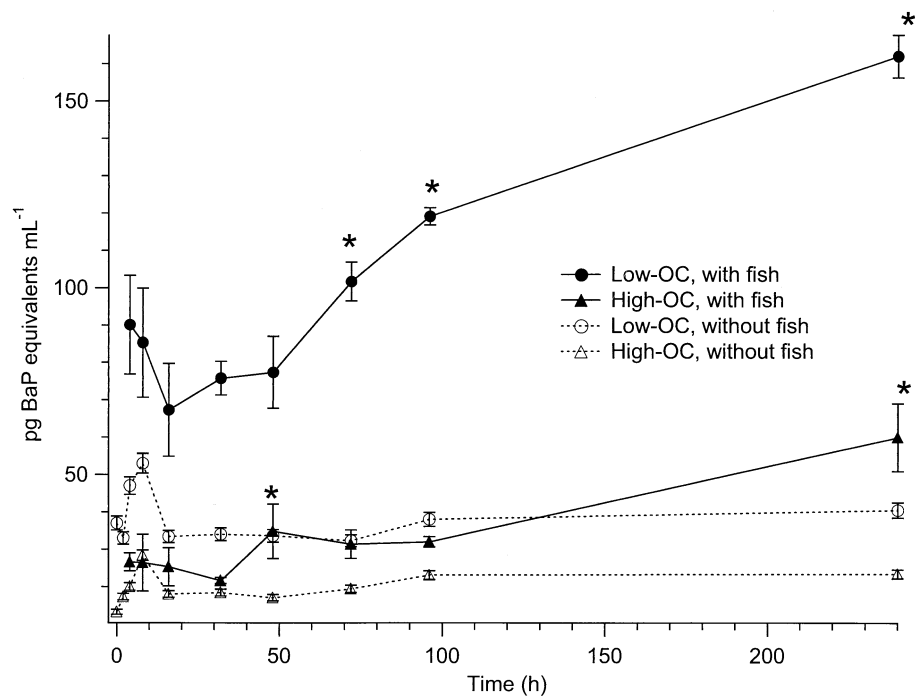
sediments is attributed primarily to lower sorptive capacity of the low-OC sediment, which resulted in higher concentrations of BaP in the centrifuged (particle-free) water (Fig. 3). Partitioning of HOCs to sediment is related to the  $f_{OC}$  of the sediment (Karickhoff 1984; Chu and Chan 2000). Within a given sediment type, contaminant concentrations in water were much greater in the aquaria containing fish compared to those without fish. Fish activity resulted in rapid resuspension of the sediment, as evidenced by the sharp increase in the concentration of  $^{141}Ce$  in noncentrifuged water (Fig. 4). The resulting turbidity was sufficient to obscure the fish. The significant increase in the concentration of dissolved BaP in the centrifuged water from the low-OC aquaria ( $p < 0.05$ ), compared to control aquaria without fish (Fig. 3), is attributed to mixing of sediment and water by the bioturbation.

The BaP concentration in the centrifuged water remained constant throughout the exposure in the control aquaria without fish (Fig. 3). Likewise, BaP concentrations do not change significantly during the early portions of the experiments in the aquaria containing fish. However, there is a significant increase in  $^{14}C$  activity after 48 h in the low-OC aquaria and after 96 h in the high-OC exposures. The increase in the  $^{14}C$  activity is postulated to be a result of the induction of the MFO system, which enhanced rates of excretion of polar metabolites by the fish during the later phases of the exposure. Desorption of BaP from the sediment is expected to maintain the concentration of

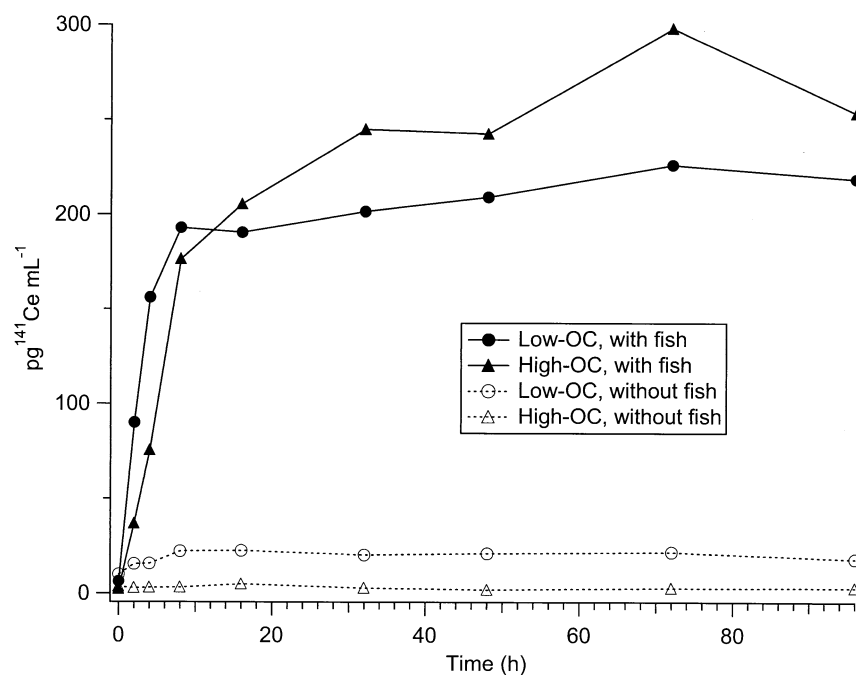
the parent compound in the water at a constant level throughout the 240-h exposure. Thus, as the dose of contaminant accumulated by the fish increases over time, their detoxification systems are induced, leading to enhanced rates of transformation and elimination. Induction of the MFO system in fish has been shown to occur within a 2–4 d timeframe (Jimenez *et al.* 1988; Jimenez and Burtis 1988; Smolowitz *et al.* 1992; Hodson *et al.* 1996), consistent with the time when elimination rates increased in this study.

The fish in the low-OC aquaria accumulated higher levels of BaP than those in the high-OC exposures (Fig. 2), and consequently achieved the dose required for induction sooner. After 48 h, the low-OC fish experience a pronounced decrease in contaminant body burdens, which is attributed to elimination of polar transformation products.

We attribute the decreased contaminant levels in the fish to an increased rate of elimination of polar transformation products. Comparison of the mass of  $^{14}C$  activity eliminated by the fish between 48 and 240 h (Fig. 2) and the  $^{14}C$  activity increase in the water during the same period (Fig. 3) is consistent with this hypothesis, as illustrated in Fig. 5. In Fig. 5, the mass of BaP equivalents (assumed here to be polar metabolites) is calculated from the change in contaminant concentrations in the fish or water between sampling events, and the mass of fish or water in the exposure aquaria during that time interval. The induction response is later and less pronounced in the fish in the



**Fig. 3.** Time course of change in dissolved concentration of  $^{14}\text{C}$ -BaP in water from aquaria containing low- and high-OC sediments. Water was centrifuged before analyses to remove particle-bound BaP. Data are shown for aquaria without fish (open symbols and dotted lines) and with fish (solid lines and symbols). Plotted values are means  $\pm$  standard error ( $n = 3$ ). Data with an asterisk indicate a significant difference in the concentration, relative to the previous observation ( $p < 0.05$ )



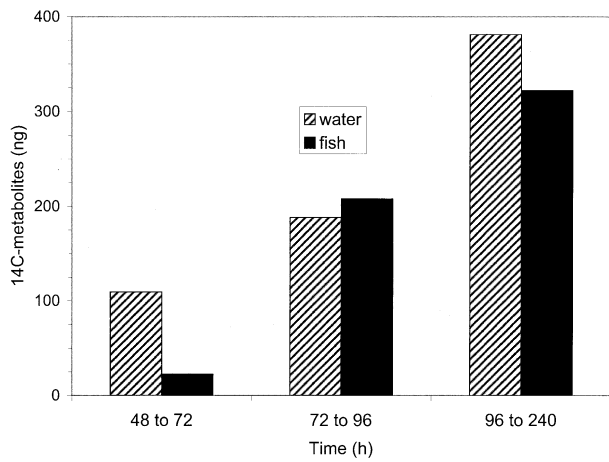
**Fig. 4.** The concentration of  $^{141}\text{Ce}$ -labeled sediment suspended in noncentrifuged water is plotted to illustrate the bioturbation of the sediment by the fish

high-OC aquaria, likely because they were exposed to a smaller dose of BaP. As was observed in the low-OC exposures, the mass of BaP metabolites lost by the fish between 96 and 240 h is similar to that observed as levels in the water increased.

#### *Ingestion of Contaminated Sediments by Fish*

The amount of  $^{141}\text{Ce}$  in the fish increased approximately linearly during the 10-d experiment (Fig. 6). Note that data were

corrected for decay of the  $^{141}\text{Ce}$  over the course of the exposure. The slope of the line corresponds to an ingestion rate of 0.0199 and 0.0069 ng  $^{141}\text{Ce}$ /g-fish/h for the low- and high-OC exposures, respectively. To confirm that all the  $^{141}\text{Ce}$  was localized in the gut and that there was no assimilation of the radiolabel into the fish tissues, fish remaining at 240 h were dissected and  $^{141}\text{Ce}$  measured in the hindgut and in the remaining carcass. The concentration of  $^{141}\text{Ce}$  in the dissected gut agrees well with that measured by gamma-counting the intact animal (Fig. 6). The slight discrepancy probably reflects dif-



**Fig. 5.** The mass of <sup>14</sup>C activity (expressed as ng of BaP or BaP metabolites) released by fish in the low-OC exposure over the indicated time intervals is compared to concomitant mass of <sup>14</sup>C activity accumulated in the water, beginning at 48 h. The mass of <sup>14</sup>C metabolites released by fish over each time interval is calculated based on the change in the mean body burdens (ng BaP-equivalents g fish<sup>-1</sup>) over each time interval, and the mass of fish present

ferences in counting geometry. These results also confirm that <sup>141</sup>Ce was not assimilated by the fish tissues.

The rate of <sup>141</sup>Ce accumulation by the fish (Fig. 6) is equivalent to a sediment ingestion rate of 1.3 and 0.6% of body weight per day, respectively, for the low- and high-OC exposures, based on the specific activity of the labeled sediment.

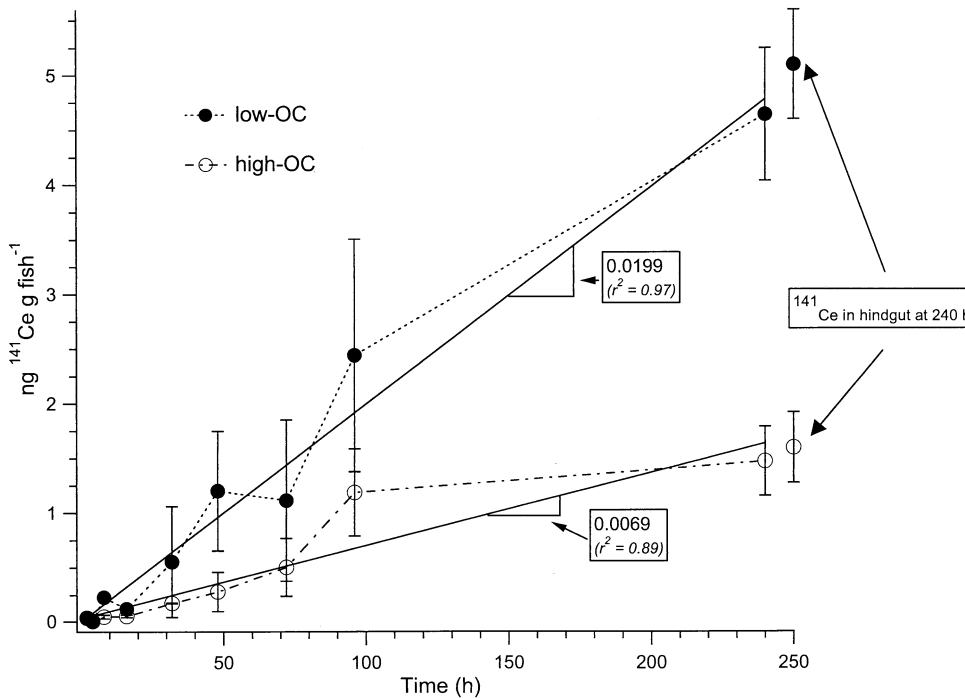
Asaeda *et al.* (2001) found that the feeding rates and swimming activity of a planktivorous fish was related to the level of

satiation, with increased satiation resulting in reduced feeding rates. Fathead minnows normally eat insect larvae and plankton in the overhead water column and the absence of these foods may have contributed to the extent of bioturbation and the sediment ingestion rates. Neither sediment provided high quality food, but the higher ingestion rates of the fish in the low-OC aquaria may be related to the lower food quality of the low-OC sediments.

Ingestion of <sup>14</sup>C-BaP-contaminated sediments constitutes only a small fraction of the total rate of <sup>14</sup>C uptake by the fish. The rate of ingestion of sediment-bound <sup>14</sup>C-BaP can be estimated from the <sup>141</sup>Ce ingestion rate (Fig. 6) and the <sup>14</sup>C:<sup>141</sup>Ce ratio. The rate of sediment ingestion estimated in this manner is approximately 40 to 60-fold lower than the total rate of <sup>14</sup>C uptake by fish, calculated as a linear approximation of the uptake curve over the first 32 h (Fig. 2). Another indication that uptake from water dominated the bioaccumulation process is the comparison of the maximum bioconcentration factor (BCF) for the different treatments (BCF = ng BaP g-fish<sup>-1</sup>/ng BaP mL<sup>-1</sup>). For the sediment exposures, the BCF calculation was based on the average aqueous concentration observed during the first 48 h of exposure. The peak BCF values for all exposure conditions were remarkably similar (2300 and 2400 for the low-OC and high-OC exposures, respectively, and 2500 for the water-only exposure). The apparent dependence of the BCF on the aqueous concentration suggests that the aqueous exposure route can account for most of the accumulation, regardless of the treatment.

**Summary and Conclusions**

This study is consistent with the expected effects of sediment organic content on aqueous concentrations of BaP, with higher



**Fig. 6.** Ingestion of <sup>141</sup>Ce-labeled sediment by fathead minnows exposed to low-OC (solid symbol) and high-OC sediments (open symbol). Solid lines are the results of a linear regression on each set of data. Points connected by lines are results of analyses of the whole fish (means ± standard error, n = 3). The concentrations of <sup>141</sup>Ce in the dissected hindgut of fish at 240 h are also shown for comparison

levels in water for aquaria with lower  $f_{OC}$  sediments. Bioturbation further increased aqueous BaP levels, likely due to the greater degree of mixing of sediment and water. Likewise, it was not unexpected that fish from the low-OC exposures had significantly higher rates of BaP accumulation, primarily as a result of the greater concentration of BaP in the water. However, the organic content of the sediment also affected the rate of sediment ingestion, so that fish ingested significantly more low-OC sediment than high-OC sediment, perhaps in response to the lower food quality of the low-OC sediment. Although bioaccumulation was initially much greater in fish exposed to the low-OC sediments, the 50% decline in body burdens between 48 and 240 h is attributed to induction of MFO enzymes in response to accumulation of BaP. A smaller effect was evident in the fish from the high-OC aquaria, consistent with the lower dose of BaP they experienced. These results illustrate the complex, and sometime counterintuitive, interactions that affect the uptake and bioaccumulation of sediment-associated contaminants.

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