Bioavailability of Sediment-Associated Benzo(a)pyrene Within Single- Versus Multiple-Species Systems

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Abstract. A series of experiments were conducted with benzo(a)pyrene (B(a)P) spiked sediments to determine if bioavailability of sediment-associated contaminants is affected by multiple species interactions. Three benthic invertebrates, Hyalella azteca, Chironomus tentans, and Lumbriculus variegatus, were exposed to sediments spiked with radiolabeled B(a)P that was aged for 60 days. Organisms were introduced into the spiked sediments in single, binary, and ternary combinations. Changes in bioavailability were then determined for each species by estimating uptake clearance coefficients \( k_s \) and bioaccumulation factors (BAFs) during 7-day exposures. In general, there was a trend toward lower \( k_s \) values in binary and ternary exposures compared to the single-species systems. In contrast, BAF estimates were more variable with fewer significant differences noted among treatments. BAF estimates were highest for \( L. \text{variegatus} \) followed by \( C. \text{tents} \) and \( H. \text{azetca} \) and appear to be dependent on specific feeding and habitat requirements as well as the relative biotransformation/elimination potential of each species. Overall, these results suggest that animal–animal interactions may be important to consider when estimating bioavailability of sediment-bound chemicals.

Bioavailability of sediment-associated contaminants can be defined as the measure to which a contaminant in the sediment is free for uptake into and accumulation by an organism (Landrum and Robbins 1990; Landrum et al. 1992). Two approaches are commonly used to assess bioavailability and include measuring bioaccumulation factors (BAFs) and uptake clearance coefficients \( k_s \). Bioaccumulation factors are determined by the ratio of the chemical concentration in the organism and the chemical concentration in the sediment assuming steady-state conditions exist between the organism and the chemical in the environment. On the other hand, uptake clearance coefficients relate the flux of contaminant into the organism to contaminant concentrations in the sediment and are determined through the use of kinetic studies (Landrum and Robbins 1990). Both methods have been extensively used to assess bioavailability of sediment-associated contaminants (Adams 1984; Landrum 1989; Lee 1991; Weston and Mayer 1998).

Animal–sediment interactions can be a complicating factor in bioavailability studies. Several authors have observed that the type and number of benthic organisms in a sediment system affect contaminant mobilization and availability (Reible et al. 1996; Means and McElroy 1997). Sediment processing by benthic organisms continuously creates new sediment interfaces for contaminants to desorb into the interstitial and overlying waters. For example, bioturbation from conveyer-belt-type feeding organisms, such as Lumbriculus variegatus, continuously transports buried sediments to the surface resulting in significant contaminant fluxes from the sediment phase to the water phase (Davis and Means 1989; Reible et al. 1996). The bioavailability of contaminants resulting from these processes then depends on such factors as the chemical partitioning among the freely dissolved, particulate sorbed, and dissolved organic carbon (DOC) sorbed phases (Leppänen and Kukkonen 1998). A number of studies have examined the possible effects of animal–sediment interactions on bioavailability (Riedel et al. 1989; Peterson et al. 1996; Wall et al. 1996; Ciarelli et al. 1999).

In addition to the potential for increased exposure to contaminants resulting from animal–sediment interactions, animal–animal interactions also may influence bioavailability. Fewer studies have focused on these types of relationships. Interactions among benthic invertebrates with similar feeding habits may lead to competition among species for food and thereby result in differences in exposure (Reynoldson et al. 1994; Kaag et al. 1997). In addition, species may compete or interfere with the ability of each organism to acquire space (Reynoldson et al. 1994) that could also result in differences of exposure. Thus, exposing more than one test species in a system may influence the bioavailability of some sediment-sorbed contaminants.

The objectives of the current study were to estimate the bioavailability of B(a)P to three invertebrate species (Hyalella azteca, Chironomus tentans, and L. variegatus) using (BAFs)
and uptake clearance coefficients \( (k_\text{s}) \), and to determine if having multiple test species in a system affects bioavailability of B(a)P to individual test species within that system.

**Materials and Methods**

**Organisms**

Three invertebrate test species, *H. azteca* (juvenile), *C. tentans* (fourth instar), and *L. variegatus* (adult), were selected to estimate bioavailability of sediment-associated B(a)P. The organisms used throughout this study were reared onsite at Wichita State University from cultures originally obtained from the U.S. EPA Environmental Research Laboratory, Duluth, Minnesota. These test species were selected because each has a different microhabitat preference within the same ecosystem and feeding habit (Ingersoll 1995) that may influence the bioavailability of contaminants in sediments (Table 1). Even though the microhabitat and feeding niches of these organisms overlap, all three organisms are deposit feeders and two of the organisms burrow into the sediment.

**Sediment Dosing**

Florissant soil (0–10 cm) was collected from Florissant, Missouri, sieved (1mm), mixed, and stored at 4°C. The soil has been characterized as a silt loam soil and previous assays have determined the total organic carbon (TOC) to be approximately 1% (Harkey et al. 1994; Schuler unpublished data). The soil was hydrated to produce a sediment with a dry:wet ratio of approximately 60%. The sediment was dosed with radiolabeled \( [3 \text{H}] \) B(a)P (84 Ci mmol\(^{-1}\)) in an acetone carrier. \( [3 \text{H}] \) B(a)P was purchased from Sigma Chemical Company (St. Louis, MO) and found to have ≥ 95% purity as determined by thin-layer chromatography and liquid scintillation counting. All scintillation counting was performed using a Packard 1900TR Liquid Scintillation Analyzer (Packard Instrument Company, Meriden, CT) equipped with automatic quench control, and each sample was counted for 10 min per vial. Sample counts were corrected for background and quench using the external standards ratio method.

Bulk sediments were prepared in 4-L glass containers. \( [3 \text{H}] \) B(a)P was added drop-wise to the water/sediment slurry and stirred continuously for 4 h at 20°C with a stainless-steel paddle driven by an overhead motor. Separately dosed sediments were combined and re-mixed to ensure an even distribution of \( [3 \text{H}] \) B(a)P. The nominal concentration of B(a)P was 100,000 disintegrations per min (DPM) \times 0.1396 μg/kg. The sediments were over lain with test water and covered with aluminum foil prior to storage. Prior to use, sediments were aged in darkness for 60 days at 4°C to allow for dissolution and partitioning of the spiked B(a)P.

**Uptake**

The uptake experiment was performed under static conditions in 600-ml beakers containing 50 g (dry weight) \( [3 \text{H}] \) B(a)P dosed sediment with 400 mL reconstituted moderately hard water. The moderately hard water was prepared in accordance with EPA protocols by the addition of the appropriate amount of salts to deionized water (US EPA 1994). The sediment was allowed to settle for 24 h prior to the addition of the organisms. The overlying water was gently aerated throughout the exposures to ensure adequate DO levels. The experiment was preformed in a Precision Scientific Dual-Jet Environmental Chamber (Grand Rapids, MI) maintained at 20°C with a 16 light:8 dark photoperiod. To ensure that organic carbon and B(a)P were not limiting factors within the system, a 50:1 ratio of organic carbon in the sediment to dry weight of organism was used (US EPA 1994). In single-species exposures (three replicates), individual organism loading was 10 mg (dry weight) per 50 g sediment. In binary (three replicates) and ternary-species exposures (five replicates), loading was determined by a dry weight ratio of the organisms to the sediment, with the total organism weight being 10 mg (dry weight) per 50 g of sediment. In multiple-species exposures the organism loading (10 mg) would be divided equally among the test species. For example, in binary tests each species would comprise one-half the total loading and in ternary tests each species would comprise one-third the total loading. The mean weights of individual test species used in the exposures were 0.20 ± 0.02 mg, 0.32 ± 0.04 mg, and 0.39 ± 0.04 mg for *Hyallela*, *Lumbriculus*, and *Chironomus*, respectively. The organisms were not fed any additional food other than the sediment to which they were exposed to during the 7-day accumulation period. This eliminated the possibility of selective feeding on uncontaminated substrate that could potentially have affected organism uptake of B(a)P from the sediment. In all exposures, the mortality was generally low (< 5%). There was no significant weight loss or gain during the 7-day experiments.

Organisms were collected from beakers at 0, 6, 12, 24, 48, 96, and 168 h. Organisms were collected from the sediment using a No. 230 (63 μm) sieve; organisms retained in the sieve were removed with a pipette, rinsed with deionized water, blotted dry, and weighed to the nearest 0.1 mg using a Mettler Balance Model H18 (Mettler, Hightstown, NJ). Samples were placed into scintillation cocktail (ScountSafe Plus 50%, Fisher Scientific), extracted by sonication using Tekmar Model 501 Sonic Processor (Cincinnati, OH) for 60 s, and stored in darkness for 24 h prior to radioactivity measurement. All preparations, experiments, and extractions were carried out under red light to minimize photodegradation of the B(a)P.

**Data Analysis**

Uptake clearance coefficients \( (k_\text{s}) \) were estimated to compare intraspecies differences due to animal interactions. An initial rates model was used to obtain the initial estimate for \( k_\text{s} \) (Lydy et al. 1994, 2000). Initial rates estimates were calculated from the slope of the tangent line using the initial portion of the uptake curve according to the following equation:

\[
C \cdot v = k_\text{s} \cdot C_\text{m} \cdot t
\]

where \( C_\text{m} \) = chemical concentration in the organism (μg chemical · kg dry organism\(^{-1}\)); \( k_\text{s} \) = uptake clearance coefficient (g dry sediment · g dry organism\(^{-1} \cdot \) h\(^{-1}\)); \( C_\text{m} \) = chemical concentration in the sediment (μg chemical · kg dry sediment\(^{-1}\)); and \( t \) = time (h).

The initial rates model assumes that elimination is not important during the initial portion of the uptake phase, that the flux of contaminant into the organisms is much greater than the flux out, and that the concentration in the sediment remains constant. The data also were fit by performing an iterative least squares fit to the following differential equation using the fourth-order Runge-Kutta

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**Table 1. Comparison of feeding requirements and microhabitat preferences for the selected test species (adapted from Ingersoll 1995)**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Microhabitat</th>
<th>Feeding Habit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. azteca</em></td>
<td>Burrow, epibenthic</td>
<td>Grazing/deposit feeder</td>
</tr>
<tr>
<td><em>C. tentans</em></td>
<td>Tube dweller</td>
<td>Suspension/deposit feeder</td>
</tr>
<tr>
<td><em>L. variegatus</em></td>
<td>Burrow, infaunal</td>
<td>Deposit feeder</td>
</tr>
</tbody>
</table>