Environmental Toxicology

TOXICOKINETICS AND TOXICITY OF SEDIMENT-ASSOCIATED PYRENE AND PHENANTHRENE IN DIPOREIA spp.: EXAMINATION OF EQUILIBRIUM-PARTITIONING THEORY AND RESIDUE-BASED EFFECTS FOR ASSESSING HAZARD

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Abstract—The amphipod Diporeia spp. was exposed to pyrene (0.14 to 1.11 μmol g⁻¹) or phenanthrene (0.08 to 0.62 μmol g⁻¹)-dosed sediments for month-long exposures. Phenanthrene was only slightly toxic with 12 ± 3% mortality at the highest sediment dose (0.62 μmol g⁻¹). Failure to attain and maintain toxic residue body burdens, based on a nonpolar narcosis concentration of approximately 6 μmol g⁻¹, accounts for the low mortality. Phenanthrene toxicokinetic parameters were essentially constant among all doses and consistent with previous measures. Sediment concentration was a poor representation of dose for mortality by pyrene. The relative pyrene distribution among the <63-μm particles increased in the smallest-sized particles at larger doses. An apparent stimulation of pyrene accumulation was observed as a peak in uptake clearance values between sediment concentrations of 0.16 and 0.26 μmol g⁻¹ dry sediment. (Uptake clearance is the amount of source scavenged of contaminant per mass of organism per time.) The pyrene particle-size distribution and the variation in kinetics with dose provide a partial explanation for the poor representation of dose by the sediment concentration. The pyrene body burdens provided a good dose response yielding LD₂₀ values of 6.3 (4.6-41.7, 95% C.I.) and 9.4 (7.9-54.2) μmol g⁻¹ for two experiments. These values are consistent with the residue concentrations for 50% mortality by a nonpolar narcosis mechanism. Comparing the experimental and predicted equilibrium partitioning-based sediment concentrations for 50% mortality, the equilibrium prediction overestimates the toxic pyrene sediment concentration by approximately a factor of ten. Diporeia behavior, differential particle-size distribution, and kinetic limitations appear as likely reasons for the variation between calculated and observed concentrations required to produce mortality.

Keywords—Pyrene Phenanthrene Sediment Residue effects Equilibrium partitioning

INTRODUCTION

Efforts to understand the factors that govern the toxicity of sediment-associated contaminants and to establish sediment quality criteria have produced several approaches for assessing contaminant hazard in sediments [1-3]. No one method has become generally accepted as the optimal approach for regulatory assessment. Evaluating effects on a bulk sediment basis is considered the most variable because of differences in contaminant bioavailability among sediments. Even with such variance, levels can be established above which effects are generally observed [2]. Thus, one of the main criteria in developing a suitable approach is the ability to account for differences in the bioavailability of sediment-associated contaminants among sediments of varying composition. The equilibrium-partitioning approach attempts to account for bioavailability differences among sediments for nonpolar compounds by normalizing for the sediment organic carbon concentration [4]. The method assumes that (a) the chemical in the sediment and interstitial water are in equilibrium and (b) the benthos are at steady state with respect to the interstitial water—that is, the dose from whole-sediment exposure is equivalent to the dose from a water-only exposure where the freely dissolved interstitial water concentration is the same as the water-only exposure concentration [4]. Thus, the dose equals the water concentration of the contaminant that elicits a specific response. Therefore, for the approach to work, the toxic dose must be within the solubility limit of the compound.

Another approach to account for bioavailability differences among sediments is to base the dose, not on the external concentration, but rather on the concentration in the organism that is associated with an effect (reviewed by [5]). The paradigm established in aquatic toxicology for water exposures has been to relate the dose in the external environment (water) to that in the organism, which is proportional to the dose at the receptor [6]. It is the dose at the receptor that determines the response. When the source of the exposure is water, this paradigm works well because there is only one route of accumulation, and behavioral and physiochemical modifications that reduce exposure are minimal. However, for sediment-associated contaminants, the scenario is much more complex. There are multiple exposure routes: ingestion of particles, exposure to overlying water, and exposure to interstitial water. The proportions of the contaminant from each route depend both on the chemistry of the contaminant and organism behavior—for example, avoidance of sediments that would reduce exposure to interstitial wa-
ter, and changes in food sources that would alter the ingestion route. With such a complex milieu, relating effects to only a single source concentration is unreasonable; therefore, evaluating effects based on body residue moves the paradigm one step closer to the site of action. With a proper database, this approach permits development of relationships between bioaccumulation and toxicity for all routes of exposure.

Accumulations of sediment-associated polycyclic aromatic hydrocarbons (PAHs) by the amphipod Diporeia spp. (formerly Pontoporeia hoyi [7]) are apparently kinetically limited by both the desorption rate to the interstitial water and the rate of accumulation through ingestion [8–12]. Because of these kinetic limitations, the sediment concentration of a PAH mixture required to produce mortality [11] is approximately 20 times greater than would be predicted by the equilibrium-partitioning approach. This toxicity was measured using a PAH mixture, so an equilibrium-partitioning evaluation may not reflect the actual toxic agent. Assuming additivity, however, the body burden required to produce 50% mortality for the mixture was 6.1 μmol g⁻¹ for the molar sum of PAHs. This concentration is similar to that required for nonpolar narcosis in a variety of aquatic animals [5]. Thus, it appeared that the tissue-residue approach would have accurately predicted the toxicity of the mixture, whereas the equilibrium-partitioning approach would overestimate it.

The present study was designed to confirm the above result by exposing Diporeia to individual PAH congeners, so that the complexities of exposure to a mixture would not confound the results. The objective was to determine the tissue effect concentration for nonpolar narcosis and to compare the toxicity with that predicted with the equilibrium-partitioning approach. Toxicity and toxicokinetics were determined in Diporeia spp. exposed to pyrene and phenanthrene sorbed to sediments. Additionally, respiration was measured as a potential physiological indicator of stress with PAH exposure.

MATERIALS AND METHODS

Collection of organisms, sediment, and water

Diporeia spp. were collected from Lake Michigan off Grand Haven, Michigan (43°01.2' N, 86°17.6' W) at a depth of 24 to 28 m with a Ponar grab sampler. Diporeia collected from this site have low background concentrations of PAHs; individual PAH congeners ranged from 0.2 to 2 μg g⁻¹ [13]. Diporeia were screened from the sediment, placed in clean lake water, and kept cold with ice during transport to the laboratory. Diporeia were held in aquaria containing 3 to 4 cm of sediment, collected from the same site as the organisms, and 7 to 10 cm of lake water at 4°C [14]. The lake water used throughout the study was Lake Michigan surface water, collected about 1 m below the surface and stored at 4°C.

Lake Michigan sediment for the toxicity studies was obtained by Ponar grab approximately 8 km off Grand Haven, Michigan (43°02.0' N, 86°21.9' W) at 45-m depth. This sediment has somewhat higher background concentrations of PAHs than does the site from which the Diporeia were collected. Individual components ranged from 40 to 200 ng g⁻¹, with phenanthrene approximately 70 ng g⁻¹ and pyrene approximately 200 ng g⁻¹ [13]. The sediment organic carbon content (0.46%) is higher than that from the site of Diporeia collection; thus, the sediment would have a more consistent food supply for longer exposures. The sediment was sieved at 1 mm to remove animals and large debris and held at 4°C until use.

Chemicals

Nonradiolabeled pyrene (99% pure), phenanthrene (98% pure), and chrysene were purchased from Aldrich Chemical Company. The pyrene and phenanthrene were used as purchased. Chrysene, for use as an internal standard, was recrystallized from acetonitrile.

The radiolabeled compounds were obtained as follows: [³H]pyrene (25.2 Ci mmol⁻¹, Chemsyn Science Laboratories); [³H]benzo[a]pyrene (69 Ci mmol⁻¹, Amersham, Corp.); [¹⁴C]phenanthrene (14 mCi mmol⁻¹, Sigma Chemical Co.); [¹⁴C]-2,4,5,2',4'-hexachlorobiphenyl (12.2 mCi mmol⁻¹, Sigma Chemical Co.); and [¹⁴C]-2,4,2',4'-tetrachlorobiphenyl (13.8 mCi mmol⁻¹, Sigma Chemical Co.). The radiopurities of all compounds were determined using thin-layer chromatography, with hexane:benzene (8:2, v/v) as the solvent, and liquid scintillation spectrometry [14] and were found to be >98% pure prior to use.

Sediment exposures

Two separate experiments were performed: one in the summer of 1988 using high-pressure liquid chromatography (HPLC) for analysis with separate exposures to phenanthrene and pyrene; and one in the summer of 1992 using isotopic dilution (ID) to analyze exposure to pyrene. All analytical and preparative work was performed under gold fluorescent lights, λ > 500 nm, to minimize potential photodegradation.

HPLC experiment. The acetone stocks for dosing the sediments contained 57 mg ml⁻¹ pyrene and 40.6 mg ml⁻¹ phenanthrene, respectively. Wet sediment (2,000 g) was suspended in 2 L of lake water for each dose. Pyrene and phenanthrene were added dropwise in an acetone carrier (0.35 to 3.5 ml) to sediment suspensions to create four doses for each compound (Table 1). Suspensions were stirred 18 h at room temperature. Control sediments were dosed similarly with 3.5 ml acetone. Suspensions were allowed to settle overnight

| Table 1. Phenanthrene and pyrene sediment concentrations (μmol g⁻¹) for the HPLC experiment (mean ± sd, n = 3) |
|---|---|---|
| | Initial | Day 8 | Day 31 |
| Phenanthrene | 0.08 ± 0.002 | 0.03 ± 0.01 | 0.03 ± 0.01* |
| | 0.18 ± 0.01 | 0.09 ± 0.01 | 0.07 ± 0.01 |
| | 0.45 ± 0.03 | 0.15 ± 0.01 | 0.16 ± 0.06 |
| | 0.62 ± 0.04 | 0.03 ± 0.03*b | 0.33 ± 0.03 |
| Pyrene | 0.16 ± 0.02 | 0.11 ± 0.01 | lost |
| | 0.16 ± 0.01*a | 0.17 ± 0.001*a | lost |
| | 0.30 ± 0.03 | 0.37 ± 0.03 | lost |
| | 0.80 ± 0.09 | 0.45 ± 0.15 | lost |

*a = 2; error represents one half of range.

*bData unexplainably low.
at 4°C; the overlying water was then decanted, and fresh lake water added and mixed with the sediment. Sediment was allowed to settle for 24 h and the overlying water was again decanted. Lake water (2 cm) was then placed over the sediment and allowed to equilibrate for 1 month at 4°C.

At the end of the equilibration period, the overlying water was removed, the sediment for each dose was stirred to visual homogeneity, and 75 g was added to each of 15 beakers per dose. Samples were taken at the beginning, middle, and end of the distribution to determine the wet-to-dry weight and sediment contaminant concentration. Lake water (400 ml) was added carefully to each beaker to minimize sediment disturbance, and the sediment was allowed to settle at 4°C overnight. After settling, Diporeia (20 juveniles, 4 to 8 mg wet weight) were added to each beaker. Triplicate beakers were sampled at 3, 7, 14, 21, and 28 d.

At each sampling period, the oxygen content of the overlying water was measured by Winkler titration [15]. Hardness and alkalinity of the overlying water were determined by standard methods [16]. Sediment samples for contaminant concentration and dry-to-wet weight ratio measurements were taken carefully, excluding any organisms, from each beaker. The remaining sediments were sieved and the number of live organisms recorded. All animals that were not recovered were presumed dead. Two organisms per beaker were removed and placed in a 60-ml BOD bottle for 24 h at 4°C to determine respiration. The remainder were blotted dry, weighed, and frozen prior to extraction.

Organisms taken for respiration were held in 60-ml BOD bottles overnight. Three controls without animals were also kept overnight. At the end of 24 h, the organisms were carefully removed, blotted dry, and weighed. The oxygen concentration was determined by Winkler titration [15].

**ID experiment.** Dosing solutions were prepared by adding the appropriate amounts of a 60-mg ml⁻¹ acetone stock solution for each dose to small vials. Subsequently, 10.8 μCi of [³H]pyrene was added and the volume adjusted to 4.0 ml acetone. Acetone (4.0 ml) was also added to the control sediment. Duplicate samples of the dosing solutions (2 μl) were taken for H activity determination to ascertain the extent of isotopic dilution. Four doses (Table 2) were created by adding the acetone solutions dropwise with stirring to a slurry of wet sediment (2000 g) in 2 L lake water for each dose. Suspensions were stirred for 4 h at room temperature and were allowed to settle overnight at 4°C; the overlying water was then decanted, and fresh lake water added and mixed with the sediment. Sediment was allowed to settle for 24 h and the overlying water was again decanted. Lake water (2 cm) was then placed over the sediment and allowed to equilibrate for 1.5 months at 4°C.

At the end of the equilibration period, the overlying water was removed, the sediment for each dose was stirred to visual homogeneity, and 75 g were added to each of 15 beakers per dose. Samples were taken at the beginning, middle, and end of the distribution to determine the wet-to-dry weight and sediment pyrene concentration and radiopurity. Lake water (500 ml) was added carefully to each beaker to minimize sediment disturbance, and the sediment was allowed to settle at 4°C overnight. After settling, Diporeia (20 juveniles, 4 to 8 mg wet weight) were added to each beaker. Triplicate beakers were sampled at 3, 7, 14, 21, and 28 d.

At each sampling period, the oxygen content for the overlying water was measured with a YSI oxygen meter. Sediment samples for contaminant concentration and dry-to-wet weight ratio measurements were taken carefully, excluding any organisms, from each beaker. The remaining sediments were sieved and the number of live organisms recorded. All animals that were not recovered were presumed dead. Two organisms per beaker were blotted dry and weighed, and H activity was measured. An additional two organisms per sample were taken from each beaker on sampling days 14 and 28 for lipid analysis. Lipids were also determined on organisms taken from the culture at the beginning of the experiment.

**Bioconcentration factor determination**

The bioconcentration factor (BCF) for Diporeia in water only exposures was determined for use in the equilibrium-partitioning approach calculations. Filtered lake water was dosed with radiolabeled compounds in pairs: [³H]pyrene (12 pmol L⁻¹) and [¹⁴C]phenanthrene (5 nmol L⁻¹). Additionally, BCFs were determined with two other pairs of hydrophobic contaminants: [³H]benzo[a]pyrene (30 pmol L⁻¹) with [¹⁴C]-2,4,5,2',4',5'-hexachlorobiphenyl (3.3 nmol L⁻¹), and [³H]pyrene (12 pmol L⁻¹) with [¹⁴C]-2,4,2',4'-tetrachlorobiphenyl (3.8 nmol L⁻¹). Diporeia were exposed in 6-L aquaria containing 4 L dosed lake water at 4°C under static conditions. Organisms and water samples were taken at 96, 216, 360, and 552 h. Diporeia were weighed wet and radioactivity was determined by placing them directly in scintillation cocktail (as described below). Water samples were also placed directly in scintillation cocktail for radioactivity determinations. Additionally, water samples were taken to determine the amount of chemical bound to dissolved organic matter by determining the activity in a sample before and after passing it through a C₁₈ reversed-phase cartridge [17]. The amount of compound passing through the cartridge is assumed to be the amount bound to dissolved organic matter. The apparent BCF was calculated as the ratio of organism concentration divided by the "freely dissolved" (total bound) water concentration. The lipid contents of the organisms were also measured at the end of the exposure by a microgravimetric method [18]. The biotransformation for the PAH congeners was assessed by the combination of thin-layer chromatography and liquid scintillation spectrometry [14].

<table>
<thead>
<tr>
<th>Table 2. Pyrene sediment concentrations (µmol g⁻¹) for the isotope dilution experiment (mean ± SD, n = 3)</th>
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<tbody>
<tr>
<td>Initial</td>
</tr>
<tr>
<td>0.26 ± 0.003</td>
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<tr>
<td>0.58 ± 0.04</td>
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<tr>
<td>0.91 ± 0.01*</td>
</tr>
<tr>
<td>1.23 ± 0.02</td>
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* n = 2; error represents half the range.
Analyses

Sediment dry weight was determined by drying aliquots at 90°C to constant weight. The volatile solids content was determined by combusting the dry sediment at 500°C for 1 h. Sediment organic carbon content was measured after removing carbonates with HCl on a Perkin-Elmer 2400 CHN analyzer. The carbonate was removed by adding 2 ml of 1 N HCl per 100 mg dry sediment. The mixture was shaken for 24 h and dried at 90°C. The fraction of fines was determined by wet sieving an aliquot of sediment. Then, both the material retained on a 63-μm sieve and that passing through were collected, dried, and weighed.

**HPLC experiment.** Sediment contaminant concentrations were determined in triplicate by reversed-phase HPLC. The chrysene internal standard was added. Sediment samples (approximately 5 to 10 g wet weight) were weighed and chrysene internal standard was added. At the same time, samples were also taken for wet-to-dry weight determination. Samples were then desiccated with anhydrous sodium sulfate and extracted by adding first 50 ml ethyl acetate, which was mixed into the sediment, and then 50 ml cyclohexane. Solvent and sediment were mixed by swirling every couple of days while standing a minimum of 1 week. (The relative recovery from the static extraction was 104% of the Soxhlet recovery for pyrene and 86.3% for phenanthrene.) The solvents were separated from the sediment by filtration, and the extraction jar and sediment were rinsed twice with 10 ml cyclohexane. The combined solvent was reduced in volume by rotary evaporation to approximately 5 ml and evaporated under a stream of nitrogen to 100 μl. Acetonitrile (2.0 ml) was added, and the samples were centrifuged to sediment obvious precipitate. After centrifugation, the acetonitrile solution was transferred to vials for HPLC analysis.

Organism samples were placed in a glass tissue homogenizer, spiked with the chrysene internal standard, and extracted twice with 20 ml ethyl acetate:acetone (4:1 v/v) and twice with 20 ml cyclohexane. Extracts were combined, dried over anhydrous sodium sulfate, and prepared for HPLC analysis as described for the sediment extracts.

Reversed-phase HPLC with UV detection at 254 nm was used for the pyrene and phenanthrene analyses. Separation was performed with a gradient elution from 40 to 100% acetonitrile in water. Flow was 1 ml min⁻¹, and the gradient program was 20 min ramp, followed by 15 min at 100% acetonitrile and 5 min equilibration delay at 40% acetonitrile between samples. Quantitation was determined by the internal standards ratio method, using chrysene as the internal standard. Relative response factors were determined daily. Quantitation was double-checked by the standard curve method.

**ID experiment.** Wet sediment samples (approximately 100 mg) were added directly to scintillation cocktail (12 ml, Research Products International, 3a70B) and sonicated with a Tekmar sonic disrupter for 2 min each. Additional samples were taken at the same time for wet-to-dry weight determination. Samples were allowed to stand for 24 h, and ³H activity was determined by liquid scintillation counting. Organisms were added directly to scintillation cocktail, sonicated for 1 min, allowed to stand for 24 h, and analyzed for ³H activity. The lipid contents of organisms were measured by a microgravimetric method [18].

Particle-size distribution of sediment mass and pyrene were determined by a modified sedimentation technique [19, 20] for both the lowest and the highest doses used in the ID experiment. In addition to these two concentrations, trace level [³H]pyrene (2.1 pmol g⁻¹) sediment was prepared and analyzed. Approximately 40 g wet sediment was wet-sieved using filtered (Gelman Sciences, glass fiber, type A/E) lake water through 420-, 105-, and 63-μm standard sieves. Materials remaining on each sieve were collected. Triplicate samples were taken for liquid scintillation counting; the remainder were dried to constant weight at 90°C for dry-weight analyses. Material passing through the 63-μm sieve was mixed at room temperature with filtered lake water (total volume 1 L) in a 1-L graduated cylinder. Samples (25 ml) from sediment suspension were taken at specific depths and times after mixing based on calculations using Stoke’s law with 2.6 g ml⁻¹ as the specific gravity of the particles [19]. Three 2-ml aliquots from each sample were analyzed by liquid scintillation counting. The remaining portion of the sample (19 ml) was dried to constant weight at 90°C for dry weight.

Counting for ³H activity was performed on a Wallach LKB 1217 liquid scintillation counter. After subtracting background, samples were corrected for quench and counting efficiency using the external standards ratio method.

Calculations

The respiration rate was calculated from the difference in concentration between the control bottles and the bottles containing Diporeia through the following equation:

\[
R = \frac{(O_2^{\text{control}} - O_2^{\text{experimental}}) \cdot (V)}{(W) \cdot (t)},
\]  
(1)

where \(R\) is the respiration rate (µg O₂ mg⁻¹ h⁻¹), \(O_2\) concentrations are in µg O₂ ml⁻¹, \(V\) is the volume of the BOD bottle (ML), \(W\) is organism weight (mg), and \(t\), time, is in hours.

The kinetics of accumulation were determined by fitting the data to a first-order rate-constant model:

\[
C_a = \frac{k_a \cdot C_0 \cdot (e^{-k_d t} - e^{-k_f t})}{(k_d - \lambda)},
\]  
(2)

where \(k_i\) is the uptake clearance coefficient (g dry sediment g⁻¹ wet organism h⁻¹), \(C_0\) is the concentration in the sediment (µmol g⁻¹) at \(t = 0\), \(\lambda\) is the rate constant for change in bioavailable concentration (h⁻¹), \(t\) is time (h), \(k_e\) is the elimination rate constant (h⁻¹), and \(C_a\) is the concentration in the organism (µmol g⁻¹) [8]. Uptake clearance is defined as the amount of the source compartment scavenged of contaminant per mass of organism per time (see [5] for discussion of uptake clearance). While the full model was required to evaluate the phenanthrene data, simplifications
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were possible with the pyrene data, where apparently \( \lambda \) equaled zero.

The time-dependent apparent bioconcentration factors (BCFs) were fit by nonlinear regression to Equation 3 to estimate the steady-state BCF:

\[
BCF = BCF_{ss}(1 - e^{-kt}),
\]

where \( k_1 \) is the apparent elimination rate constant (h\(^{-1}\)), and \( t \) is time (h).

Mortality data were fit to the probit model [21] for determining the LC50 and LD50 values. Means and slopes were compared using Student’s \( t \) test. Statistical differences were considered significant if \( p < 0.05 \).

**RESULTS**

**Experimental characteristics**

**HPLC experiment.** The volatile-solids contents of the sediments were 1.3 ± 0.2% (phenanthrene, mean ± sd, \( n = 3 \)) and 1.4 ± 0.3% (pyrene, \( n = 4 \)). Percent fines (<63 \( \mu \)m) were 39 ± 0.2% (phenanthrene, \( n = 5 \)) and 38 ± 1.2% (pyrene, \( n = 5 \)). The sediment organic carbon measurements were nearly twice the volatile-solids values. These invalid (i.e., impossible) measurements probably reflect incomplete removal of the carbonates.

The extraction recovery, based on total compound dosed to the sediment slurry, was 65 ± 10% (\( n = 3 \)) for static extraction and 75 ± 6% (\( n = 3 \)) for Soxhlet extraction for phenanthrene, and 63.4 ± 3.9% (\( n = 3 \)) and 60.5 ± 3.1% (\( n = 3 \)), respectively, for pyrene. The amounts found on the sediment relative to expected concentrations were not statistically different for the two methods, which indicates that the static extraction was adequate for the experiment. Recoveries of 65 and 60% for phenanthrene and pyrene, respectively, were similar to previously reported results for these compounds when dosed into sediments via aqueous suspensions [8]. The concentrations of phenanthrene declined significantly, approximately 50%, over the course of the experiment (Table 1), with most of the change occurring in the first 8 d. Pyrene concentrations remained essentially constant for the first 8 d (Table 1). Samples for the last day of the experiment were lost.

The overlying water was examined for changes in quality characteristics throughout the HPLC experiment. At the beginning of the experiment, the pH was 8.0 (\( n = 2 \)), 8.01 ± 0.03 (\( n = 20 \)) after 2 weeks, and 8.0 ± 0.02 (\( n = 20 \)) at the end of the experiment. Initial alkalinity was 112 ± 0.6 mg CaCO\(_3\) L\(^{-1}\) (\( n = 3 \)) and increased slightly to 128 ± 4.5 (\( n = 14 \)) by the end of the experiment. Hardness was essentially constant, 168 ± 2.1 mg CaCO\(_3\) L\(^{-1}\) initially, and 173 ± 14 (\( n = 16 \)) at the end of the experiment. Oxygen concentration was 8.8 ± 0.73 \( \mu \)g O\(_2\) ml\(^{-1}\) after 24 h and 8.6 ± 0.23 (\( n = 15 \)) at the end of the experiment. Because water quality was so stable during the HPLC experiment, only oxygen was monitored for the ID experiment.

Respiration rates for Diporeia were measured at each sampling point, and no differences were found among doses at any sampling time. Averages were 0.41 ± 0.29 \( \mu \)g O\(_2\) mg\(^{-1}\) h\(^{-1}\) (\( n = 71 \)) for the phenanthrene experiment and 0.24 ± 0.13 \( \mu \)g O\(_2\) mg\(^{-1}\) h\(^{-1}\) (\( n = 44 \)) for the first 8 d of the pyrene experiment. Respiration rates for later sampling times in the pyrene experiment could not be determined because samples had O\(_2\) concentrations as high or higher than the control water. However, the amount of thiosulfate titrant required for each sample indicated no significant variation among the doses. Respiration rates were similar to those measured previously for Diporeia [22].

**ID experiment.** The percent fines were 42.8% (\( n = 2 \)) for the high dose and 42.1% (\( n = 1 \)) for the low dose. The volatile-solids content increased significantly from 1.54 ± 0.11% (\( n = 12 \)) to 2.09 ± 0.02% (\( n = 12 \)) by the end of the second week. While the volatile-solids content increased significantly, there were no differences among the different doses for any one sample period. The sediment organic carbon content was determined at the beginning (0.46 ± 0.04%, \( n = 14 \)) and at the end (0.40 ± 0.07%, \( n = 11 \)) of the experiment. No significant differences existed in the sediment organic carbon content, either among doses or over time from the beginning to the end of the experiment. Overall, the volatile-solids content tended to increase, while the sediment organic carbon content did not change.

The extraction efficiency for pyrene was essentially quantitative. The amount dosed and that found on the sediments were essentially the same, average accountability 109 ± 5%. Pyrene concentrations were essentially constant over the course of the experiment (Table 2).

Sediment from the Lake Michigan 45-m station is dominated by particles ranging in size from 420 \( \mu \)m down to 45 \( \mu \)m. These particles make up about 80% of the total dry weight of the sediment (Fig. 1). There was no difference in particle-mass distributions between different pyrene doses. However, pyrene distribution in these three samples was different (Fig. 1). At the high pyrene concentration, small particles tended to sequester a greater proportion of pyrene (23.1 ± 1.3% of total, \( n = 2 \)) than at the trace-level concentration (10.7 ± 0.6% of the total, \( n = 2 \)). Particles between 63 \( \mu \)m and 43 \( \mu \)m bound 26.1 ± 7.7% (\( n = 2 \)) at the highest pyrene dose and 43.1 ± 8.2% (\( n = 2 \)) at the trace-level concentration. The lowest ID experiment concentration (0.26 \( \mu \)mol g\(^{-1}\)) was intermediate between these two concentrations (Fig. 1). The relative concentrations of pyrene (pyrene concentration in a fraction divided by pyrene concentration in the bulk sediment) in different fractions also suggest that pyrene tends to concentrate on the smaller particles at high doses (Fig. 2A). With all doses, the relative pyrene concentrations were less than 1 in coarse fractions (>63 \( \mu \)m, Fig. 2A). Thus, the material in the large particles does not bind pyrene as efficiently as those of the smaller particles. At the trace-level concentration, pyrene relative concentration was between 3 and 4.5, suggesting a uniform distribution within the smaller (<43 \( \mu \)m) particles. At the highest dose, particles <10 \( \mu \)m had 7 to 10 times higher pyrene concentrations than the bulk sediment (Fig. 2A). Further, differences in the relative concentration among particle sizes remain even with carbon normalization (Fig. 2B).

Oxygen concentration was 8.4 ± 1.4 (\( n = 15 \)) \( \mu \)g O\(_2\) ml\(^{-1}\) at the end of 1 week and ranged from 10 to 12 \( \mu \)g O\(_2\) ml\(^{-1}\) over the remainder of the experiment. Diporeia health was
Fig. 1. Particle mass (A) and pyrene distribution (B) in Lake Michigan sediment at three different doses of pyrene. Values shown represent the mean of two replicates.

monitored by measuring the lipid content on a dry-weight basis. Initial lipid content was 25.9 ± 9.0% (n = 14), and 22.2 ± 5.6% (n = 15) at the end of the experiment. No trend in lipid content was noted with exposure.

Experiment comparisons. The percent fines were similar in both the HPLC and ID experiments and, initially, the volatile solids were similar as well. Therefore, the fraction of sediment organic carbon was presumed to be similar, particularly because the sediments came from the same station. The sediment characteristics were also similar to those determined previously for sediments collected at the same station [23]. The amount of carrier was not expected to affect the partitioning of the PAH congeners to the sediment for either experiment because the aqueous cosolvent effect of water-soluble solvents at the fraction employed to dose the sediment (2%) shows minimal effect on partitioning to aqueous suspensions of soils [24]. Additionally, the decanting and addition of fresh lake water should have removed essentially all the carrier prior to the exposures.

Toxicokinetics

Accumulation of phenanthrene by Diporeia was fit to the first-order model presented above (Eqn. 2). Uptake clearance coefficients (k, g dry sediment g⁻¹ wet organism h⁻¹) were essentially constant with increasing dose, as were k₆ and λ, except for the 0.45-pmol g⁻¹ dose where both k₆ and λ increased (Table 3).

Uptake and elimination coefficients for pyrene (Table 4) were determined by fitting the accumulation data to the two-compartment model with λ = 0, because these data exhibited apparent saturation curves (Fig. 3) except for the 0.52-μmol g⁻¹ dose in the ID experiment where the model would not converge. For the 0.52-μmol g⁻¹ dose, the model was further simplified because the accumulation was appar-
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Fig. 2. Relative concentrations of pyrene in sediment particle-size fractions at three different concentrations (A) on a particle-mass basis and (B) on an organic carbon-normalized basis. The relative concentration in each fraction was calculated by dividing the pyrene concentration in the fraction by the pyrene concentration in the bulk sediment on a mass or organic carbon basis.

Fig. 3. Mean concentrations of pyrene in Diporeia: For the HPLC experiment (A) where the sediment doses are ■ = 0.14 μmol g⁻¹; ■ = 0.16 μmol g⁻¹; and ▲ = 0.62 μmol g⁻¹, and for the ID experiment (B) where the sediment doses are ■ = 0.26 μmol g⁻¹; ■ = 0.52 μmol g⁻¹; ■ = 0.86 μmol g⁻¹; and ▲ = 1.11 μmol g⁻¹.

Table 4. Toxicokinetic parameters for pyrene accumulation by Diporeia

<table>
<thead>
<tr>
<th>Sediment dose (μmol g⁻¹)</th>
<th>kₐ (g⁻¹ h⁻¹)</th>
<th>kₗ (h⁻¹)</th>
<th>kₐ (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC experiment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.14</td>
<td>0.030 ± 0.005</td>
<td>0.002 ± 0.0007</td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>0.049 ± 0.009</td>
<td>0.0023 ± 0.0009</td>
<td></td>
</tr>
<tr>
<td>0.31</td>
<td>0.044 ± 0.005</td>
<td>0.0031 ± 0.0006</td>
<td></td>
</tr>
<tr>
<td>0.62</td>
<td>0.019 ± 0.004</td>
<td>0.002 ± 0.0008</td>
<td></td>
</tr>
<tr>
<td>ID experiment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.26</td>
<td>0.048 ± 0.003</td>
<td>0.0016 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>0.52</td>
<td>0.018 ± 0.0011</td>
<td>ND²</td>
<td></td>
</tr>
<tr>
<td>0.86</td>
<td>0.018 ± 0.002</td>
<td>0.0012 ± 0.0003</td>
<td></td>
</tr>
<tr>
<td>1.11</td>
<td>0.017 ± 0.001</td>
<td>0.0022 ± 0.0002</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SE.

Sediment contaminant concentrations (Table 1 and Table 2) in the two experiments were designed to yield accumulated concentrations in the Diporeia of approximately 6 μmol g⁻¹ based on the accumulation kinetics [11]. However, there was evidently linear [8]. The pyrene kₐ values exhibited a peak at about 0.21 μmol g⁻¹ and remained fairly constant between 0.5 and 1.1 μmol g⁻¹ (Fig. 4).

Toxicity

Sediment contaminant concentrations (Table 1 and Table 2) in the two experiments were designed to yield accumulated concentrations in the Diporeia of approximately 6 μmol g⁻¹ based on the accumulation kinetics [11]. However, there was
Fig. 4. Uptake clearance coefficients for exposure to pyrene only (●) and to a mixture of PAHs (■) where the total PAH concentration was similar on a micromolar basis. Error bars represent standard error from regression.

Fig. 5. Mean accumulation of phenanthrene by Diporeia over the 31-d exposure to spiked sediments of the following doses: ■ = 0.08 μmol g⁻¹; ● = 0.18 μmol g⁻¹; ▲ = 0.45 μmol g⁻¹; and ◆ = 0.62 μmol g⁻¹.

Fig. 6. Percent mortality versus Diporeia pyrene concentration for the HPLC experiment (A) and for the ID experiment (B).

essentially no toxicity with the phenanthrene exposures. Although the doses were designed to produce significant mortality, only 12 ± 3% mortality was observed at the highest dose by the end of the experiment, which reflects the failure of the Diporeia to accumulate and maintain toxic body burdens of phenanthrene (Fig. 5).

While pyrene was toxic to Diporeia, LC50 values could only be estimated for the HPLC experiment (147 μg g⁻¹, 0.73 μmol g⁻¹). No confidence intervals for the LC50 value could be calculated owing to the variability of the data. In the ID experiment, no LC50 value could be determined because toxicity was lower at the highest dose (22 ± 6%, n = 3, mean ± SD) than at the next lower dose (33 ± 10%, n = 3). Calculating toxicity on the basis of body residue at the end of the experiment yielded a dose–response relation-ship from which 95% confidence intervals and median toxicity values were obtained (Fig. 6). The LC50 for pyrene in Diporeia was 6.3 (4.6–41.7) μmol g⁻¹ for the HPLC experiment and 9.4 (7.9–54.2) μmol g⁻¹ for the ID experiment. These values were not statistically different.

Bioconcentration factors

The BCFs were determined to permit equilibrium-partitioning calculations. The BCF for pyrene was near steady state by 552 h (Fig. 7) and the curves for the other compounds were similar, but some of the more hydrophobic compounds were not as near steady state at the end of 552 h as was the pyrene. The BCF values did not track with the log Kow values directly or on a lipid-normalized basis (Table 5). Bioconcentration factors were determined using radiolabeled compounds, and if biotransformation occurred the BCF values for the parent compound could be biased compared to that determined for the radiotracer. The measured amount of potential metabolites was low (3.9 ± 0.3% for benzo[a]pyrene, 7.6 ± 4.9% for phenanthrene, and 7.8 ± 4% for pyrene). This is consistent with previous studies where biotransformation of PAH congeners by Diporeia spp. was low to nondetectable for anthracene [14] and benz[a]pyrene at 4°C in experiments extending to 56 d [23] or at an elevated
Pyrene toxicity in Diporeia

![Graph](image)

**Fig. 7.** Time course for the mean bioconcentration factors in Diporeia. TCBP = tetrachlorobiphenyl; PY = pyrene; PHE = phenanthrene; HCBP = hexachlorobiphenyl; BAP = benzo[a]pyrene.

The temperature of 10°C out to 14 d [26]. Thus, the failure of the BCF to track with log K<sub>ow</sub> is not caused by PAH biotransformation. Biotransformation would also not be expected to account for the reversal of the BCF values relative to log K<sub>ow</sub> for the two chlorinated biphenyls since these compounds are known to be poorly metabolized by nearly all organisms.

**DISCUSSION**

**Toxicity**

A major purpose of these experiments was to evaluate the toxicity of selected PAH congeners dosed into sediments and to compare these results with responses to a mixture of PAHs [11]. Assays using Diporeia and sediments dosed with a mixture of PAHs showed sediment concentrations about equally useful for describing toxicity as the concentration in the organism [11]. The toxicokinetics from the mixture experiment were used to calculate doses for the HPLC experiment that would produce 6 μmol g<sup>-1</sup> in Diporeia after 28-d exposure. The kinetics for these concentrations were calculated doses for the HPLC experiment that would produce 6 μmol g<sup>-1</sup> in Diporeia after 28-d exposure. The kinetics for these experiments come from the equation for the two chlorinated biphenyls since these compounds are known to be poorly metabolized by nearly all organisms.

**Table 5. Bioconcentration factors for selected PAH and PCB congeners in Diporeia on both a wet-weight basis (BCF) and a lipid-normalized basis (BCF)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log BCF&lt;sub&gt;w&lt;/sub&gt;</th>
<th>Log BCF</th>
<th>K&lt;sub&gt;ow&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenanthrene</td>
<td>5.28 (5.11–5.37)</td>
<td>4.18</td>
<td>4.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pyrene</td>
<td>5.83 (5.78–5.87)</td>
<td>4.72</td>
<td>5.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>5.43 (5.37–5.48)</td>
<td>4.33</td>
<td>5.98&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hexachlorobiphenyl</td>
<td>6.33 (6.24–6.40)</td>
<td>5.23</td>
<td>6.92&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tetrachlorobiphenyl</td>
<td>6.71 (6.62–6.79)</td>
<td>5.62</td>
<td>5.85&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>BFC was calculated from the mean lipid content of 29.7 ± 6.7% of dry weight and the dry-to-wet weight ratio of 0.269 [38].

<sup>b</sup>Numbers are the mean and range for 1 SD for the 552-h sample.

<sup>c</sup>Numbers in parentheses are 95% confidence intervals from the regression equation.

<sup>d</sup>[38].

<sup>e</sup>[39].

expected to be 0.60 μmol g<sup>-1</sup> dry sediment with a confidence interval of 0.17 and 0.86 μmol g<sup>-1</sup>. Similar calculations for phenanthrene placed the dose for 50% mortality between 0.13 and 0.62 μmol g<sup>-1</sup> dry sediment.

The reason for the low phenanthrene toxicity, even at the highest sediment dose, seems clear. On average, the body burden remained lower than the anticipated 6 μmol g<sup>-1</sup> required to produce 50% mortality, and only approached this concentration for a short period of time (Fig. 5). Two sources appear to account for reduced accumulation compared to projections: (a) The sediment was stored for 1 month prior to testing, and sediment aging is known to reduce the bioavailability of phenanthrene [8,10]; and (b) the measured concentrations declined significantly over the course of the experiment. A decline in phenanthrene concentration was not totally unexpected, as a similar decline had been observed previously for this compound [9,11]. The magnitude of the decline was, however, greater than expected although similar to that observed in one other study where the phenanthrene-dosed sediment had been aged 3 d [9]. Why phenanthrene concentration declines in some experiments rather sharply (this study, [9]) and not so sharply in others [8,11] remains unknown. Because phenanthrene is so soluble, it may be more readily available for microbial biodegradation, or it may intercalate into particles more easily and become less extractable and bioavailable.

Pyrene toxicity could not be described as a dose–response relationship based on the sediment concentration in either experiment. Although an LC50 could be estimated in the HPLC experiment, the data were not good enough to permit calculation of confidence intervals. The failure to obtain a classical dose–response relationship when sediment concentrations were employed may result from either biological or chemical factors. Sediment composition can be ruled out as a variable, because the sediment was the same for all doses. However, the particle-size distribution of pyrene varied with dose. At trace levels, pyrene was distributed more evenly among fine particles of different sizes (<63 μm) than at the high dose, where the pyrene concentration on the finest particles was 10 times higher than in the bulk sediment (Fig. 2).

Some of the variation among the particle-size fractions can be attributed to the different organic carbon content in the fractions. But even the organic carbon–normalized relative concentrations show the same trend, thus carbon normalization does not remove the difference between pyrene doses (Fig. 2B). Further, compound associated with the <20-μm fraction of some sediments is apparently not available via ingestion for Diporeia [27].

Biological factors that contribute to the failure of the sediment concentration to describe mortality include the observed peak in K<sub>ow</sub> values. Such stimulation was also observed for pyrene and other PAH congener kinetics in the mixture exposure. This apparent stimulation may be the result of increased feeding, which can lead to increased accumulation [27]. Further, as the organisms became intoxicated, the feeding rate may decline, producing lower bioavailability at the highest doses.

Finally, the body burden was adequate to describe the dose response. The LD50 values were 6.3 and 9.4 μmol g<sup>-1</sup>
for the HPLC and ID experiments, respectively. These values are not statistically different and are equivalent to the concentration of PAHs as the molar sum of the added congeners that produced toxicity in the mixture [11]. These concentrations are in a range similar to that for other organisms exhibiting mortality due to nonpolar narcosis, as reviewed in Landrum et al. [5]. This study confirms that for Diporeia exposed to PAHs the narcotic effect depends on attaining a certain molar concentration in the organism and that, for a mixture of PAHs, molar additivity is a reasonable approximation of the dose. This finding encourages continued development of a residue-based approach for hazard evaluation [5].

Physiological responses

Measures of respiration were expected to produce an earlier and more sensitive response to the PAHs than measures of mortality. The PAH congeners, for example, naphthalene and phenanthrene, are known to reduce respiration at solution concentrations in the mg L\(^{-1}\) range [28-34]. Further, changes in respiration were expected because toxicokinetic changes have been seen with PAHs at concentrations sufficient to produce mortality ([11], this study). Changes in toxicokinetics are thought to result, at least in part, from increased feeding rates, which lead to increased accumulation [27,35], and in turn, presumably, to increased respiration. However, even when the PAH congener pyrene was at toxic concentrations based on the mortality response, no difference in the respiration rate was observed. Thus, at levels required to produce a narcotic effect in Diporeia, the concentrations are not sufficient to reduce or stimulate respiration at a magnitude observable by the techniques used.

The lipid content of Diporeia in the ID experiment did not significantly change over the course of the study. This result is similar to that of other toxicity assays performed with these sediments [36]. Thus, the energy requirements of Diporeia are apparently being met, and their metabolism is unchanged despite the presence of toxic concentrations of PAHs. Both the respiration and lipid data would suggest that the animals’ health remained generally good.

Toxicokinetics

The uptake clearances for phenanthrene were essentially constant across all doses. When changes in \(k_r\) for phenanthrene have been observed, they have been associated with toxic responses [11]. The fact that little mortality was observed and that the internal dose only approached the toxic range on a transient basis suggests why the kinetics did not change with dose. Accumulation kinetics for phenanthrene were similar to those observed for Diporeia exposed to a mixture of PAHs at the same concentration range based on the molar sum of the PAHs [11]. The \(k_v\) value was approximately twice that observed for Diporeia exposed to sediment collected from the same station and aged for a similar length of time at lower dose (0.17 mmol g\(^{-1}\) dry sediment) [10]. Thus, for phenanthrene the higher doses seem to be more available even after aging. The values for \(k_v\) and \(\lambda\) were generally twice those observed for Diporeia exposed at low concentrations [8,37]. Overall, the kinetics seemed to respond to the greater exposure, but not in a manner producing any predictable dose–response relationship.

The trend in pyrene uptake kinetics was similar among the HPLC and ID experiments. Both showed a peak in \(k_v\) values with increasing dose. A similar response was observed for pyrene in the PAH mixture, although the \(k_v\) values were somewhat larger in the mixture experiment than were values over a similar range of total PAH concentrations (Fig. 4) [11]. The \(k_v\) values found by nonlinear regression of the data were similar to those found previously at trace concentrations [37]. A nonlinear model was suitable for all concentrations except the 0.52-\(\mu\)mol g\(^{-1}\) concentration in the ID experiment for which a linear model was most appropriate. Accumulation data did not exhibit changes in the bioavailable concentration over the course of the experiments. These results differ from studies employing pyrene at trace doses [8]. The chemistry observed for trace exposures may be influenced more than at higher doses by different particle characteristics. As described previously, the particle-size distribution of pyrene at trace levels differs from that at the more concentrated doses. The factors that caused these changes in bioavailability at low doses may have been masked at the high doses in the HPLC experiment by analytical variability and in the ID experiment by isotopic dilution. Additionally, the process observed at trace levels can perhaps only accommodate a maximal mass; thus, the fractional change at high doses would not be observed.

Comparison of observed pyrene toxicity with sediment-quality assessment values

The very crude LC50 estimates make comparison with any sediment-quality assessment value difficult. However, if the LC50 is considered to be between 147 and 223 \(\mu g\) g\(^{-1}\) (0.72-1.1 \(\mu\)mol g\(^{-1}\)), as suggested by the limited data available, then the whole sediment–estimated LC50 was well above the effects range median presented by Long and Morgan [2]. This suggests that Diporeia is much less sensitive to pyrene than other aquatic organisms, yet this conclusion is not supported by the tissue residue values. If the inherent sensitivity of Diporeia for nonpolar narcosis is not different from other organisms, then the greater concentration required to produce mortality compared to the effects range median may be related to the rate or extent of accumulation (bioavailability) issues. These bioavailability questions continue to promote discussion of appropriate approaches and values for sediment-quality criteria.

One approach for normalizing apparent differences in bioavailability of sediment-associated contaminants is the equilibrium-partitioning approach [4]. If one considers the BCF for Diporeia, and the requirement of 6.3 \(\mu\)mol g\(^{-1}\) as the internal dose required to produce 50% mortality (HPLC experiment), then the apparent sediment concentration required to produce 50% mortality can be calculated from equilibrium partitioning. To accomplish this, the partition coefficient between the sediment and the interstitial water must be established on an organic carbon–normalized basis. Employing the relationship between log \(K_{oc}\) and log \(K_{BCF}\) from Di Toro et al. [4], and using a log \(K_{oc}\) of 5.2 [38], the
resultant log $K_{oc}$ value would be 5.11. This value is consistent with the log $K_{oc}$ measured for pyrene dosed to sediment from the same station (range 4.76–5.05) [11]. The calculated sediment concentration to produce an LC50 can then be determined by first calculating the water concentration required to achieve $6.5 \, \mu mol \, g^{-1}$, based on the BCF (Table 5): $1.2 \times 10^4 \, \mu mol \, ml^{-1}$, or 24 ng ml$^{-1}$. This concentration does not exceed the water solubility of pyrene, which is 134.7 ng ml$^{-1}$ [38]. Converting to the sediment concentration using the $K_{oc}$ and calculated water concentration suggests a concentration of 3,092 $\mu g \, g^{-1}$ organic carbon. With an organic carbon content of 0.46%, the sediment concentration would be projected to be 14.2 $\mu g \, g^{-1}$ sediment. Based on these calculations, the Diporeia in this study do not appear to be close to equilibrium. These sediments may not have attained equilibrium in the 1 to 1.5 months between dosing the sediments and initiating the exposures. However, since the partitioning continues to increase over time [9], the compounds should be more bioavailable than if the sediments had set longer. Thus, the difference between the calculated value and the measured estimate of the whole-sediment LC50 could have been greater had the sediments been at complete equilibrium. Further, the measures of PAH congener concentrations by two separate methods, namely HPLC analysis and radiotracer analysis, yielded similar results; therefore, the methodology for analysis did not bias the findings. Thus, the comparison of the calculated values with the estimated LC50 for the HPLC experiment suggests that, for Diporeia exposed in freshwater sediment, the equilibrium-partitioning approach overestimates the toxicity of sediment-associated pyrene by a factor of approximately 10.

One of the predominant assumptions of the equilibrium-partitioning approach is that the animals are at steady state. The kinetics suggest that the organisms in the HPLC experiment were at essentially 82% of the steady-state concentration. Thus, the assumption of steady state was nearly met in the exposures and should only account for a small amount of the difference between the calculated concentration and the measured concentration for 50% mortality. The partitioning to sediment from this station is nearly the same as the calculated value, and the organisms are nearly at steady state. Thus, the assumptions of the equilibrium-partitioning approach method do not seem to be violated. Consequently, it appears that kinetic limitations to bioavailability and biological modification of exposure remain as possible explanations for the difference between the predicted equilibrium-partitioning value and the observed LC50 values.

CONCLUSIONS

Sediment concentrations, even with no change in sediment composition among doses, were not a reliable measure of dose to Diporeia. The variation in pyrene distribution with dose among the <63-μm particle-size fraction of sediment appeared to provide a partial explanation. The stimulation in $k_x$ values also partially accounts for the failure to observe a classical dose response. Thus, for Diporeia both chemical and biological factors appear to alter the dose response anticipated by sediment concentration. The equilibrium-partitioning approach calculations appear to overestimate the toxicity of pyrene by a factor of about 10. The amount of accumulated compound, as a representation of the internal dose, was a useful representation of toxic response, yielding LD50 estimates for pyrene of 6.3 and 9.4 $\mu mol \, g^{-1}$. These LD50 estimates are similar to the concentration required to produce nonpolar narcosis in other aquatic organisms. Thus, organism sensitivity does not account for the difference between observed and calculated concentrations required to produce mortality. Overall, behavioral modification of exposure and/or kinetic limitations to exposure offer the best possible explanation for the difference between calculated and observed results. Respiration, as a second potential toxicity end point, was not useful for pyrene or phenanthrene exposures.

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REFERENCES


of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod Diporeia sp. Environ. Toxicol. Chem. 10:35–46.


