

ACCUMULATION AND TOXICOKINETICS OF FLUORANTHENE IN WATER-ONLY EXPOSURES WITH FRESHWATER AMPHIPODS

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Abstract—Two 10-d water-only toxicity tests with radiolabeled fluoranthene were conducted with two species of freshwater amphipods, *Hyalella azteca* and *Diporeia* sp. For *H. azteca*, 10-d median lethal concentrations were 564 nmol/L and 481 nmol/L. Tentative median lethal doses, determined from the regressions of body burden of remaining live *H. azteca* versus survival, were 5.6 and 3.6 nmol fluoranthene/kg wet weight tissue. *Diporeia* appeared to be less sensitive, because survival in *Diporeia* was greater than 84% after 10 d exposures. Elimination rates determined for *Diporeia*, ranging from 0.0011 to 0.0042/h (half-lives of 7–26 d), were much slower than rates determined for *H. azteca* of 0.128 to 0.188/h (half-lives of 4–6 h). Faster elimination in *H. azteca* may be related to its greater ability to metabolize fluoranthene. For *H. azteca*, an average of 17% of its body burden was present as metabolites after 24 h of exposure to radiolabeled fluoranthene, as compared to 5% for *Diporeia*. For *Diporeia*, exposure to various water concentrations of fluoranthene for various lengths of time resulted in declines in the conditional uptake clearance rates (ml water cleared/g wet weight tissue/h). A similar, although less dramatic trend was observed for conditional uptake clearance rates in *H. azteca*.

Keywords—Fluoranthene Amphipods Toxicokinetics Water Critical body burden

INTRODUCTION

The toxicity of organic contaminants to aquatic invertebrates has long been employed as an important test of the effects of chemicals released to the environment. In particular, Section 304(A) of the Clean Water Act requires that the U.S. Environmental Protection Agency (EPA) establish water quality criteria (WQC) for certain priority pollutants. These criteria, based on water-only toxicity tests, establish final chronic values (FCVs), or concentrations of individual pollutants in ambient water that, when not exceeded, will ensure a water quality sufficient to protect a specified water use [1]. The EPA has also examined a variety of approaches for the establishment of sediment quality criteria (SQC), numeric concentrations of individual chemicals that are predictive of biological effects. Sediment quality criteria have been proposed for several organic contaminants, including polycyclic aromatic hydrocarbons (PAHs), using the equilibrium partitioning (EqP) approach [2]. The EqP approach uses the FCV determined for water-only exposures, to establish sediment organic-carbon-normalized SQC.

A variety of toxicokinetic models are also used to predict the bioaccumulation and effects of organic contaminants. These models, which were developed to more accurately predict non-steady-state and non-equilibrium situations, depend on the measurement of uptake clearance and elimination rate constants to describe the kinetics of accumulation. First-order rate coefficient models, and especially clearance volume models, have been used extensively in aquatic toxicology [3]. (Uptake clearance rates are defined as the volume or mass of a source compartment that is cleared of contaminant per mass of organism per unit time.) In addition, the critical body residue

hypothesis [4] is presently being evaluated as a means to interpret and assess the potential toxicity of compounds that are bioaccumulated. This approach predicts that the potency of chemicals that act by a narcotic mechanism (most nonpolar organics) should be essentially constant for similar organisms [5,6], and acute narcosis will occur at body burdens of 2 to 8 mmol/kg wet weight. In this approach, the internal body burden, rather than the external water or sediment concentration, is used as a surrogate for dose at the site of toxic action.

For any approach or criteria to be effective, the assumptions of the model and the data used to make predictions must continue to be tested and refined. Many of the approaches previously mentioned incorporate exposure and accumulation from water as a parameter in modeling uptake of contaminants. One goal of the present study was to measure important toxicokinetic parameters such as conditional uptake clearance rate and elimination rate for two standard sediment toxicity test organisms, the freshwater amphipods *Hyalella azteca* and *Diporeia* sp. These experiments test the assumption that the rate of uptake of contaminant is independent of the water concentration and duration of exposure to the contaminant. We hypothesized that exposure to a hydrophobic organic compound, such as the PAH fluoranthene, would result in narcosis and a subsequent decline in the uptake clearance rate of fluoranthene. These experiments also determine a median lethal concentration (LC50) for fluoranthene based on measured water concentrations and a median lethal dose (LD50) based on tissue concentrations for these species. Ability to biotransform fluoranthene was also examined.

MATERIALS AND METHODS

Chemicals

[¹⁴C]Fluoranthene with specific activities of 45 and 55 Ci/mol was obtained from Sigma Chemical Co. (St. Louis,

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MO, USA). Unlabeled fluoranthene was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). [^{14}C]Fluoranthene was tested for purity prior to use by thin-layer chromatography on silica plates (Alltech Associates, Deerfield, IL, USA) using hexane:benzene (8:2, v/v), and found to be 98% pure.

Spiking

Water used in these experiments included both Lake Michigan and Huron River water, which closely matches Lake Michigan water in terms of hardness (165 mg/L total hardness as calcium carbonate), alkalinity (250 mg/L total alkalinity as calcium carbonate), and pH (8.2). Test solutions were prepared by adding appropriate amounts of an unlabeled fluoranthene stock solution (1 mg/ml in acetone) to 4 L of filtered (0.45 μm Fin-L-Filter[®], Cole Palmer Co., Niles, IL, USA) water. Control test solutions were prepared with similar amounts of acetone (<0.5 ml/L). The concentration of fluoranthene in the unlabeled stock solution was confirmed by gas chromatography/mass spectrometry (GC/MS) as previously described [7]. Some test solutions spiked with unlabeled fluoranthene (including control solutions) were also spiked with trace amounts of [^{14}C]fluoranthene. Prior to use in experiments, the concentration of [^{14}C]fluoranthene in each radiolabeled test solution was measured in triplicate (1 ml) by liquid scintillation counting (LSC) on a Tri-Carb Liquid Scintillation Analyzer (Model 2500 TR, Packard Instrument Co., Meriden, CT, USA). Samples were corrected for quench using the external standards ratio methods after subtracting background. The nominal specific activity (mCi [^{14}C]fluoranthene added/mmol total fluoranthene added) for each test solution was used to calculate the concentration of total fluoranthene in all water and tissue samples. Beakers containing radiolabeled test solution were sampled (1 ml) for LSC every day, before and after renewal of test solutions. Two thirds of the overlying water in each beaker was replaced every day with fresh test solution.

Organisms

The epibenthic detritivore *H. azteca* is widely found in the surface sediments of shallow, freshwater lakes and streams throughout North and Central America, where it reaches maturity rapidly (within 30 d) under optimal environmental conditions (see references in [8]). *Hyaella azteca* used in the present experiments were received from C. Ingersoll of the National Biological Survey, Columbia, Missouri, USA. Animals were generally of a size that passed through a 1-mm sieve, but were retained on a 500- μm sieve (2–3 weeks old). *Diporeia* were collected from Lake Michigan as previously described [9] and held in the lab for less than 1 month prior to use. *Diporeia* spp., standard organisms for sediment toxicity tests [10], are the dominant macrobenthic invertebrates of the Great Lakes, where they tend to feed on bacteria-rich sediments. Previously known as *Pontoporeia hoyi*, a recent taxonomic reassessment transferred this amphipod to the new genus *Diporeia* [11]. The exact number of species in this genus is uncertain, but at least eight species are known [11]. Life span ranges from 1 to 3 years [12]. *Diporeia* from the Lake Michigan site have previously been shown to have low tissue concentrations of PAHs [13]. All experiments were conducted under constant dim yellow light ($\lambda > 500 \text{ nm}$) to avoid photooxidation of the compound and photoinduced toxicity.

Water-only toxicity tests

Two water-only toxicity experiments were carried out for each species. Animals were exposed to test solutions at am-

bient temperatures (4°C for *Diporeia* and room temperature for *H. azteca*). Each day for 10 d, the number of live and dead amphipods from replicate beakers (five beakers/treatment) was recorded and dead animals were removed. For the second *Diporeia* experiment and for both *H. azteca* experiments, live animals from separate beakers were sampled at various time points for tissue concentration. Animals were blotted dry, weighed, transferred to 12-ml scintillation cocktail (3a70b, Research Products International, Mt. Prospect, IL, USA), and sonicated for 1 min with a Tekmar (Cincinnati, OH, USA) high-intensity probe (375 W at 20% power). After subsidence of chemiluminescence (24 h), radioactivity was quantified by LSC. The total amount of fluoranthene (both radiolabeled and unlabeled) in each sample was calculated using the nominal specific activity of the test solution. For all experiments (except biotransformation determinations), tissue concentrations represent total fluoranthene equivalents (parent compound and metabolites on a molar basis). Because *Diporeia* has a limited ability to metabolize PAHs [14], body burdens are predominantly parent compound. *Hyaella azteca* has greater ability to metabolize PAHs [15] and, therefore, body burdens of fluoranthene equivalents represent unknown portions of parent compound and metabolites.

Experiments were conducted with *H. azteca* starting on December 1, 1994, and March 14, 1995. After gradual acclimation (2 d) to local Huron River water, animals were exposed to test solutions (200 ml) in 400-ml beakers, 20 animals per beaker, five beakers per concentration. Nominal concentrations of radiolabeled test solutions were 0, 80, 320, 470, and 630 nmol/L for the first *H. azteca* experiment and 0, 80, 320, 630, and 1,270 $\mu\text{g/L}$ for the second *H. azteca* experiment. Each beaker contained a 1-cm square of sterile cotton surgical gauze for substrate that was presoaked for 48 h in filtered river water. Possible adsorption of compound to the gauze was accounted for because measured rather than nominal concentration of radiolabeled compound in the beakers was used to determine the concentration of total fluoranthene (as described above). *Hyaella azteca* were fed 0.5 ml YCT yeast-cerophyl-trout chow (YCT) per beaker every other day [8]. For both experiments, remaining live animals were sampled on day 10 for tissue concentration.

Experiments were conducted with *Diporeia* starting on August 2, 1994 and May 12, 1995. Animals were exposed to test solutions (300 ml) in 600-ml beakers, 20 animals per beaker, five beakers per concentration. Nominal concentrations of radiolabeled test solutions were 0, 320, 630, 950, and 1,270 nmol/L for both experiments. Live animals were not sampled in experiment 1 for body burden. In experiment 2, live animals were sampled on day 10 for determination of tissue concentrations.

For the first water-only toxicity experiment with *Diporeia*, data were also taken on the number of animals that appeared to be narcotized. Narcosis is defined as the inability to maintain an upright body orientation. Another definition of narcosis used in some studies is lack of movement. Lack of movement was an unsatisfactory descriptor in these experiments, because the animals were capable of some movement, but did not exhibit normal upright orientation. Because *H. azteca* held fast to the substrate (gauze) in the beaker even after death, narcosis could not be observed in this species.

Elimination

Hyaella azteca were exposed at 22°C to 200 ml of radiolabeled test solution in 400-ml beakers, 20 animals per beaker,

for 24 h. Nominal concentrations of radiolabeled test solutions were 80, 320, 630, and 1,270 nmol/L. Each beaker contained a 1-cm square of presoaked surgical gauze for substrate. After exposure, *H. azteca* were transferred to unlabeled water (with clean gauze) and fed 1 ml of YCT per beaker. Animals were sampled from replicate beakers ($n = 3$) before transfer to unlabeled water ($t = 0$) and at approximately 1, 4, and 8 h after transfer. Tissue concentrations were determined by LSC as described above. Exact times were used for calculating elimination rates.

Diporeia were exposed at 4°C to 300 ml of test solution in 600-ml beakers, 20 animals per beaker, for 24 h. Nominal concentrations were 80, 320, 630, and 1,270 nmol/L. After exposure, animals were transferred to 600-ml beakers containing 200 g wet weight Lake Michigan sediment and 400 ml of clean, filtered Lake Michigan water. Animals were sampled from replicate beakers ($n = 3$) before transfer to unlabeled sediment ($t = 0$) and 1, 2, 4, 7, 10, and 14 d after transfer. At each time point, animals were sampled for tissue concentration as described above. The rate of elimination for both species was estimated from a linear regression of the natural log (ln) of tissue concentration versus time.

Biotransformation

Hyalella azteca and *Diporeia* were exposed to [¹⁴C]fluoranthene (approx. 1.5 nmol/L) in water for 24 h. Samples of *H. azteca* (200 mg wet weight) and *Diporeia* (100 mg wet weight) were ground with a mortar and pestle and 2.5 g anhydrous sodium sulfate and extracted with chloroform, methanol, and water using a modification of a lipid extraction method [16], adapted for the analysis of PAHs and metabolites [17]. The extraction protocol separates nonpolar compound from aqueous metabolites. Radioactivity in duplicate subsamples (1 ml) of the aqueous phase were determined by LSC. Duplicate subsamples (1 ml) of the organic extract were dried and resuspended in scintillation cocktail for analysis by LSC. The remaining organic phase was extracted with hexane, potassium hydroxide, and dimethyl sulfoxide as previously described [17,18] to separate parent compound from polar metabolites. After extraction, an aliquot of the remaining salt and tissue pellet was sonicated with scintillation cocktail. After subsidence of chemiluminescence (24 h), radioactivity was quantified by LSC. Unextractable radioactivity associated with the tissue pellet was presumed to reflect electrophilic primary metabolites of fluoranthene that were covalently bound to cellular macromolecules in the tissue. An aliquot of the [¹⁴C]fluoranthene standard stock without tissue was also extracted as described above to assess the extraction protocol.

Accumulation with and without preexposure to fluoranthene

Prior to determination of the rate of accumulation of [¹⁴C]fluoranthene, animals were preexposed for various times to test water spiked with unlabeled fluoranthene (0, 320, 630, or 1,270 nmol/L, nominal concentrations), with daily renewal. Both *Diporeia* (10 animals per beaker) and *H. azteca* (20 animals per beaker) were exposed to 200 ml of spiked water in 400-ml beakers. During preexposure, *H. azteca* were fed every other day with 0.5 ml YCT [8]. *Diporeia* were not fed. After preexposure for 1, 2, 5, or 10 d, preexposure water in each beaker was replaced with test water containing the same concentration of cold fluoranthene as listed above and a trace amount of [¹⁴C]fluoranthene. Other animals were exposed di-

rectly to radiolabeled test water without preexposure to measure the decline in water concentration. Radiolabeled water from each beaker was sampled at the beginning and end of each 6-h uptake exposure. Animals were sampled from triplicate beakers after 1, 2, 4, and 6 h in radiolabeled test solution in order to determine the uptake clearance rate. Four animals were sampled from each beaker at each time point, blotted dry, weighed, and transferred to scintillation cocktail. Samples were sonicated for 1 min and held overnight prior to quantification by LSC. Nominal specific activities for each stock solution were used to calculate the concentration of total fluoranthene in each sample.

Modeling accumulation

Because there were substantial declines in the water concentration over the course of the 6-h uptake experiments, and because the mass-to-volume ratio changed as animals were removed, estimation of uptake clearance rates employed a model that specifically includes a term for the decline in water concentration. Assuming a linear decline in water concentration over time, conditional uptake clearance rates (ml water cleared of fluoranthene/g wet weight tissue/h) were estimated by nonlinear regression to the following model:

$$C_a = (k_u/k_e) \{ [C_w^0 + (m/k_e)] [1 - \exp(-k_e t)] - mt \}$$

where C_a = the concentration of fluoranthene in the tissue (pmol/g wet weight), k_u is the conditional uptake rate constant (ml/g/h), k_e is the rate constant for the elimination of fluoranthene from the tissue (h⁻¹), C_w^0 is the concentration in the water at $t = 0$, m is the slope of the decrease in the water concentration (pmol/ml/h), and t = time (h). Average (SD) percent decrease in water concentration over 6 h was 24% (3.8%, $n = 12$) for *Diporeia*, and 31% (9.7%, $n = 21$) for *H. azteca*. Mean measured values for m (ranging from 0.048 to 44.5 pmol/ml/h), C_a , and C_w^0 , were used in all calculations. Previously determined mean measured elimination rates of 0.15/h for *H. azteca* and 0.0021/h for *Diporeia* were used in all calculations.

Statistics

Nonlinear regression of accumulation data was modeled using *SYSTAT for Windows, Version 5* (SYSTAT, Evanston, IL, USA). Linear regressions for elimination data were modeled with *SAS[®]/STAT, Version 6*, 4th edition (SAS Institute, Cary, NC, USA). Student's t test was used when comparing means or slopes of regression lines. Differences were considered significant when $p < 0.05$. Mortality data were analyzed by the trimmed Spearman-Kärber method, using *Statistical Methods and Software for Toxicological Data Analysis* (B.A. Zajtlik, University of Waterloo, Waterloo, ON, Canada, and M. Newman, Savannah River Ecology Lab, Aiken, SC, USA). Confidence limits for the LD50, determined from the regression of survival versus body burden, were calculated from formulas in Sokal and Rohlf [19].

RESULTS

Toxicity experiments

Water concentrations. In the 10-d mortality studies, water concentrations measured at the start of the experiments were in general agreement with nominal concentrations, except for the first *Diporeia* experiment (Table 1). Measured concentrations for the first *Diporeia* experiment were unexpectedly high and may represent an error in the amount of radiolabeled com-

Table 1. Nominal and mean measured water concentrations, measured body burdens, percent narcosis of surviving 10-d animals, and percent survival in 10-d water-only toxicity tests

Organism	Experiment 1					Experiment 2			
	Nominal water concn.	Mean (SD) measured water concn. (nmol/L) (n = 5)	Mean (SD) measured 10-d body burden (mmol/kg)	10-d % Narcosis	Mean (SD) 10-d % survival (n = 5)	Nominal water concn.	Mean (SD) measured water concn. (nmol/L) (n = 3)	Mean (SD) measured 10-d body burden (mmol/kg)	Mean (SD) 10-d % survival (n = 5)
<i>Diporeia</i>	0	ND ^a	ND	8	96 (4.2)	0	ND	ND	99 (2.2)
	320	326 (25)	ND	10	97 (4.5)	320	311 (15)	2.9 (1.1)	92 ^{ab} (2.7)
	630	781 (49)	ND	15	87* (7.6)	630	677 (20)	4.9 (1.3)	84* (6.5)
	950	1,200 (59)	ND	26	91 (6.5)	950	979 (30)	5.0 (1.1)	91* (4.2)
	1,270	1,920 (54)	ND	15	93 (5.7)	1,270	1,350 (30)	6.0 (1.5)	90* (5)
<i>Hyalella azteca</i>	0	ND	ND	ND	98 (2.7)	0	ND	ND	99 (2.2)
	80	69 (3)	0.5 (0.5)	ND	100 (0)	80	59 (2)	0.2 (0.004)	99 (2.2)
	320	272 (5)	2.1 (1.1)	ND	79* (12)	320	217 (5)	0.3 (0.06)	100 (0)
	470	440 (15)	3.5 (1.4)	ND	80* (18)	630	420 (5)	1.6 (0.31)	67* (15)
	630	593 (15)	5.0 (1.1)	ND	48* (14)	1,270	880 (30)	ND	4* (9)

^a ND = not determined.

* * significantly different from the control ($p < 0.05$).

pound that was added and in the nominal specific activities used to calculate total fluoranthene for that experiment. Measured concentrations of radiolabeled compounds in that experiment averaged approximately 25% greater than the nominal concentration; therefore, we estimate that reported measured concentrations for the first *Diporeia* experiment may be overestimated by approximately 25%. Before renewal, water concentrations in the beakers declined by an average of 25.4% per day (SD = 15.1, $n = 75$, experiment 2) for *Diporeia* and 18.1% (SD = 5.7, $n = 47$, experiment 1) and 17.6% (SD = 15.1, $n = 61$, experiment 2) for *H. azteca*.

Mortality. Although *Diporeia* exposed to fluoranthene showed signs of narcosis (discussed below), percent survival at day 10 was generally greater than 90%, and an LC50 could not be established (Table 1). Note that in both experiments, maximum mortality was observed in *Diporeia* that were exposed to intermediate water concentrations. For *H. azteca*, percent survival in the first experiment ranged from 98% in controls to 44% at the highest water concentration, 593 nmol/L (Table 1). In the second experiment, *H. azteca* were exposed to higher water concentrations and 10-d survival dropped to near zero at the highest concentration, 880 nmol/L. Ten-day LC50 values for *H. azteca* are 564 nmol/L (524–603 nmol/L, 95% CI) for the first experiment and 481 nmol/L (448–516 nmol/L, 95% CI) for the second experiment.

Narcosis. For *Diporeia*, percent of remaining live animals that were narcotized on day 10 ranged from 8% for controls to a maximum of 26% for animals exposed to the second highest water concentration (1,200 nmol/L) (Table 1). Maximum percent narcosis at intermediate water concentrations was also observed at earlier time points in the first experiment. This result agrees with the observation that maximum mortality was typically observed at intermediate water concentrations.

Body burdens. For *H. azteca*, the relationship between percent survival versus tissue concentration of surviving amphipods on day 10 (taken from all water concentrations) yielded significant linear regressions for both experiments (Fig. 1). On the basis of the relationship determined for the first experiment, the estimated tissue concentration associated with 50% mortality (LD50) was 5.6 mmol/kg wet weight (2.6–9.2 mmol/kg wet weight, 95% CI) (Fig. 1b). For the second experiment,

tissue concentrations for animals that were exposed to the highest water concentration were not included in the regression because nearly all animals were dead by the 10-d time point (Table 1 and Fig. 1). The body burden for animals exposed to comparable water concentrations in the second experiment was less than that in the first experiment (Table 1). Only a rough estimate of 3.6 mmol/kg (0.47–7.2 mmol/kg, 95% CI) can be established for the LD50 in experiment 2.

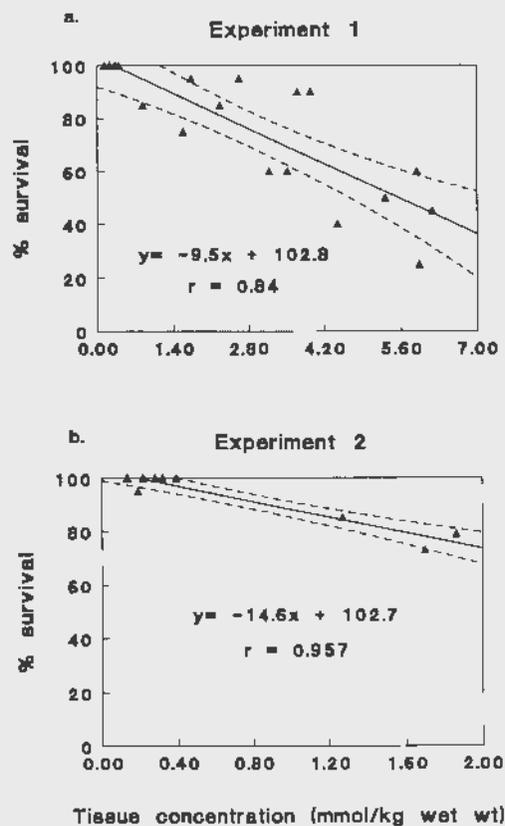


Fig. 1. Concentration of fluoranthene in *Hyalella azteca* tissue versus percent survival, after 10-d water-only exposures. For experiment 1, $n = 19$, representing five samples/treatment with one missing data point. For experiment 2, $n = 9$, representing three samples/treatment. Dashed lines represent 95% confidence intervals.

Table 2. Elimination rate (k_{el}) of fluoranthene after preexposure to various concentrations of fluoranthene ($n = 15$)

Organism	Nominal preexposure dose (nmol/L)	k_{el} (h^{-1})	SE	r^2
<i>Diporeia</i>	80	0.0037	0.0007	0.573
	320	0.0011	0.0007	0.114
	630	0.0042	0.0006	0.690
	1,270	0.0021	0.0006	0.386
<i>Hyalella azteca</i>	80	0.147	0.0204	0.838
	20	0.136	0.0154	0.885
	630	0.188	0.0184	0.912
	1,270	0.128	0.0296	0.653

Because survival of *Diporeia* was generally greater than 90% at all doses (Table 1), no regression relationship could be established for the estimation of a water-only LD50 in this species. In experiment 2, average (SD) tissue concentrations ($n = 7$ per dose) of live amphipods measured on day 10 were 2.9 (1.1), 4.9 (1.3), 5.0 (1.1), to 6.0 (1.5) mmol/kg, for lowest to highest water concentrations, respectively.

Elimination experiments

Estimated elimination rates for *Diporeia* ranged from 0.0011 to 0.0042/h, corresponding to half-lives of 7 to 26 d (Table 2). Elimination rates in *H. azteca* were much faster, ranging from 0.128 to 0.188/h, with corresponding half-lives of 4 to 6 h. Elimination did not appear to change in a dose-dependent manner in either species.

Biotransformation

For *H. azteca*, average (SD, $n = 2$) percent of total [^{14}C]fluoranthene body burden was 83.2% (0.6) parent compound, 1.1% (0.01) polar metabolites, 8.8% (0.07) aqueous metabolites, and 7.0% (0.5) residual or unextractable. For *Diporeia*, average (SD, $n = 2$) percent compound in each class was 95.0% (0.6) parent compound, 0.65% (0.2) polar metabolites, 1.3% aqueous metabolites, and 3.2% (0.4) residual. The average (SD, $n = 2$) percent of the [^{14}C]fluoranthene standard associated with each phase was 96.8% (1.1) parent compound, 1.7% (1.1) polar phase, 0.3% (0.3) aqueous phase, and 1.0% (0.1) residual. These results suggest that *H. azteca* has a greater ability to biotransform fluoranthene than does *Diporeia*.

Accumulation experiments

Accumulation of fluoranthene was well described by the model described in Equation 1. To examine the model's predictive ability, estimated conditional uptake clearance rates were used in Equation 1 to compare model predictions of body burden to actual data. Comparison of actual body burdens to model predictions (solid line) demonstrates reasonable agreement of the model to the data in these two typical examples (Fig. 2).

Conditional uptake clearance rates were estimated by non-linear regression to the model described in Equation 1, using measured values for body burden, elimination rate, initial water concentration, and decrease in water concentration. When *Diporeia* were not preexposed, conditional uptake clearance rates declined in a dose-dependent manner (Fig. 3). For example, the highest uptake rate (210 ml/g/h) was measured for animals in trace levels of fluoranthene, and the lowest rate (59 ml/g/h)

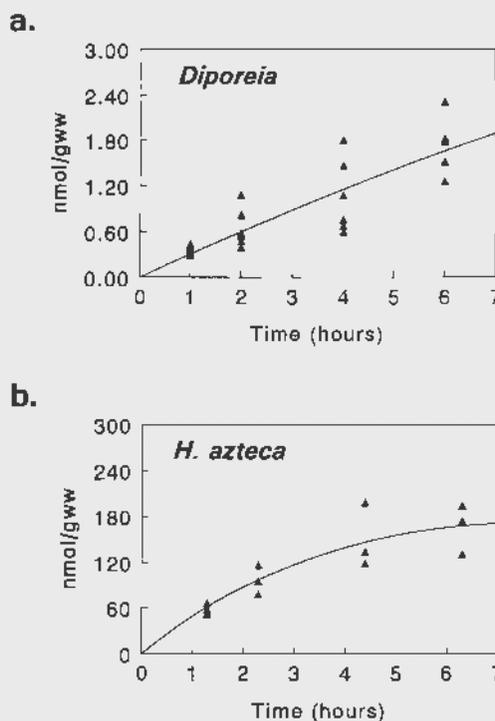


Fig. 2. Typical uptake curves for accumulation of fluoranthene from water. Curves represent model predicted values.

was measured for animals in the highest water concentration (1,260 nmol/L). This result suggests that for at least 6 h (the time over which the uptake rate was measured), exposure to high concentrations of fluoranthene reduced the uptake clearance rate in *Diporeia*. After 1 or 2 d of preexposure to various concentrations of fluoranthene, uptake clearance rates for *Diporeia* appeared to be reduced in most cases ($k_{el} = 56$ –134 ml/g/h), in comparison to animals that were exposed for comparable times to water without fluoranthene ($k_{el} = 156$ –158 ml/g/h) (Fig. 3). However, the reduction was not as great as that seen when rates were measured without preexposure. After 5 or 10 d of preexposure to water without fluoranthene, conditional uptake clearance rates measured in trace amounts of radiolabeled fluoranthene were generally lower (141–149 ml/g/h) than rates measured for control animals without preexposure (210 ml/g/h). This result suggests that conditional uptake clearance rates were reduced in animals held for 5 to 10 d in water only, without sediment or fluoranthene. The rate measured after 10 d of preexposure to the highest doses ($k_{el} = 90$ ml/g/h) was not as low as the rate measured for exposure to the highest dose without preexposure ($k_{el} = 59$ ml/g/h). This result suggests that some recovery or adaptation to the compound may have occurred over the course of the 5- to 10-d preexposures to unlabeled fluoranthene.

When *H. azteca* were not preexposed, measured conditional uptake clearance rates ranged from 284 to 439 ml/g/h and did not appear to decline when the *H. azteca* were exposed to high concentrations of fluoranthene, as was observed in *Diporeia* (Fig. 3). After 5- or 10-d exposures to the highest water concentration (980 nmol/L), all test organisms were dead, body burdens were not measured, and uptake rates could not be determined. After 2, 5, or 10 d of preexposure, conditional uptake rates calculated for the three highest concentrations (270–980 nmol/L) ranged from 67 to 194 ml/g/h, values that were slightly lower than rates calculated for animals that were

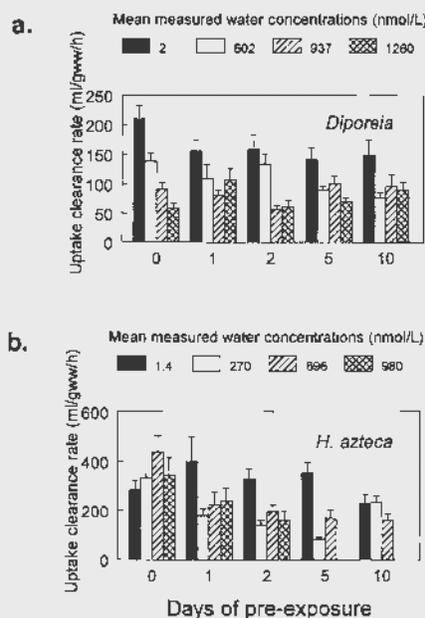


Fig. 3. Uptake clearance rates (k_u , = ml water cleared of fluoranthene/g wet weight tissue/h) for accumulation of radiolabeled fluoranthene. Rates were measured over a period of 6 h, after preexposure to various concentrations of unlabeled fluoranthene for various lengths of time (0–10 d). Error bars represent 95% confidence limits of the regression estimate.

held for comparable times in water without fluoranthene (226–353 ml/g/h). This result suggests that long exposure to high concentrations of fluoranthene may result in a decrease in the water-only conditional uptake clearance rate in *H. azteca*.

DISCUSSION

Toxicity and critical body burdens

In these experiments, *H. azteca* appeared to be more sensitive than *Diporeia*, because an average 10-d water-only LC50 could be determined for *H. azteca* (522 nmol/L), but survival for *Diporeia* was greater than 84% even at the highest water concentrations (Table 1). The LC50 determined for *H. azteca* in these experiments is somewhat higher than previously determined 10-d LC50s for fluoranthene in this species of 221 nmol/L [20] and 299.6 nmol/L (B. Suedel, personal communication), and considerably greater than the final acute value (FAV = 166 nmol/L) established from the geometric mean of acute LC50s determined for 13 freshwater species [21].

In contrast, *H. azteca* was less sensitive than *Diporeia* (based on sediment concentration) in 28-d fluoranthene sediment exposures [22] and in comparative 28-d survival sediment bioassays with field-collected sediments [23]. Because *H. azteca* is more sensitive than *Diporeia* on the basis of body burdens measured in water-only exposures, we conclude that low mortality of *H. azteca* in fluoranthene sediment experiments [22] is due to reduced accumulation of the compound from the sediment exposures, rather than an overall lack of sensitivity to the compound.

The maximum measured 10-d body burden for *Diporeia* in the water-only experiment, 6.0 mmol/kg, was associated with only 10% mortality (Table 1). This was surprising, because related work with *Diporeia* found body burdens of 2.7 mmol/kg (0.9–12.9 mmol/kg, 95% CI) (10 d) and 6.5 mmol/kg (3.4–25.3 mmol/kg, 95% CI) (30 d) to be associated with 50% mortality in two separate sediment bioassays with fluoranthene

[22] and estimated 30-d LD50s were 6.3 mmol/kg (4.6–41.7 mmol/kg, 95% CI) and 9.4 mmol/kg (7.9–54.2 mmol/kg, 95% CI) for pyrene sediment bioassays [24] and 6.1 mmol/kg (3.7–21.3 mmol/kg, 95% CI) for a mixture of PAHs at 26 d [25]. However, factors other than fluoranthene exposure may have contributed to the low LD50 of 2.7 mmol/kg in one of the previous sediment experiments. We believe that the population of animals collected in December for that sediment experiment probably contained more senescent animals than populations collected for other experiments and therefore the critical body residue (CBR) of 2.7 mmol/kg should not be considered representative of the typical sensitivity of *Diporeia*. The results from the present experiment suggest that the critical body burden is greater than 6.0 mmol/kg in this species.

Although the importance of lipid normalization for the interpretation of CBRs has yet to be resolved, it has been hypothesized that sequestration of a compound in storage lipid would remove it from the site of action (membranes for narcotics), and a higher lipid content might be considered protective [26–28]. Recent work found that 50% of the intraspecific variation in CBR for single compounds in fathead minnows could be attributed to the lipid content of the individual animals [29]. Because changes in percent lipid content or size of the animals are hypothesized to contribute to intra- and interspecific differences in lethal body burdens, seasonal differences in sensitivity of field-collected *Diporeia* to organic contaminants are presently being examined in water and sediment bioassays in our lab. Because lipid content of *Diporeia* is known to be maximal in May to June (up to 54% of their ash-free dry weight) and minimal in December to March (21% of the ash-free dry weight) [30], we can speculate that the average body burden of 6.0 mmol/kg in the present toxicity tests (conducted with *Diporeia* in mid-May) might have been too low to produce significant mortality because of a high seasonal lipid content. Alternatively, longer exposures (>10 d) to an internal body burden of 6.0 mmol/kg may be required to produce lethality in the water-only exposures, because doses of this magnitude produced 50% mortality only after longer, 30-d exposures in previous sediment experiments [22]. Also note that although actual mortality was low in both water-only toxicity tests with *Diporeia*, animals exposed to higher concentrations of fluoranthene exhibited acute narcosis more often than did control animals (unpublished data and Table 1) and were obviously affected by the exposure.

For *H. azteca*, estimates of body burdens associated with 50% mortality in the water-only exposures were 5.6 mmol/kg (2.6–9.2 mmol/kg, 95% CI) and 3.6 mmol/kg (0.47–7.2 mmol/kg, 95% CI) for experiments 1 and 2, respectively. These values are in agreement with values of 2 to 8 mmol/kg predicted by the CBR hypothesis to be associated with acute narcosis. Seasonal variation in lipid content is not expected for *H. azteca* (which has about half the lipid content of *Diporeia*) because the organisms used in these assays were raised in culture rather than collected from the field. When comparing the effective dose between two species, the relative lipid content may be important.

Biotransformation

Preliminary results from these experiments suggests that *H. azteca* has a greater ability to metabolize fluoranthene than does *Diporeia*. After a 24-h exposure, only 83% of *H. azteca*'s body burden was present as parent compound. The portion of total body burden present as metabolites should increase over

time, especially if the rate of elimination of metabolites is slow, as has been seen in other species of aquatic invertebrates [31,32]. At present, the relative contribution of parent compound and metabolites to overall toxicity is unknown and body burdens are presented as total fluoranthene equivalents.

As observed in other species of amphipods and crustaceans, variation between species in ability to metabolize organic xenobiotics may be reflected in differences in sensitivity to these compounds [31,32]. For example, another comparative study of two species of amphipods found the more sensitive species to have a greater ability to metabolize the aromatic hydrocarbon benzo[a]pyrene to potentially toxic intermediates [33]. Alternatively, ability to metabolize, if coupled with rapid elimination of parent compound and metabolites, may be protective. The overall advantages and disadvantages of the ability to metabolize PAHs are not yet well understood for aquatic invertebrates.

Greater ability of *H. azteca* to metabolize fluoranthene probably contributes in part to the more rapid elimination rate in this species (0.128–0.188/h), in comparison to *Diporeia* (0.011–0.0042/h), and respective elimination half-lives of 3 to 6 h and 7 to 25 d. Higher ambient temperature and lower lipid content in *H. azteca* may also contribute to its faster elimination rate in comparison to *Diporeia*.

Uptake clearance rates

Reduction in conditional uptake clearance rate at increased fluoranthene concentrations and length of exposure has important implications for our understanding of the factors that affect bioaccumulation and bioavailability. Reduction in the uptake clearance with increasing exposure invalidates the usual toxicokinetic models because these terms are implicitly assumed to remain constant over the course of the study. Such changes would make predictions of bioaccumulation and steady state impossible unless the effect on the rate coefficients were known. The 6-h measurements made in this study are the first to attempt to define these potential changes.

For compounds like fluoranthene, uptake from water apparently occurs from transfer across the respiratory membrane and across the chitinous exoskeleton of *Diporeia* [34]. Similar mechanisms of uptake from water are likely to occur in *H. azteca*. In the present water-only exposures, reductions in conditional uptake clearance rates are probably the result of changes in physiology (such as narcosis) that reduce movement through the water and rate of respiration, and thus the volume of water encountered. The absence of a clear reduction in conditional uptake clearance for *H. azteca* (Fig. 3) until substantial preexposure (2 d, more than five half-lives and sufficient time for *H. azteca* to reach steady state) may reflect the protective biotransformation of fluoranthene to more polar compounds. Biotransformation would reduce the effective narcotic potency of the fluoranthene because less of the compound will partition into membranes due to lower partition coefficients of the metabolites.

Reductions in conditional uptake clearance rate will be expressed as a reduction in the flux of compound into the organism, assuming a constant fluoranthene concentration in the exposure medium. Similar reductions in the uptake flux of contaminants have been observed for exposures in sediments. In sediment exposures, a factor (λ) was included in the bioaccumulation models to account for the apparent reduction in bioavailability [9]. The reduction in bioavailability was hypothesized to reflect the rate at which a contaminant moves

into an unavailable pool, and to be similar to changes observed in chemical extractability for compounds distributing between reversible/slowly reversible pools within the sediment [35]. However, in water-only exposures, observed declines in the uptake clearance rates for *Diporeia* at high doses of compound are less likely to reflect changes in the physical/chemical bioavailability of the compound. Although physical/chemical changes may have a dominant effect on the bioavailability of sediment-associated compounds, particularly when concentrations in the sediments are very low relative to those required for toxic effects [9,24], other factors may also contribute to λ , the parameter used to model the reduction in the uptake flux into the organism. Alternative mechanisms, such as the physiological effects of narcotic compounds hypothesized for water-only exposures, could account in part for changes observed in uptake kinetics in sediment exposures. Thus, the mechanism underlying differences in sediment uptake rates may include physiological changes in the organisms, as well as differences in the chemical/physical partitioning of the compound in the sediment.

CONCLUSIONS

Interspecific differences in metabolic ability and toxicokinetic rate coefficients may contribute to differences in sensitivity to organic contaminants. *Hyalella azteca*, which does metabolize fluoranthene, appears to be more sensitive than *Diporeia*, which does not. Measured water-only 10-d LD50s for *H. azteca* (3.6 and 5.6 mmol fluoranthene equivalents/kg tissue) were within the range of values predicted by the CBR hypotheses to be associated with acute narcosis (2–8 mmol/kg). The CBR is probably higher for *Diporeia* than for *H. azteca*, because little mortality was observed for *Diporeia* at body burdens as high as 6 mmol/kg in 10-d tests. Conditional uptake rates for *Diporeia* varied over time of exposure and for various concentrations of compound. Dose-dependent changes in conditional uptake rates may affect predictions of steady-state body burdens and associated toxicity based on toxicokinetic models.

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