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## COMPARISON OF WHOLE-SEDIMENT, ELUTRIATE AND PORE-WATER EXPOSURES FOR USE IN ASSESSING SEDIMENT-ASSOCIATED ORGANIC CONTAMINANTS IN BIOASSAYS

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**Abstract**—Bioassays have frequently been used as tools to simulate exposure of benthos to sediment-associated contaminants in hazard assessments. Due to the problems involved with estimating bioavailability in whole-sediment bioassays, aqueous fractions such as elutriates and pore water have been substituted for whole-sediment exposures. The objective of this research was to compare and evaluate the bioavailability of representative neutral hydrophobic contaminants in whole sediments and in aqueous extracts of whole sediment (elutriate and pore water) in simultaneous bioassays, using three representative indicator species, *Diporeia* spp., *Chironomus riparius* larvae, and *Lumbriculus variegatus*. Aqueous extracts of whole sediment did not accurately represent the exposure observed in whole sediment. Generally, the aqueous extracts underexposed organisms compared to whole sediment, even after adjusting accumulation to the fraction of organic carbon in the test media. Accumulation comparisons among whole-sediment, elutriate, and pore-water exposures depended on sampling time. At some sampling times for some contaminants, differences in accumulation between a particular aqueous extract and whole sediment were not significant; however, these similarities were not observed for all species at the particular sampling time. Bioaccumulation and contaminant clearance data suggest that a number of factors such as the indicator species, exposure media, and chemical/physical properties of individual contaminants are responsible for the accumulation differences observed among the tested media. Normalizing bioaccumulation to the amount of organic carbon in a source compartment adjusted for bioavailability differences of only some contaminants. We suggest that the bioavailability of contaminants such as those tested cannot be accurately predicted in bioassays that expose organisms to aqueous representations of whole sediment.

**Keywords**—Bioaccumulation Sediment Elutriate Pore water Bioassay

### INTRODUCTION

Contaminated sediments are a recognized problem in many coastal areas and are presently a significant regulatory issue. The bioassay approach has been used to assess the toxicity of such sediments [1-4]. In the bioassay approach, organisms are exposed to whole sediments or aqueous extracts of whole sediments to evaluate and estimate contaminant concentrations that produce effects. Although whole-sediment exposures may be the most realistic lab simulation of in situ exposure to indicator species, aqueous extracts of whole sediment (i.e., pore water) have been used to circumvent problems that occur when whole sediments are diluted and in situations that require identification of soluble toxicants via toxicity identification evaluation [5-7]. Elutriate fractions, although originally intended to mimic the open-water disposal of dredged material, have also been used to determine toxicity of contaminated sediments [1,8]. To date, few studies have directly compared exposure of contaminants in whole sediments and their extracts by using the same species in simultaneous exposures. Of these studies, all comparisons of the various exposure types have used only toxicity as an end point [3,9,10]. However, toxicity is strongly influenced

by factors other than the amount of toxicant available to the organism; for example, oxygen concentration, habitat, temperature, and food. Thus, evaluating the bioaccumulation of toxicants by a variety of indicator species exposed to whole sediments and their aqueous extracts at nonlethal concentrations would increase our understanding of the basic processes involving exposure in these various representations of sediment and would permit better interpretation of toxicity bioassays. Furthermore, bioaccumulation assays that compare the uptake of sediment-associated contaminants in whole sediments and their aqueous extracts would be useful in identifying the most appropriate organisms and conditions for evaluating sediment contamination.

In this study, a number of hydrophobic organic contaminants were used to compare temporal accumulation in three indicator species exposed to whole sediment and pore water and elutriates obtained from the same contaminated sediment. *Chironomus riparius* larvae, *Diporeia* spp., and *Lumbriculus variegatus* differ in physiology and behavior, and represent a range of uptake strategies (e.g., feeding strategies and behavior) in freshwater benthic invertebrates. Midge larvae of *C. riparius* are considered nondiscriminant infaunal filter feeders [11-13]. Larvae spend only a portion of their life cycle in the aquatic environment, with pupation and adult emergence occurring 15-21 d after hatching in room-

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temperature lab cultures [14]. In contrast, both the amphipod *Diporeia* spp. and the oligochaete *L. variegatus* complete their life cycles in the benthic environment. Infaunal *Diporeia* spp. are intermittent, selective feeders [15–17]. Their high lipid content, reaching as much as 50% (dry-weight basis) [18], makes them an optimal species for studies involving the bioaccumulation of lipophilic organic contaminants. Conversely, *L. variegatus* is an infaunal sediment ingester [4,19] that has demonstrated continuous feeding behavior in the lab. All three species have been used in sediment toxicity and bioaccumulation tests, and have the potential to be used as indicator species for assessing hazard of sediment-associated contaminants.

Two main questions were posed in this study: Do pore-water and/or elutriate extracts of whole sediment adequately represent whole-sediment exposures? And does accumulation of hydrophobic organic contaminants depend on the indicator species? To answer these questions, each of the three species was exposed to lab-dosed sediments and their generated pore water and elutriates containing sublethal concentrations of selected PAHs and organochlorine insecticides. Accumulation of the contaminants was monitored to determine differences in the rates and routes of exposure among the three species. The objectives of the study were to measure accumulation kinetics for compounds possessing different physical and chemical properties, such as octanol/water partition coefficients, as a means of comparing bioavailability from the various representations of contaminated sediments. In addition, changes in the bioavailability of selected contaminants resulting from the length of sediment/contaminant contact time were detected by exposing organisms to the media after various intervals of sediment storage.

## METHODS

### Chemicals

The studied compounds included  $^{14}\text{C}$ -radiolabeled *trans*-chlordane (13.7 mCi/mmol, Velsicol Chemical Co., Memphis, TN),  $^{14}\text{C}$ endrin (8.4 mCi/mmol, Sigma Chemical Co., St. Louis, MO),  $[4,4'\text{-}^{14}\text{C}]$ DDT (11.8 mCi/mmol, Sigma Chemical Co),  $^{14}\text{C}$ benzo[*a*]pyrene (BaP; 16.2 mCi/mmol, Sigma Chemical Co.),  $^3\text{H}$ BaP (40.0 Ci/mmol, Sigma Chemical Co.; 69.0 Ci/mmol, Amersham Ltd., Amersham, UK),  $^3\text{H}$ chrysene (340.0 mCi/mmol, Chemsyn Science Laboratories, Lenexa, KS), and  $^3\text{H}$ pyrene (25.2 Ci/mmol, Chemsyn Science Laboratories). All compounds were dissolved in an acetone carrier. Compound radiopurity was >97% for all compounds before use, as determined by TLC, using either benzene:ethyl acetate (3:1, v/v; endrin, DDT, and *trans*-chlordane) or hexane:benzene (8:2, v/v; BaP, chrysene, and pyrene) and liquid scintillation counting (LSC). All solvents were of HPLC grade. Analytical procedures were performed under gold fluorescent light ( $\lambda > 500$  nm) to minimize the photodegradation of the PAHs.

### Sediment dosing and manipulation

Sediment was collected at a 45-m depth in Lake Michigan by Ponar grab approximately 8 km off the coast of Grand Haven, Michigan. The sediment was passed through

a 1-mm screen sieve to remove debris and indigenous organisms. A sediment-water slurry was made by diluting wet sediment with Lake Michigan water in a 1:4 (w/v) sediment:water ratio. Radiolabeled chemicals were added drop by drop in a minimal amount of acetone carrier (<1 ml) to the slurry while being stirred on a mechanical stirrer at room temperature for 4 h. Sediments for most assays were prepared with dual-labeled compounds in the following combinations: BaP/*trans*-chlordane, endrin/pyrene, DDT/chrysene, and *trans*-chlordane/pyrene, in which one compound was  $^3\text{H}$  labeled and the other was  $^{14}\text{C}$  labeled. Two sediments were single labeled with pyrene and BaP. After stirring, sediment slurries were left to settle at 4°C for 48 h. After the settling period, overlying water was decanted, and the sediment was washed with another volume of lake water. The mixture was again stirred by mechanical stirrer at room temperature for another 4 h, then left to settle for 48 to 96 h. The overlying water was again decanted, and portions of the sediment were either used to prepare elutriates or centrifuged to obtain pore water. All contaminants in the prepared sediment and their corresponding elutriate and pore water were at concentrations considered to be nonlethal to indicator species, except for the endrin assay described below (Table 1). In assays that used stored sediment, overlying water was decanted after the 96-h settling period and was replaced with approximately 2 L fresh Lake Michigan water. The prepared sediment was then stored in the dark at 4°C.

Due to the potentially toxic concentrations of endrin to midge larvae used in the first endrin/pyrene assay, leftover sediment was diluted for the second endrin/pyrene assay. Sediment was diluted with two additional volumes of wet 45-m Lake Michigan sediment. Enough  $^3\text{H}$ pyrene was added to bring the sediment-pyrene concentration to that of the previous assay, and the sediment was mixed for 4 h. The mixture was allowed to settle for 48 h before decanting overlying water and preparing elutriate, pore-water, and whole-sediment exposures for the second assay.

### Elutriate preparation

A 1:4 ratio (sediment:water, v/v) of prepared dosed sediment was used to prepare elutriates [20]. The sediment-water mixtures were placed into 250-ml Teflon® centrifuge bottles, placed on a lab rotator at 200 rpm for 30 min, then allowed to settle for 1 h. The unsettled portions were decanted into clean Teflon centrifuge bottles and were centri-

Table 1. Mean contaminant concentrations in prepared sediment, pore water, and elutriates used in the assays

Compound	Whole sediment (ng/g dry wt.)	Elutriate (ng/ml)	Pore water (mg/ml)
<i>trans</i> -Chlordane	124.61–1,405.90	0.395–4.835	1.492–18.630
Benzo[ <i>a</i> ]pyrene	0.27–80.90	0.001–0.262	0.004–0.913
Pyrene	0.14–0.87	0.001–0.002	0.001–0.016
Chrysene	34.21–34.42	0.173–0.174	0.305–0.306
Endrin	526.23–527.51	2.890–2.991	3.050–3.764
DDT	367.28–367.30	0.916–0.917	3.280–3.290

fuged at 2,000 g at 10°C for 30 min. The elutriate fractions were decanted and stored for no longer than 24 h at 10°C before use.

#### *Pore-water preparation*

Dosed prepared sediment was placed into 250-ml stainless-steel centrifuge bottles and centrifuged at 4,000 g for 30 min at 10°C. Supernatant from the bottles was decanted into 250-ml Teflon centrifuge bottles and spun at 2,000 g for 30 min at 10°C. The supernatant from all bottles was decanted and stored for no longer than 24 h at 10°C before use.

#### *Dosed lake-water preparation*

All water used in the study was Lake Michigan surface water collected about 1 m below the surface and stored at 4°C until used. Quantities of lake water were filtered through 0.45- $\mu$ m glass-fiber filters to remove particles. Containers of lake water were placed on a lab stirrer upon the addition of radiolabeled compounds in acetone carrier. Compounds were added as either dual- or single-radiolabels with the same compound combinations as prepared dosed sediments. Mixtures were stirred at room temperature for at least 1 h and stored overnight in the dark at 10°C before use.

#### *Organisms*

Juvenile *Diporeia* spp., approximately 3 to 5 mm in length, were collected from surficial sediment at a water depth of 29 m in Lake Michigan in the fall, spring, and summer of 1991 to 1993. Organisms were screened from the sediments, transported to the lab in cool lake water, and kept in holding aquaria containing 3 to 4 cm lake sediment overlaid with 10 cm lake water at 4°C in the dark. The lake sediment provided sufficient food for the animals, and no additional food was provided in the holding aquaria. Three days before the start of an assay, animals were removed from the sediment and placed into acclimating aquaria containing the same substrate as the holding aquaria. *Diporeia* spp. were kept in the dark and acclimated to 10°C by elevating the temperature by 2°C/d.

Midge larvae were reared in the lab at ambient temperature (22–25°C) on a substrate of shredded brown paper towels and a diet of ground TetraMin® (TetraWerke, Germany) and Cerophyl® (AgriTech, Kansas City, MO) [14]. Fourth-instar larvae, 15 to 20 d old, were removed from the culture aquaria and placed in environmental chambers with the same photoperiod (18:6 h light:dark) and feeding schedule as the original culture aquaria. Environmental chambers were equipped with yellow lights to minimize photodegradation of the contaminants. Larvae were acclimated to 10°C by lowering the temperature by not more than 2°C in a 24-h period.

Adult *L. variegatus* (3–6 mg wet weight per individual) were reared in the lab at room temperature on a substrate of shredded brown paper towels and a diet of Purina Trout Chow® (St. Louis, MO) according to Phipps et al. [4]. Animals were removed from the culture aquaria and acclimated to 10°C on the same 18:6-h light:dark photoperiod as *C. riparius*.

#### *Experimental procedure*

Each assay exposed one of the indicator species to three replicates of whole sediment, elutriate, and pore water for predetermined, timed intervals. In addition, replicates of dosed, filtered Lake Michigan water were used as reference aqueous exposures to establish baseline rates of uptake for species exposed to aqueous-phase contaminants. Three beakers of whole sediment, pore water, elutriate, and dosed lake water were initially set up for each sampling point in all assays. To more accurately compare contaminant uptake rates, all assays were conducted at 10 ± 2°C. Preliminary studies indicated relatively short time intervals were required to achieve steady-state contaminant body burdens for *C. riparius* and *L. variegatus*, but longer intervals were required for *Diporeia* spp.

Each whole-sediment exposure consisted of 30 g prepared wet sediment overlaid with 20 ml Lake Michigan water in 50-ml beakers, which was allowed to settle overnight before the addition of animals. Two animals were added to each beaker, then kept in an environmental chamber with an 18:6-h light:dark photoperiod when testing *C. riparius* and *L. variegatus*. Environmental chambers were equipped with gold fluorescent lights ( $\lambda > 500$  nm) to avoid PAH photodegradation. All beakers were loosely covered with plastic food wrap to minimize water evaporation during the assays. *Diporeia* spp. were kept in darkness throughout the exposures. Animals from three replicate beakers were removed after 1, 6, 24, 48, 72, and 96 h. Additional replicates were prepared for 7-d *L. variegatus* exposures and 7, 10, and 14 d exposures for *Diporeia* spp. In some assays, only two replicate beakers were set up for pore-water exposures. Elutriate, pore-water, and dosed lake-water exposures were set up similarly to whole-sediment exposures, using 20 ml aqueous material in each 50-ml beaker. Two animals were added to each beaker and placed in the same environmental chamber as whole-sediment exposures.

Fifty-milliliter glass centrifuge tubes were prepared for each assay (one tube each for the first, middle, and last exposure period; tubes were used so that pore water could be obtained without disturbing sediment before centrifugation) containing 30 g wet dosed sediment and 20 ml filtered lake water with no added organisms to act as controls for the whole-sediment and pore-water exposures. These tubes were placed in the same environmental chamber as the exposure beakers. At the completion of each timed interval, the control tube was centrifuged, and pore water and sediment were assayed for contaminant concentration and compared to concentrations in whole-sediment and pore-water exposure beakers.

DO and pH were monitored as indicators of overlying water quality at the start and end of individual assays.

#### *Analyses*

At the end of each exposure interval, individual animals were removed from the test media, rinsed in distilled water, blotted dry, weighed, and directly placed into xylene-based scintillation cocktail (3a70b; Research Products Inter-

national, Inc., Mt. Prospect, IL). Animals were not purged of gut contents, because previous work demonstrated no significant increase of total radioactivity from nonpurged vs. purged animals (J. Kukkonen, personal communication) [17]. Organisms were left to stand for at least 24 h for direct extraction of the contaminant(s) before analysis of radioactivity on an LKB 1217 liquid scintillation counter.

Triplicate sediment samples were taken for dry-to-wet-weight ratios and contaminant concentration. Aliquots of sediment were placed on foil and weighed, then dried at 90°C to constant weight for determination of moisture content. Contaminant concentration in the sediment samples was determined by placing approximately 100 mg wet sediment directly into scintillation cocktail and sonicating the sample for 2 min using a Tekmar (Cincinnati, OH) high-intensity ultrasonic processor (extraction recovery 82–92% for BaP and 93–99% for *trans*-chlordane) [17]. Samples were left to stand in scintillation cocktail for at least 48 h before determining activity. Amount of contaminant per gram dry weight sediment was calculated against moisture content of the samples.

Aqueous samples (pore water, elutriate, and dosed lake water) for contaminant analysis were removed from exposure beakers in 2-ml aliquots and placed directly into 12 ml scintillation cocktail; radioactivity then was determined via LSC. All samples analyzed via LSC were corrected for quench using the external standards ratio method after subtracting background.

TOC content of sediment samples was determined by drying the sediment to constant weight, treating with 1 N HCl to remove carbonates, redrying, and assaying organic carbon on a Perkin Elmer (Norwalk, CT) 2400 CHN elemental analyzer. Samples were measured against an acetanilide standard. Triplicate analyses yielded differences of 0 to 3%.

Replicate samples of elutriate, pore-water, and dosed lake-water exposures were obtained for TOC determination at the beginning, middle, and end of each assay. Unfiltered, 2-ml samples were placed into precombusted ampules and frozen for later TOC determination. TOC was performed on an Oceanography International (College Station, TX) carbon analyzer after persulfate digestion [21]. Triplicate analyses yielded differences of 1 to 10%. In some aqueous exposures, TOC decreased significantly and linearly with time. In these cases, TOC concentration for individual exposure intervals was extrapolated from the regression produced from TOC analyzed at the beginning, middle, and end of the assay.

Biotransformation of pyrene, BaP, and *trans*-chlordane was examined in each of the indicator species after exposure in dosed lake water or whole sediment for 2 to 14 d. Ten to 20 animals were removed from the exposure media, rinsed in distilled water, blotted dry, and placed in a Ten Brock tissue homogenizer. Samples were acidified with five drops concentrated HCl and extracted with 2 × 5 ml ethyl acetate:acetone (4:1, v/v) and 1 × 5 ml benzene. The extracts were combined and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and the volume was reduced to approximately 100 μl on a Zymark Turbo-Vap® evaporator. The extracts were analyzed by TLC [22]. Extracts analyzed for *trans*-chlordane and pyrene along with standard parent compounds were developed in pentane:diethyl ether (9:1, v/v). Developed plates were divided

into 2-cm sections, scraped from the TLC plates into scintillation cocktail, and activity determined. Percentage parent compound was determined by the amount of extract corresponding to the R<sub>F</sub> of the parent compound. Analysis of BaP was determined by two-dimensional TLC with pentane:diethyl ether (9:1) and toluene:methylene chloride:methanol (25:10:1). The spots were identified by UV light and quantified by LSC. Standards of BaP metabolites for TLC chromatography were obtained from the National Cancer Institutes' Standard Chemical Reference Repository.

Contaminant purities of a dual-labeled *trans*-chlordane/BaP-spiked sediment and a single-labeled pyrene-dosed sediment were analyzed after standing in the dark at 4°C for 6.5 months (*trans*-chlordane/BaP) and two months (pyrene). Replicates of approximately 3 g wet sediment were placed into 50-ml centrifuge tubes, and the samples were acidified with 10 drops concentrated HCl. Samples were extracted with 2 × 30 ml ethyl acetate:acetone (4:1, v/v), followed by a final extraction with 30 ml benzene. Samples were pulse sonicated for 3 min after each extraction, then centrifuged. The supernatant was combined and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and the volume was reduced by rotary flash evaporation followed by evaporation under a stream of nitrogen to approximately 500 μl. The extracts were analyzed by TLC using hexane:benzene (8:2, v/v, BaP) or pentane:diethyl ether (9:1, v/v, *trans*-chlordane) and LSC as described above.

Lipid contents of *Diporeia* spp., *C. riparius*, and *L. variegatus* were analyzed after exposure of 3- to 14-d intervals in dosed lake water, pore water, elutriate, or whole sediment. To accurately determine changes in lipid content during exposures, lipid analysis was also performed on animals directly obtained from culture aquaria or, for *Diporeia* spp., holding tanks. A microgravimetric procedure with a chloroform/methanol extraction was used for all lipid determinations [18].

#### Comparison evaluation

To compare the rate of contaminant uptake among whole-sediment, elutriate, pore-water, and dosed lake-water exposures, the concentration of contaminant readily available to the organism must be known. For aqueous fractions, this "freely dissolved" fraction has been accurately measured, using various partitioning methods such as equilibrium dialysis [23], C<sub>18</sub> reversed-phase separation [24,25], or water solubility enhancement [26]. However, no chemical measures currently exist to predict bioavailability of sediment-bound fractions. Consequently, no accurate comparison regarding bioavailability of contaminants in whole sediment and aqueous extracts can be made by simply determining total contaminant concentration in the exposure media. However, hydrophobic organic contaminants are known to partition strongly to organic carbon [27,28]. If one assumes that the contaminants used in this study partition to organic carbon similarly in whole-sediment, elutriate, and pore-water exposures, then uptake and accumulation comparisons can be made among the different exposure media, based on the available form of the contaminant from the organic carbon fraction alone. Therefore, by normalizing contaminant concentration in the various media to the amount of TOC in the exposure system, we may estimate the relative bioavailabil-

ity among whole sediment and aqueous extracts of whole sediment.

Carbon-normalized concentrations of contaminants in aqueous media (pore water, elutriate, and dosed lake water) were calculated according to the equation

$$\frac{\text{aqueous concentration (ng/ml)}}{\text{TOC concentration in aqueous media (ng/ml)}}$$

Carbon-normalized concentrations of contaminants in whole sediment were calculated according to the equation

$$\frac{\text{wet-weight sediment concentration (ng/ml)}}{\text{TOC concentration in wet sediment (ng/ml)}}$$

Wet-weight sediment concentrations were based on the average dry-to-wet ratios obtained from individual assays that ranged from 0.59 to 0.69.

The units used to compare bioaccumulation in aqueous fractions were defined as

$$\frac{\mu\text{g contaminant/g wet weight animal}}{\text{ng/ml contaminant in media} \cdot \text{ng/ml TOC in media}^{-1}}$$

and are the units used to describe accumulation values throughout this work.

#### Kinetics models

Uptake rate coefficients were determined using either one- or two-compartment models. The one-compartment model is described as

$$C_a = K_{\text{uoc}} C_m t,$$

where

$C_a$  = concentration in the animal ( $\text{ng g}^{-1}$ )

$K_{\text{uoc}}$  = conditional uptake rate coefficient, ( $\mu\text{g organic carbon cleared of contaminant/g organism wet weight/h}$ )

$C_m$  = organic-carbon-normalized concentration of contaminant in the media (units as above)

$t$  = time.

This model assumes that the concentration of contaminant in the media remains constant over the interval of the assay. For whole-sediment exposures, contaminant concentration, as well as TOC concentration, did not change, and the one-compartment model was used. However, both the TOC content and the total concentration of contaminant changed in a linear fashion over time in most aqueous exposures. In these cases, it was necessary to incorporate the media concentration changes into the model:

$$C_a = K_{\text{uoc}} \left[ C_{w_1} \left( t - \frac{s}{2} t^2 \right) \right]$$

where

$C_{w_1}$  = organic-carbon-normalized concentration of contaminant in the media at  $t = 1$  h (units as above)

$s$  = slope of the regression line of organic-carbon-normalized media concentration with time.

Both models assume that elimination is unimportant over the period for which the uptake rate coefficient is calculated, and any biotransformation and/or elimination of contaminants will be sufficiently diluted by the exposure media as not to result in a significant reuptake of metabolite. In studies where a significant amount of contaminant was biotransformed in a short time, estimates of reuptake of metabolites were determined and found to be <2% of the total body burden.

#### Statistics

Two-factor ANOVA was used to test for significant differences in contaminant accumulation among media (whole-sediment, elutriate, and pore-water exposures) and exposure intervals after log-transforming the data [29]. Student's  $t$  tests were used for overall significant differences in contaminant accumulation among whole-sediment and aqueous phases at individual exposure intervals using Data Desk® Professional software [30]. Differences were considered significant between the test categories at the 0.05 probability level. Rate coefficients and linear regression analyses were calculated with the regression packages used in SAS® and Microsoft® Excel [31].

## RESULTS

Contaminant accumulation between whole-sediment and aqueous exposures differed significantly ( $p > 0.05$ ) for at least one sampling time in all assays. Representative assays showed that the ratios of mean elutriate accumulation to mean whole-sediment accumulation and the ratios of mean pore-water accumulation to mean whole-sediment accumulation differed among sampling times, and generally displayed upward or downward trends throughout the sampling times (Figs. 1–6). Accumulation C.V. in individual assays ranged from 6.8 to 90.7%; however, most values were closer to 25% C.V. [32]. In repeat experiments in which the same contaminants and species were used, accumulation ratios followed the same patterns through time and differed by not more than a factor of three for any exposure period. Two-factor ANOVA showed contaminant accumulation to be significantly affected by the exposure media, as well as the length of exposure for all assays except when *Diporeia* was exposed to chrysene. Of the studied contaminants, aqueous fractions containing pyrene appeared to represent exposure to whole sediment most closely (Fig. 1a and b), whereas aqueous fractions containing *trans*-chlordane represented a significantly lower exposure than that represented by whole sediment (Fig. 6a and b). However, both higher and lower accumulations from elutriate and pore-water vs. whole-sediment exposures were observed and were dependent on the sampling times as well as the indicator species. Animal survival was 90 to 100% for all assays.

#### Pyrene accumulation

Duplicate assays that used each of the indicator species showed elutriates most closely reflected the exposure of

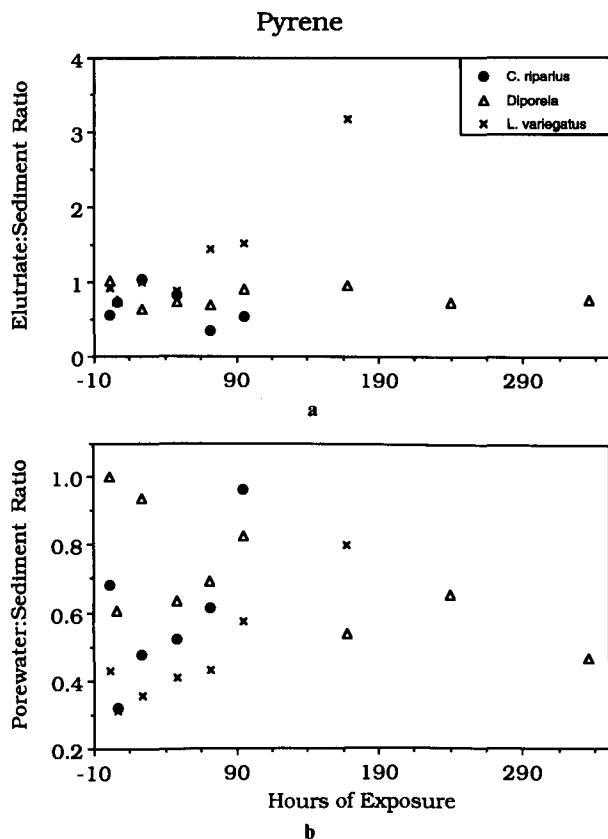


Fig. 1. Ratio of (a) elutriate-to-sediment and (b) pore-water-to-sediment bioaccumulation of pyrene from 1 to 96 h (*Chironomus riparius*), 1 to 168 h (*Lumbriculus variegatus*), and 1 to 336 h (*Diporeia* spp.). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, pore-water, and whole-sediment exposures.

pyrene-dosed sediment, with mean elutriate-to-sediment ratios of approximately one for all indicator species, except *L. variegatus* at 7 d (Fig. 1a). For the assay shown in Figure 1a, mean pyrene accumulation was only significantly lower ( $p < 0.05$ ) in elutriates than in whole sediment at 1, 72, and 96 h (*C. riparius*), and 6 h (*Diporeia* spp. and *L. variegatus*; Fig. 1a). Pyrene accumulation from elutriates was greater than that from whole sediment in *L. variegatus* for all exposures observed after 48 h (Fig. 1a).

Accumulation of pyrene from pore-water exposures was lower than accumulation from whole sediment (Fig. 1b). No significant differences between pyrene accumulation from pore water and whole sediments were observed at 1, 24, 96, and 240 h (*Diporeia* spp.); 1, 72, and 96 h (*C. riparius*); and one week (*L. variegatus*). Uptake rate coefficients consistently demonstrated less pyrene uptake from either aqueous extract than from whole sediment (Table 2).

Pyrene uptake was greatly enhanced when it was used in a dual-labeled assay with [ $^{14}$ C]endrin at nontoxic concentrations (e.g., no mortality or apparent behavioral changes). Pyrene  $K_{uoc}$  values for *C. riparius* exposed to dual-labeled media were  $936.9 \pm 164$  (SE)  $\mu\text{g}$  organic carbon cleared  $\text{g}^{-1}$

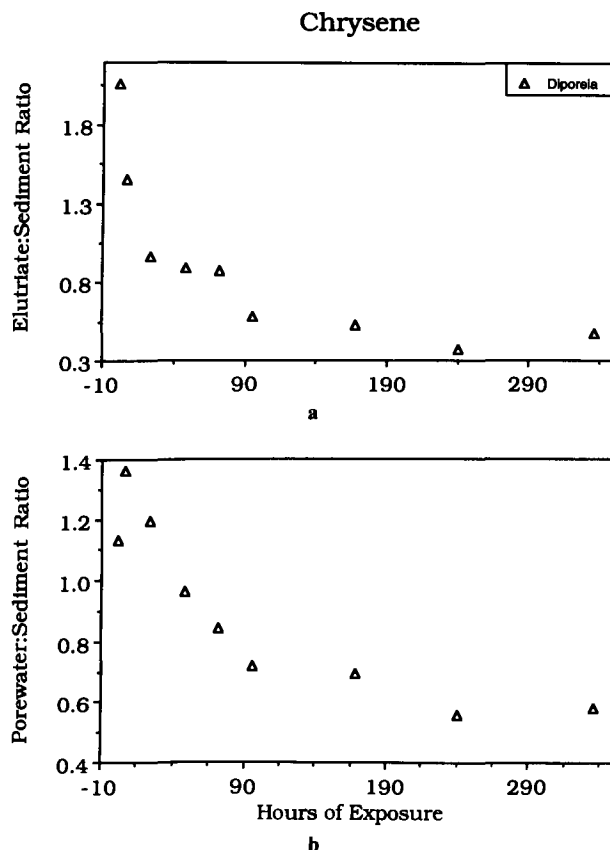


Fig. 2. Ratio of (a) elutriate-to-sediment and (b) pore-water-to-sediment bioaccumulation of chrysene from 1 to 336 h (*Diporeia* spp.). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, pore-water, and whole-sediment exposures.

$\text{h}^{-1}$  (whole sediment),  $142.0 \pm 23$  (elutriate), and  $346.2 \pm 35$  (pore water), compared to  $K_{uoc}$  values of  $165.9 \pm 9$  (whole sediment),  $61.4 \pm 4$  (elutriate), and  $85.9 \pm 7$  (pore water) found for single-labeled exposures.

#### Chrysene accumulation

A single assay was performed with *Diporeia* spp. exposed to chrysene. No significant accumulation differences were seen among exposure media (ANOVA;  $F = 0.39$ ;  $d.f. = 2, 99$ ;  $p = 0.681$ ), although accumulation was significantly different among exposure intervals. Using Student's  $t$  tests, accumulation of chrysene was significantly greater in elutriate exposures than whole sediment after 1 h at the  $p < 0.05$  level but was not significantly different between 6- and 72-h periods. After 72 h, accumulation of chrysene from elutriate was significantly lower than from whole sediment, and the elutriate-sediment accumulations followed a downward curve over time (Fig. 2a). A similar curve was observed for pore-water-to-sediment accumulation ratios (Fig. 2b). However, pore-water accumulation was significantly lower than whole-sediment chrysene accumulation only after 96 h of exposure ( $t = 2.009$ ;  $d.f. = 10$ ;  $p = 0.038$ ). Uptake rate coeffi-

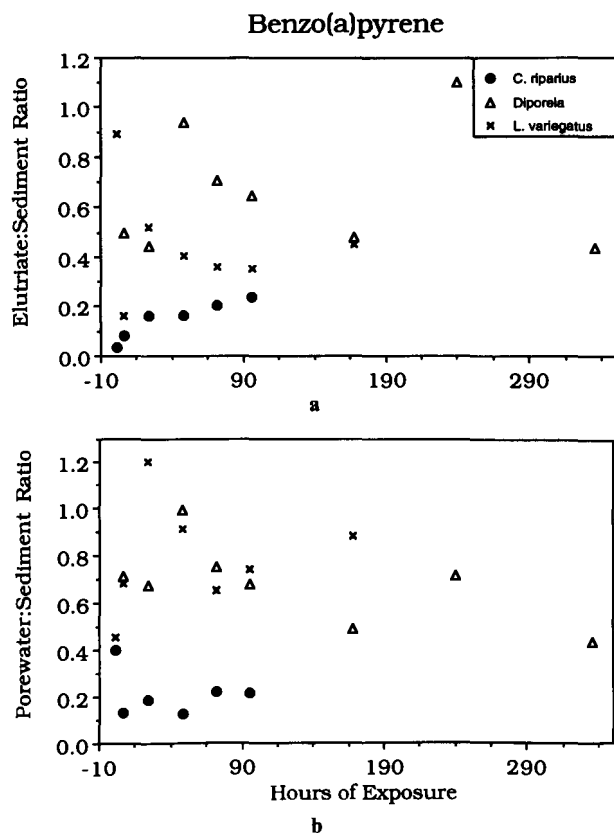


Fig. 3. Ratio of (a) elutriate-to-sediment and (b) pore-water-to-sediment bioaccumulation of benzo[a]pyrene from 1 to 96 h (*Chironomus riparius*), 1 to 168 h (*Lumbriculus variegatus*), and 1 to 336 h (*Diporeia* spp.). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, pore-water, and whole-sediment exposures.

cients for *Diporeia* spp. exposed to chrysene were highest in pore water (244.3  $\mu\text{g}$  organic carbon cleared  $\text{g}^{-1} \text{h}^{-1}$ ) and lowest in elutriate (55.2  $\mu\text{g}$  organic carbon cleared  $\text{g}^{-1} \text{h}^{-1}$ ; Table 3).

#### BaP accumulation

Duplicate, triplicate, and single assays were run with *C. riparius*, *Diporeia* spp., and *L. variegatus*, respectively. Accumulation differences between BaP-dosed whole-sediment and aqueous extracts appeared to be a function of the examined species. *Chironomus riparius* larvae accumulated considerably less BaP in elutriate and pore water than in whole sediment over all exposure intervals (Fig. 3a and b). In most cases, accumulation in midge larvae from elutriates was comparable to that from pore water. BaP accumulation in *Diporeia* spp. was also generally lower in elutriate and pore water than in whole sediment. However, for a few sampling times (48 and 240 h in elutriate, Fig. 3a; 6, 48, and 96 h in pore water, Fig. 3b), mean accumulation in *Diporeia* spp. was not different from that in whole sediment ( $p < 0.05$ ). Accumulation of BaP in *L. variegatus* was generally lower in aqueous extracts compared to whole sediment, and *L. va-*

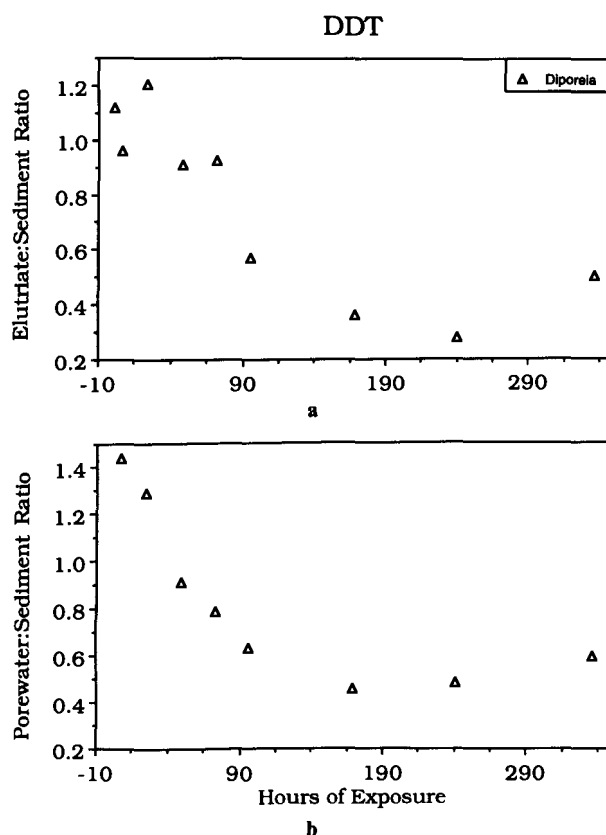


Fig. 4. Ratio of (a) elutriate-to-sediment and (b) pore-water-to-sediment bioaccumulation of DDT from 1 to 336 h (*Diporeia* spp.). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, pore-water, and whole-sediment exposures.

*riegatus* accumulation from whole sediment was greater than elutriate accumulation by a factor of approximately 6 after 6 h (Fig. 3a). For *L. variegatus* exposures in pore water, accumulation was not different from accumulation from sediment, except at 96 h, whereas pore-water accumulation was significantly lower than that from sediment ( $t = 2.730$ ;  $d.f. = 10$ ;  $p = 0.011$ ).

Uptake rate coefficients for BaP were the lowest of any for the studied contaminants and ranged from 11.0 (*Diporeia* spp.) to 53.5 (*L. variegatus*) in sediment, and from 2.7 (*C. riparius*) to 30.4 (*L. variegatus*) in aqueous extracts (Table 2). Of the three examined species, *L. variegatus* consistently cleared BaP from organic carbon most rapidly, whereas *C. riparius* and *Diporeia* spp. tended to clear approximately the same amounts of contaminant from organic carbon per time.

#### DDT accumulation

The accumulation of [ $^{14}\text{C}$ ]DDT by *Diporeia* spp. was monitored along with [ $^3\text{H}$ ]chrysene in a single assay. Accumulation of DDT was not significantly different between sediment and elutriate through 96 h [31]. Pore-water/sediment accumulation comparisons appeared similar to elutriate/

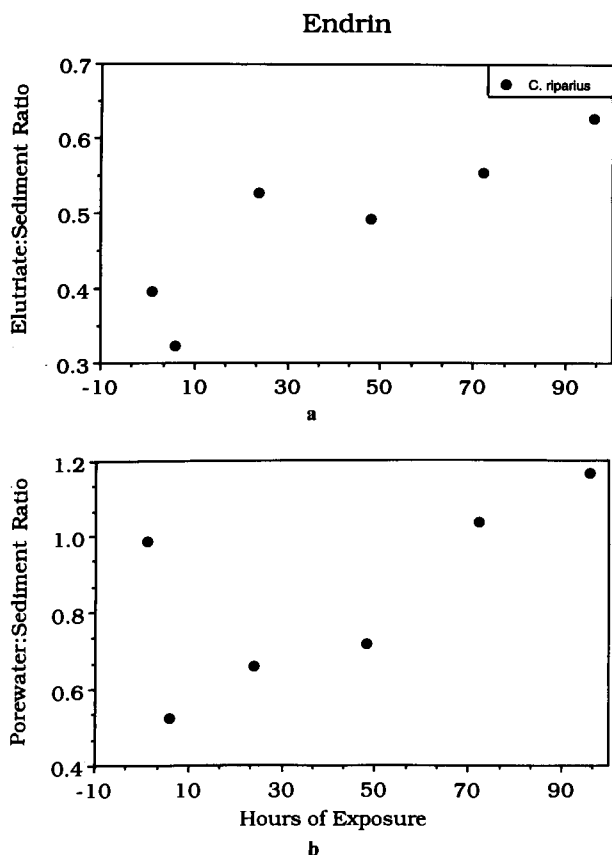


Fig. 5. Ratio of (a) elutriate-to-sediment and (b) pore-water-to-sediment bioaccumulation of endrin from 1 to 96 h (*Chironomus riparius*). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, pore-water, and whole-sediment exposures.

sediment comparisons, except that mean pore-water accumulation was significantly greater than whole-sediment accumulation at the first sampling time (1 h;  $t = 3.161$ ;  $d.f. = 9$ ;  $p = 0.006$ ). Both elutriate and pore-water DDT accumulations were significantly lower than whole-sediment accumulation after 96-h exposures and showed a downward trend (decreasing elutriate-to-sediment and pore-water-to-sediment accumulation ratios) from 24 to 240 h (Fig. 4a and b). Uptake rate coefficients for DDT in *Diporeia* spp. were highest in elutriate and pore-water exposures, whereas sediment  $K_{uoc}$  was less than half that obtained for pore water (Table 3). No differences in DDT accumulation between elutriate and whole-sediment exposures and between pore-water and whole-sediment exposures were observed for most of the early sampling times ( $p < 0.05$ ; Fig. 4a and b).

#### Endrin accumulation

The first endrin assay conducted with *C. riparius* produced significant mortality. Survival of *C. riparius* was 100% in whole-sediment exposures up to 72 h but dropped to 50% at 96 h. Aqueous exposures appeared even more toxic, with

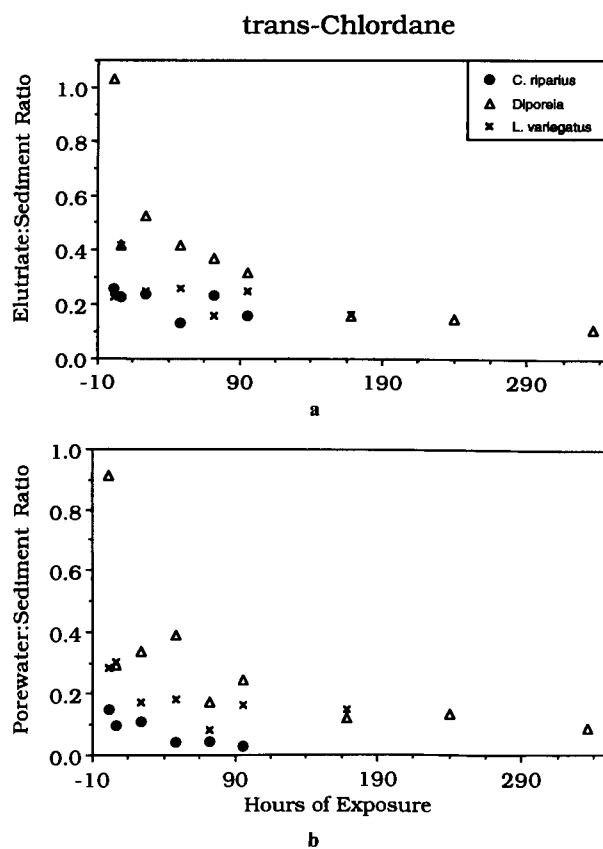


Fig. 6. Ratio of (a) elutriate-to-sediment and (b) pore-water-to-sediment bioaccumulation of trans-chlordane from 1 to 96 h (*Chironomus riparius*) 1 to 168 h (*Lumbriculus variegatus*), and 1 to 336 h (*Diporeia* spp.). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, pore-water, and whole-sediment exposures.

100% survival to 24 h in both elutriate and pore water declining to 66% at 48 h, 33 and 66% at 72 h (elutriate and pore water, respectively), and 50 and 83% at 96 h (pore water and elutriate, respectively). In a second assay, unused sediment from the first assay was diluted with clean 45-m Lake Michigan sediment, so that the endrin concentration dropped from 1.5  $\mu\text{g/g}$  dry weight (326  $\mu\text{g/g}$  organic carbon) to 0.5  $\mu\text{g/g}$  dry weight (109  $\mu\text{g/g}$  organic carbon). Survival of *C. riparius* was 100% in assay 2.

In assay 2, mean elutriate-to-sediment accumulation ratios were all less than one (Fig. 5a). Mean pore-water accumulation was significantly less than whole-sediment accumulation at 6-, 24-, and 48-h exposure periods via Student's  $t$  tests (Fig. 5b). An upward trend in elutriate-to-sediment and pore-water-to-sediment accumulation ratios after 6 h was apparent (Fig. 5a and b). Uptake rate coefficients were substantially lower in assay 2 than in assay 1 (Table 4), whereas the calculated  $K_{uoc}$  in whole sediment was over nine times greater in the first assay than in the second. Uptake rate coefficients from assay 1 data in elutriate and pore water were much lower than those calculated in whole sediment (Table 4). Elu-



Table 2.  $K_{uoc}$  values in *Diporeia* spp., *Chironomus riparius*, and *Lumbriculus variegatus* for contaminants in whole sediment, elutriate, pore water, and reference Lake Michigan water exposures

Compound	Whole sediment			Elutriate			Pore water			Lake Michigan water		
	<i>Diporeia</i>	<i>C. rip.</i>	<i>L. var.</i>	<i>Diporeia</i>	<i>C. rip.</i>	<i>L. var.</i>	<i>Diporeia</i>	<i>C. rip.</i>	<i>L. var.</i>	<i>Diporeia</i>	<i>C. rip.</i>	<i>L. var.</i>
<i>trans</i> -Chlordane												
Assay 1	246.7 <sup>a</sup> (18.0) <sup>b</sup>	358.1 (25.0)	355.6 (41.6)	58.7 (3.9)	66.6 (5.7)	117.9 (3.9)	51.0 (3.5)	80.1 (14.8)	57.5 (3.6)	236.0 (2.7)	30.6 (3.3)	159.8 (16.1)
Assay 2	216.8 (12.6)	ND <sup>c</sup>	356.6 (29.0)	46.9 (3.2)	ND	72.8 (4.7)	70.9 (4.1)	ND	63.3 (4.5)	224.8 (14.9)	ND	159.6 (19.5)
Assay 3	252.7 (16.0)	ND	ND	55.1 (4.3)	ND	ND	74.7 (4.5)	ND	ND	ND	ND	ND
Benzo[ <i>a</i> ]pyrene												
Assay 1	15.3 (2.6)	15.7 (1.4)	53.5 (5.0)	8.7 (0.2)	2.7 (0.3)	8.1 (0.7)	12.5 (0.6)	3.2 (0.2)	30.4 (2.0)	242.9 (50.0)	41.3 (5.4)	176.8 (9.2)
Assay 2	19.6 (1.5)	15.0 (1.4)	ND	3.9 (0.2)	4.4 (0.2)	ND	6.9 (0.4)	7.6 (0.6)	ND	184.7 (16.3)	24.2 (1.6)	ND
Assay 3	11.0 (1.4)	ND	ND	4.3 (0.2)	ND	ND	14.2 (1.0)	ND	ND	ND	ND	ND
Pyrene												
Assay 1	319.1 (28.0)	165.9 (9.4)	185.2 (12.8)	134.1 (12.6)	61.4 (3.6)	95.5 (4.8)	250.0 (21.9)	85.9 (7.1)	90.7 (4.9)	231.7 (38.8)	40.1 (6.3)	181.0 (9.9)
Assay 2	176.0 (11.7)	ND	153.2 (23.5)	158.2 (14.1)	ND	82.0 (4.8)	133.3 (10.1)	ND	55.0 (2.9)	276.9 (20.9)	ND	159.9 (19.5)

<sup>a</sup>Units for  $K_{uoc}$  are  $\mu\text{g}$  organic carbon cleared/g organism/h.

<sup>b</sup> $\pm 1$  SE.

<sup>c</sup>Not determined.

tri- and pore-water uptake rate coefficients in assay 2 were also reduced from those observed in assay 1 but were comparable to that of the  $K_{uoc}$  calculated for whole sediment (Table 4).

#### *trans*-Chlordane accumulation

Duplicate, triplicate, and single assays were run with *L. variegatus*, *Diporeia* spp., and *C. riparius*, respectively. Accumulation of *trans*-chlordane was generally greater from whole-sediment exposures than from the aqueous extracts for all of the tested species. *Diporeia* spp. accumulation from the aqueous extracts was comparable to whole-sediment only at the first sampling time (Fig. 6a and b). However, accumulation of *trans*-chlordane was greater in sediment than in

either pore water or elutriate at 1 h for another assay that used *Diporeia* spp. [32]. Mean elutriate-to-sediment and pore-water-to-sediment accumulation ratios were all substantially less than one for *L. variegatus* and *C. riparius* (Fig. 6a and b). These differences were reflected in  $K_{uoc}$  values that were highest in whole-sediment exposures for all three species, ranging from 46.9 to 117.9 in elutriate and pore water to 216.8 to 358.1 in whole sediment (Table 2). Accumulations of *trans*-chlordane in *C. riparius* and *Diporeia* spp. were comparable in elutriate and pore water. Accumulation from elutriate was generally greater than that from pore water in *L. variegatus* over the course of the assays, whereas mean elu-

Table 3.  $K_{uoc}$  values in *Diporeia* spp. for DDT and chrysene in whole-sediment, elutriate, and pore-water exposures

Compound	Whole sediment		Elutriate		Pore water	
	$K_{uoc}$ <sup>a</sup>	SE <sup>b</sup>	$K_{uoc}$	SE	$K_{uoc}$	SE
DDT						
Assay 1	91.0	(5)	120.5	(7)	205.7	(43)
Chrysene						
Assay 2	128.5	(7)	55.2	(13)	244.3	(33)

<sup>a</sup>Units for  $K_{uoc}$  are  $\mu\text{g}$  organic carbon cleared/g organism/h.

<sup>b</sup> $\pm 1$  SE.

Table 4.  $K_{uoc}$  values in *Chironomus riparius* for contaminants in endrin-dosed sediment, elutriate, pore-water, and reference Lake Michigan water exposures<sup>a</sup>

	Whole sediment		Elutriate		Pore water		Lake Michigan water	
	$K_{uoc}$ <sup>b</sup>	SE <sup>c</sup>	$K_{uoc}$	SE	$K_{uoc}$	SE	$K_{uoc}$	SE
Endrin								
Assay 1	1,726.9	(308)	511.4	(47)	298.3	(93)	40.5	(2)
Assay 2	189.2	(21)	113.8	(12)	128.2	(9)	ND <sup>d</sup>	

<sup>a</sup>Endrin-dosed sediment at 1,477 ng/g dry weight in assay 1 was diluted with clean sediment to a concentration of 527 ng/g dry weight in assay 2.

<sup>b</sup>Units for  $K_{uoc}$  are  $\mu\text{g}$  organic carbon cleared/g organism/h.

<sup>c</sup> $\pm 1$  SE.

<sup>d</sup>Not determined.

triate accumulation was approximately twice that of pore-water accumulation in one *L. variegatus* assay [32].

#### Dosed lake-water accumulation

Accumulation of *trans*-chlordane, BaP, and pyrene from dosed lake-water exposures was generally greater than accumulation from aqueous and whole-sediment exposures but varied greatly among species and among sampling times. Uptake rate coefficients for BaP were higher in dosed lake-water exposures than in any of the test phases for all three species, but were either greater, less, or comparable to  $K_{uoc}$  values calculated for *trans*-chlordane and pyrene in the various media (Table 2). Uptake rates from dosed lake-water exposures replicated well among multiple assays for all three of the indicator species (e.g., uptake rate coefficients varied by less than a factor of two among assays for a single species; Table 2). Based on these reproducible results, dosed lake water was considered a valid reference material for these studies. Due to depletion of the  $^{14}\text{C}$  radiotracer in the first assay and its unavailability for further studies, a second dosed lake-water set of exposures for *C. riparius* with endrin was not run (Table 4). Also, the dosed lake-water exposures that used dual tracers for the DDT/chrysene study with *Diporeia* spp. showed a particularly high mortality (100% mortality by 96 h); this data set was not used to determine uptake rate coefficients.

#### Pore-water and sediment control data

Centrifuge tubes containing dosed whole-sediment controls showed pore-water contaminant concentrations to be comparable to test pore-water contaminant concentrations in early exposure periods. However, pore-water contaminant concentrations in test beakers tended to decrease from those in control tubes after 48 h in most assays, likely due to contaminant accumulation in the organisms, volatility, and/or differential sorption to the exposure beakers over the course of the assays.

TOC content in whole-sediment exposures did not change significantly over the time intervals of any studies. TOC in the Lake Michigan sediment ranged from 0.45 to 0.52% in all assays. Organic carbon in some of the aqueous exposures decreased appreciably during some of the assays, and adjustments for these decreases were made in accumulation calculations.

Overlying water, elutriate, and pore-water pH ranged from 6.5 to 7.2 in all assays. DO of aqueous media was >60% saturation in all assays.

#### Total lipid and biotransformation studies

Total lipid content of the three indicator species was determined to better describe possible differences in accumulation among the studied organisms and to determine if lipid content changed during the course of the bioassays. For example, an appreciable amount of lipid lost during a bioassay might indicate a deterioration in general health of the species during the assays.

Baseline lipid content (percentage dry animal weight) in *C. riparius* averaged  $3.72 \pm 2\%$  (SD) in animals taken directly from culture aquaria. After exposure to the various assay me-

dia (sediment, dosed lake water, elutriate, or pore water) for 3 d, lipid content ranged from 1.25 to 8.31%, with a mean of 4.73%, not significantly different from baseline content. Baseline lipid content in *Diporeia* spp. taken directly from holding tanks averaged  $33.91 \pm 9.4\%$  (SD). After two weeks' exposure in sediment or dosed lake water, no change in *Diporeia* spp. lipid was observed, whereas total lipid ranged from 23.7 to 44.49% and averaged 33.5% lipid.

Total lipid content in *L. variegatus* tended to decrease upon exposure to the media. Baseline lipids in this species taken from culture media averaged  $10.09 \pm 4.4\%$  (SD). After 3- to 7-d exposures in either pore water, dosed lake water, or whole sediment, lipid content declined to 5.27 (pore-water exposures) to 8.78% (sediment exposures), with an average of 5.43% lipid [32].

Biotransformation studies showed that of the three species tested with *trans*-chlordane, BaP, and pyrene, only *C. riparius* was able to biotransform BaP and pyrene to any extent [32]. After 4-d exposures in whole sediment at 10°C, *C. riparius* larvae biotransformed 96.9% of the parent BaP and 71.5 to 73.6% of the parent pyrene to less hydrophobic metabolites, but transformed none of the *trans*-chlordane in dosed sediment. Neither *Diporeia* spp. nor *L. variegatus* was able to metabolize any of the three contaminants, and over 90% of the parent contaminants were detected in animal tissue after 4- to 14-d exposures in dosed lake water or whole sediment.

Studies on stored sediment showed no significant transformation of the parent contaminants. After 6.5 months, the sediment still contained an average 92.8% parent BaP and 93.5% parent *trans*-chlordane, and after two months, single-labeled pyrene-dosed sediment contained an average 97.0% parent compound.

#### Accumulation from stored sediments

In assays that used *C. riparius*, contaminant accumulation appeared to either decline or stay the same with the amount of sediment/contaminant contact time and was dependent on the contaminant. Mean accumulation of *trans*-chlordane from whole-sediment exposures after an eight-week storage period declined to about half that of sediment that had been stored for one week, even though sediment concentrations were similar [32]. Mean accumulation from whole sediment was comparable between eight- and 26-week storage periods and did not vary by more than 32% for individual exposure periods. Also, *trans*-chlordane accumulation from elutriate prepared from sediment stored from one to 26 weeks stayed relatively constant and varied by not more than 31% for individual exposure intervals. However, pore-water accumulation from sediment stored one week was much lower than that produced from sediment stored 26 weeks [32]. For example, mean accumulation from pore water obtained from sediment stored one week was  $1,327.7 \pm 336$  (SD) after 72 h exposure, compared to  $3,018.2 \pm 702$  for pore water obtained from sediment stored 26 weeks. In addition, uptake rates of *trans*-chlordane from whole sediment stored one, eight, and 26 weeks appeared to decline in a linear fashion, whereas uptake from the aqueous extracts did not appear to change significantly with sediment storage (Table 5).

Table 5.  $K_{uoc}$  values for *Chironomus riparius* exposed to sediment stored from one week to 6.5 months and aqueous extracts generated from stored sediment

Compound	Medium	Storage interval					
		1 week		8 weeks		26 weeks	
		$K_{uoc}^a$	SE <sup>b</sup>	$K_{uoc}$	SE	$K_{uoc}$	SE
<i>trans</i> -Chlordane	Whole sediment	358.0	(25)	233.5	(16)	139.1	(13)
	Elutriate	66.6	(6)	24.9	(2)	60.9	(6)
	Pore water	80.1	(15)	40.4	(3)	60.5	(4)
Benzo[a]pyrene	Whole sediment	15.73	(1.4)	23.49	(3.0)	12.57	(1.4)
	Elutriate	2.72	(0.3)	1.20	(0.2)	6.64	(1.0)
	Pore water	3.25	(0.2)	11.18	(0.6)	0.86	(0.1)

<sup>a</sup>Units for  $K_{uoc}$  are  $\mu\text{g}$  organic carbon cleared/g organism/h.

<sup>b</sup> $\pm 1$  SE.

Uptake rate coefficients of BaP did not reflect the accumulation decline from whole-sediment exposures observed between one- and eight-week storage periods for *trans*-chlordane (Table 5). Mean accumulation of BaP declined by a factor of three in *C. riparius* exposed to sediment stored one week vs. that stored eight weeks. For example, mean *C. riparius* accumulation from whole sediment stored for one week was  $1,030.3 \pm 273 \mu\text{g g}^{-1}$  (SD) after 72 h exposure. Accumulation from sediment stored for eight weeks before the study averaged  $353.1 \pm 123$  after 72 h exposure. However,  $K_{uoc}$  was  $15.73 \pm 1.4$  (SE) (one week's storage) vs.  $23.49 \pm 3.0$  (eight week's storage; Table 5). Also, accumulation of BaP from pore water that had been generated from sediment stored for 26 weeks was generally greater than pore water generated from sediment stored for one or eight weeks. However, uptake rate coefficients for pore water declined with sediment storage from  $3.25 \pm 0.2$  (SE) (one week) to  $0.86 \pm 0.1$  (26 weeks; Table 5). Uptake rate coefficients for BaP from elutriates generated from stored sediment followed trends in accumulation upon sediment storage, whereas both accumulation and  $K_{uoc}$  doubled with sediment stored one and 26 weeks, respectively (Table 4) [32].

Uptake rate coefficients for *C. riparius* exposed to pyrene that used pore water generated from stored sediment increased with sediment storage. The  $K_{uoc}$  values were  $85.9 \pm 7$  (SE) and  $131.5 \pm 20$  for pore-water exposures generated from sediment that had been stored one and four weeks, respectively. No significant changes in pyrene uptake were observed for whole sediment and elutriate exposures. The  $K_{uoc}$  values for whole sediment were  $165.9 \pm 9$  (SE) and  $227.1 \pm 50$ , and  $61.4 \pm 4$  and  $55.7 \pm 5$  for elutriates produced from sediment stored one and four weeks before assays, respectively.

## DISCUSSION

### Exposure comparisons

Data from this study show that aqueous extracts of whole sediment did not accurately represent the exposure observed in whole sediment. Generally, the aqueous extracts of whole sediment underexposed organisms, compared to whole sediment, even after adjusting accumulation to the fraction of organic carbon contained in the test media. However, at

some sampling times for some contaminants, differences in accumulation between a particular aqueous media and whole sediment were not significant, as was shown with nearly all pyrene-dosed elutriate exposures. For most other contaminants, accumulation from aqueous test fractions, although statistically different from whole-sediment accumulation, agreed within a factor of two, if exposures  $\leq 96$  h are considered. Sampling time was a significant factor for bioaccumulation relationships among whole-sediment and aqueous media, exemplified by DDT accumulation in *Diporeia* spp. DDT accumulation from pore-water and elutriate exposures approximated accumulation from whole sediment early in the assays. However, after 72-h exposure intervals, both aqueous exposures underestimated DDT accumulation from whole sediment. Sampling time should be considered when determining the suitability of aqueous extracts of sediments as substitutes for whole-sediment exposures, such as in toxicity identification evaluations. In aqueous exposures, a steady-state contaminant concentration between the organism and its environment may never be attained, because lack of substrate or limited nutrients may dictate the length of time that the exposure interval can be maintained before organism die-off. Therefore, an important factor to consider in method development is the length of exposure interval that produces the most reliable results, which will most likely vary with the indicator species. In preliminary studies, detrimental changes (i.e., loss of color and decreased movement) were observed in *C. riparius* after 96-h exposures in aqueous media. However, *Diporeia* spp. could be exposed for over two weeks in pore water or elutriate without obvious effects. Thus, exposure times should be determined in accordance with individual species tolerances to exposure conditions.

In most assays, elutriate-to-sediment and pore-water-to-sediment accumulation ratios followed obvious trends over sampling intervals. Accumulation ratios usually decreased with exposure time. However, trends increased with time in some assays (e.g., *C. riparius* exposed to endrin, *L. variegatus* exposed to pyrene). One explanation for the contaminant in pore water and/or elutriate becoming less available relative to whole sediment may be that the available fraction of the contaminant became depleted in aqueous media after a

short time whereas source contaminant in sediment exposures remained relatively constant. While the total contaminant concentrations in the sediment stayed constant, total concentrations decreased appreciably in pore water and elutriate over the course of the assays, and it is likely that the bioavailable concentrations in these media also decreased.

This fails to explain the upward trend in temporal accumulation ratios in other assays. A number of factors may have contributed to this apparent increase in aqueous bioavailability relative to contaminant availability in whole sediment. The accumulation data were normalized to the total amount of organic carbon (both DOC and particulate organic carbon) contained in bioassay media, because the activity of hydrophobic contaminants is controlled primarily by their interaction with organic carbon [33,34]. By normalizing to organic carbon, we directly accounted for the carbon-bound fraction of contaminant and thus indirectly accounted for the freely dissolved concentrations in aqueous phases (e.g., isolated pore water or elutriates), or pore water in whole sediment. This was done because, first, an adequate approximation of the amount of bioavailable (e.g., freely dissolved fraction) contaminant could not be made from whole-sediment exposures, and, second, in preliminary studies with aqueous media, bioaccumulation appeared to be much greater than that contributed by the freely dissolved fractions alone. Although such normalizing techniques may work well for some contaminants of low solubility that are tightly bound to carbon, it may not be as useful for other contaminants that are more water soluble and less tightly bound to organic carbon. Pyrene and endrin were the most water-soluble contaminants used (pyrene, 135.0  $\mu\text{g/L}$  [35]; endrin, 230.0  $\mu\text{g/L}$  [36]) and were the only contaminants that demonstrated a significant upward trend in accumulation ratios over time for *L. variegatus* (pyrene exposures) and *C. riparius* (endrin exposure). This upward trend demonstrates increased exposure of the contaminants with time, as may happen when less hydrophobic compounds slowly desorb from organic carbon to the surrounding aqueous medium.

Differential exposure among whole sediment, elutriate, and pore water was clearly demonstrated in the uptake clearances, where, for most contaminants,  $K_{\text{uoc}}$  levels in aqueous extracts were lower than those obtained from whole-sediment exposures by at least a factor of two. The only exceptions were with *Diporeia* spp. exposed to DDT and chrysene in a dual-labeled assay. In that assay,  $K_{\text{uoc}}$  values in pore-water exposures were approximately two times greater than those in whole sediment. Reasons for this phenomenon are unknown, and further assays that test these contaminants separately may indicate why the uptake from pore water was greater than uptake from whole sediment.

#### Species effects

In this study, sediments possessing the same chemical and physical characteristics and containing the same organic carbon content were used. All studies were conducted at 10°C so that an adequate comparison of contaminant uptake could be made among all three of the indicator species. However, variations in uptake and accumulation among the species were still apparent. Where direct comparisons in accumula-

tion among all three species could be made (pyrene, BaP, and *trans*-chlordane), elutriate-to-sediment and pore-water-to-sediment accumulation ratios generally varied both in magnitude for a single sampling time as well as temporally. In most cases, *C. riparius* had lower accumulation ratios than either of the other two species, whereas *Diporeia* spp. generally showed the greatest accumulation for a given length of exposure (Figs. 1, 3, and 6). However, contaminant clearances among the species were generally comparable among species for a single contaminant in sediment, elutriate, or pore water (Table 2).

Increases or decreases in contaminant accumulation among the three species probably resulted from a combination of feeding selectivity, lipid content, and physiological/metabolic variation. Contaminant uptake from both gill ventilation and ingestion of food is thought to contribute to contaminant body burden [37–39]. A greater contaminant accumulation contributed from food ingestion may occur with selective feeding habits, as seen with *Diporeia* spp. If contaminant differentially sorbs onto particles that *Diporeia* preferentially ingests, exposure is increased and accumulation increases accordingly. Also, the higher lipid stores in *Diporeia* may increase its ability to accumulate lipophilic compounds. *Diporeia* spp. contained an average 10 times more lipid on a dry-weight basis, compared to *C. riparius*. Because hydrophobic contaminants partition preferentially to lipid, the potential for *Diporeia* spp. to accumulate these compounds was expected to be much greater than for either of the other two assayed species. In some cases, this was true (e.g., pyrene accumulation from sediment, elutriate, and pore water). However, in other cases accumulation was greatest in *L. variegatus* (e.g., accumulation of *trans*-chlordane in sediment), where this species' continuous feeding habits probably contributed to the significantly higher accumulation of BaP from whole sediment compared to that observed for the other two species.

Differences in metabolism among the indicator species may also have been responsible for the differential accumulation observed for a single contaminant. Metabolic studies were conducted on the three species primarily to determine if reuptake of metabolite(s) from the exposure media was a significant source of contaminant in the organisms. Although reuptake was not considered to be a significant contributor to overall accumulation, only a portion of the accumulated pyrene and BaP in *C. riparius* was parent compound. We assumed that the rate of biotransformation in *C. riparius* remained the same in all exposure media. The accumulation differences observed in *C. riparius* exposed to BaP and pyrene in pore water, elutriate, and whole sediment are valid only if this assumption is true. However, the pyrene and BaP elutriate-to-sediment and pore-water-to-sediment accumulation ratios obtained for *C. riparius* tend to agree with those we obtained for nonmetabolized *trans*-chlordane; all of these ratios were generally lower than those obtained for *Diporeia* spp. or *L. variegatus*. Therefore, PAH metabolites in *C. riparius* apparently did not alter bioaccumulation among the exposure media, and the clearance and accumulation comparisons that we make among the exposure media are valid.

Physiological differences among the species, including the

ability to survive in the various exposure media for extended periods, may have contributed to the differential bioaccumulation of single contaminants. Abnormal stresses were placed on the organisms during exposures in aqueous media. All three species normally inhabit sediments, so lack of a suitable substrate was expected to change behavior and possibly the rate of contaminant uptake. Previous studies showed that toxicity of an oil leachate to burrowing mayfly larvae decreased when artificial burrows were supplied in exposure chambers [40]. The authors suggested that the increased toxicity seen in chambers without burrows was due to thigmotactic stress created from lack of a suitable substrate. *Chironomus* spp. normally build larval cases on or within the substrate in which they live [13]. However, no cases were constructed in aqueous exposures because no suitable material was available. This may have acted to either raise or lower the contaminant exposure from the surrounding water to the larvae. Cases tend to separate the organism from the surrounding sediment in natural situations and are expected to reduce the organism's direct exposure to pore water [41]. In aqueous exposures, the organisms were no longer sheltered from contaminant contact provided by the case, and exposure was raised. However, exposure may also have been lower than when cases were present if the animals lowered their feeding rates in aqueous media. The stress brought about by an unsuitable substrate likely affected *L. variegatus*, where mean lipid content decreased to half that of organisms taken from culture media after 3- to 7-d exposures.

A relatively wide variation of contaminant uptake within individual animals at individual sampling times occurred in the assays. Individual variation in contaminant uptake is indicative of differential exposure of organisms to the contaminants, due to normal biological variation in feeding rates, respiration, age, and general metabolic rates among organisms. Even though test organisms were carefully selected for uniformity before being placed into exposures, we did not determine the exact age or sex of individuals. Fourth-instar *Chironomus* larvae may be at very different stages of development [42] and may likely slow their feeding late in this period. *Diporeia* spp. are intermittent feeders and normally show individual variation in feeding rate [43,44]. Finally, previous accumulation assays showed that contaminant uptake naturally varies among individual *L. variegatus* and that for whole-sediment accumulation tests, both longer exposures and more sample replicates should be used [4].

#### Compound effects

The relative magnitudes of the uptake rate differences observed among contaminants such as *trans*-chlordane and BaP are attributable to differential bioavailability inherent to the studied compounds. Uptake rate coefficients in whole-sediment exposures were highest for *trans*-chlordane, ranging from 216.8 (*Diporeia* spp.) to 358.0 (*C. riparius*) and were lowest for BaP, ranging from 11.0 (*Diporeia* spp.) to 53.5 (*L. variegatus*). Differential bioavailability may arise from the contaminant partitioning to different particle sizes within the sediment or to different types of organic carbon (i.e., colloids, microparticulates). This was apparent in studies with *Diporeia* spp., where differential association of hexa-

chlorobiphenyl and BaP to TOC within the same sediment led to differential contaminant exposure [44].

The high uptake observed in endrin exposures conducted at effect-level doses was probably due to stimulatory reactions (e.g., greater metabolic rates) of *C. riparius* inherent to the contaminant [45]. We diluted the high-dose sediment to an assumed no-effect level of contaminant (e.g., no mortality, behavioral changes), and  $K_{uoc}$  decreased from 1,726.9 to 189.2 in whole-sediment exposures. Even though mortality was not observed at the lower endrin concentration, pyrene uptake in this dual-labeled assay was over four times greater than when *C. riparius* was exposed to pyrene in single-labeled assays, indicating that even at seemingly no-response levels, organism metabolic rate was affected, probably by the endrin. Other studies showed that the toxicity of endrin to *Hyalomma azteca* was not affected by the amount of TOC in the exposure and that for this contaminant, normalization to organic carbon is of limited use [46].

We found the bioavailability of contaminants from whole sediment, pore water, and elutriate to decrease, increase, or stay the same with the length of sediment/contaminant contact time. Bioavailability, as measured by  $K_{uoc}$ , appeared to be a function of the exposure media as well as the studied contaminant. Uptake of *trans*-chlordane by *C. riparius* in all three media appeared to decline with the age of sediment contamination. These data agree with previous partitioning studies that showed the freely dissolved fraction of *trans*-chlordane to decline in elutriate and pore water generated from sediments stored from 7 to 60 d [47]. However, those partitioning studies, as well as other studies that measured uptake clearances, did not establish clear linear relationships between sediment storage and the amount of biologically available BaP and pyrene [48]. The mechanism for these changes in contaminant bioavailability is not known, and further studies engaging lab-dosed and field-collected sediments are needed before cause-and-effect relationships can be established. However, the effect of storage on bioavailability is an important variable and should be considered when determining hazards represented by lab-dosed sediments as well as in-place pollutants.

Our data demonstrate the difficulties encountered when comparing the bioavailability of a number of hydrophobic organic contaminants in whole sediment and aqueous fractions of whole sediment, and the assumptions that must be made pertaining to partitioning contaminants to organic carbon. Although normalizing the data to the fraction of TOC in the exposure media is considered to compensate for differences in bioavailability [49,50], our data suggest that normalization to TOC may be more appropriate for some contaminants than for others. In addition, the differential bioavailability of contaminants arising from sediment/contaminant contact time or sediment dilution may affect organic carbon/contaminant partitioning and weakens the theory that increased levels of TOC mediate the bioavailability of organic contaminants.

Our data also cast doubt on using benthic invertebrates in aqueous media to simulate whole-sediment exposure. Whereas these species may be adequate indicators of hazards in substrate similar to their natural benthic environments,

other species native to water-column or epibenthic environments may be more suitable for aqueous studies. However, water-column species such as *Daphnia* or *Ceriodaphnia* spp. may not be appropriate for assessing the toxicity of sediment-associated contaminants. Certainly, trade-offs will have to be made between which species will "work" and which species is most indicative of hazard represented by sediment-associated contaminants, if future bioassay techniques that use aqueous fractions of whole sediment are to be developed.

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