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CHARACTERIZATION OF THE ACUTE TOXICITY OF PHOTOACTIVATED FLUORANTHENE TO GLOCHIDIA OF THE FRESHWATER MUSSEL, *UTTERBACKIA IMBECILLIS*

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Abstract—The acute photoactivated toxicity of fluoranthene to the glochidial larvae of the paper pondshell, *Utterbackia imbecillis*, was characterized in the laboratory using three sets of experiments. Toxicokinetic studies revealed that glochidia rapidly bioaccumulated fluoranthene, reaching an apparent steady state in 4 h. Based on a two-compartment model, uptake (K_u) and depuration (K_d) rate constants were 1394 ml/g/h and 0.769/h, respectively. However, experimental data suggested the presence of a fast and slow depuration compartment with a K_d of 0.290 and 0.031/h, respectively. Replicate 24-h acute toxicity tests designed to determine the overall sensitivity of glochidia to photoactivated fluoranthene were conducted under simulated sunlight (ultraviolet [UV]-A = 69.0 ± 1.0 μW/cm²) (mean ± standard deviation [SD]). Mean median lethal concentrations (LC50) of fluoranthene at 8, 16, and 24 h were 5.59 ± 0.59, 4.09 ± 0.57, and 2.45 ± 0.45 μg/L, respectively. Mean median lethal doses (LD50) at the same time periods were 14.76 ± 2.17, 11.66 ± 2.82, and 6.98 ± 1.31 μg/g dry weight, respectively. Acute toxicity tests designed to elucidate the relationship between the rate of mortality and UV intensity were conducted under one of four different UV intensities (UV-A = 15, 31, 50, and 68 μW/cm²). Regression analysis revealed that the time-dependent mortality of glochidia was inversely related to the product of initial tissue residue of fluoranthene and UV intensity. These findings suggest that glochidia of freshwater mussels are among the most sensitive organisms tested to date to photoactivated fluoranthene and the time-dependent mortality of glochidia can accurately be predicted through evaluation of the product of fluoranthene tissue residue and the light intensity to which the glochidia is exposed.

Keywords—Photoinduced toxicity Freshwater mussels Polycyclic aromatic hydrocarbons Glochidia

INTRODUCTION

Native freshwater mussels (Bivalvia; Unionidae) are among the most imperiled fauna in the United States [1,2]. During the past 40 years, numbers of both individuals and species diversity have precipitously declined. The decline of many species has been primarily attributed to the habitat destruction and degradation associated with anthropogenic activities, such as the impounding and channelization of rivers [2]. Another causative factor in the decline of some species has been the introduction of environmental contaminants into these habitats. For example, acid mine runoff was implicated in the loss of mussel fauna from several streams in western Pennsylvania, USA [3]. Salt water and waste from oil and gas wells have been implicated in the destruction of many mussel beds in Texas, USA [4]. The loss of more than 6 miles of mussel beds in the lower Tennessee River (Kentucky, USA) has been linked to industrial pollution [5]. More recently, cholinesterase poisoning, probably resulting from an organophosphate or carbamate pesticide, was implicated as a causative factor in a die-off of the endangered Tar spinymussel (*Elliptio steinstansana*) in North Carolina, USA [6].

Contaminants in the environment appear to be causes not only of immediate mussel die-offs but may have a more subtle, but equally negative, impact on mussel recruitment. In fact, studies suggest that the populations of several species of freshwater mussels are currently experiencing recruitment failures [7,8]. Recruitment in most species of freshwater mussels is dependent on the successful transformation of a unique larval stage called the glochidium (reviewed in [9]). Glochidia develop within the gills of females, which act as marsupial brood pouches. When mature, glochidia (ranging in size from 50 to 400 μm, depending on species) are released from the female into the water column, where they can remain for up to 10 to 14 d. In order to complete their larval development, the glochidia of most species are obligate ecto- or endoparasites on host-specific fish. The time to juvenile metamorphosis and excystment is species dependent, ranging from 6 to 160 d. When the glochidium has finalized its transformation process into a juvenile mussel, it breaks out of the cyst and drops to the bottom sediments and begins its benthic existence.

The sensitivity of released glochidia has been evaluated for a few environmental toxicants, particularly metals [10,11] and pesticides [12,13]. To date, no studies have been performed to characterize the sensitivity of glochidia to polycyclic aromatic hydrocarbons (PAHs). Polycyclic aromatic hydrocarbons represent a major class of environmental contaminants that are ubiquitous in aquatic environments [14]. The primary routes of PAHs into aquatic environments include petroleum spillage, domestic or industrial effluents, surface runoff, and atmospheric deposition. Although PAHs as a group have a wide range of solubilities, they are generally considered to be both hydrophobic and lipophilic [15]. As a consequence, PAHs accumulate to high concentrations in sediments and the tissues of aquatic organisms, especially in areas near the PAH sources [16]. The toxicity of some PAHs is known to increase by an order of magnitude or more in the presence of solar ultraviolet light (reviewed by [17]). This UV-induced, or photoinduced, toxicity has been reported to a variety of freshwater and marine

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species including annelids, crustaceans, insects, fish, and amphibians [18–21]. Conceptual models have been developed that suggest PAH phototoxicity in animals is a direct function of both PAH tissue levels and the intensity of UV to which the organism is exposed [19,22].

The objective of the current study was to characterize the acute toxicity of photoactivated fluoranthene to the glochidia of a common freshwater mussel, *Utterbackia imbecillis*. Fluoranthene was used throughout this study because, in freshwater ecosystems, it often comprises the largest fraction of PAHs in surface waters, sediments, and biota [23,24]. In addition, the phototoxic properties of fluoranthene have been well documented [19,25–27]. Since PAH phototoxicity in animals is directly related to internal PAH dose or tissue residue [19,22], a basic understanding of the uptake and depuration processes is essential in characterizing its overall hazard to an organism or to a particular life stage of an organism. Toward this end, the first set of experiments in this study was toxicokinetic studies designed to determine the first-order conditional uptake and depuration constants for waterborne fluoranthene in glochidia. The second set of experiments was designed to assess the overall sensitivity of glochidia, both in terms of fluoranthene water and tissue concentrations, using replicate 24-h acute toxicity tests. The third set of experiments was designed to investigate the degree of reciprocity between fluoranthene dose and UV intensity for photoactivated fluoranthene in glochidia using a series of four acute toxicity tests conducted at one of four different intensities of UV.

MATERIALS AND METHODS

Test organism

The freshwater mussel used in these experiments was the paper pondshell, *Utterbackia imbecillis*. This species has a widespread distribution throughout the eastern United States, and its distribution and abundance are currently considered stable [2]. Adult mussels were obtained from wild populations located in a pond in rural Scatter Branch (Hunt County, TX, USA). This pond receives no urban stormwater runoff, and presumably the only source of PAHs into the pond is atmospheric deposition. Mussels were collected by hand, transported to the lab, and maintained in a flow-through system with a water temperature of 20 ± 2°C and a photoperiod of 14 h light:10 h darkness.

Techniques for the collection of mature *U. imbecillis* glochidia have previously been reported [12]. Briefly, the maturity of the glochidia within the gills of brooding females was deduced by color. Gills containing mature glochidia are enlarged and brown in color, whereas enlarged beige or white gills contain immature glochidia. Mature glochidia were collected by excising the gills containing glochidia and manually shaking them in water. Glochidia were then allowed to settle to the bottom of the container, and the supernatant was removed. Mature glochidia settled to the bottom, whereas the immature ones remained in supernatant. This washing process was repeated at least three time to remove debris and immature glochidia. The remaining mature glochidia were then used immediately in the experiments.

Test compound

Solutions of fluoranthene (molecular weight = 202.26, 98% purity, Aldrich Chemical, Milwaukee, WI, USA) in water were obtained by passing carbon-filtered, dechlorinated tap water (toxicokinetic study) or moderately hard reconstituted water (acute toxicity tests) once through an elution column filled with fluoranthene-coated sand. This avoided the use of carrier solvents in the experiments. Fluoranthene-contaminated water was eluted from the column at aqueous solubility (~180 μg/L at 24°C) and diluted to the appropriate concentrations prior to being used in the experiments. Control water was also passed through a sand column prepared as above with no fluoranthene added.

Lights and light measurement

Ultraviolet and visible light were provided in the laboratory using a bank of fluorescent bulbs designed to simulate natural sunlight (Vita-Lite, Duro-test, Bloomfield, NJ, USA). These lamps have a spectral distribution very similar to that of natural sunlight [27]. Ultraviolet-A (320–400 nm) was quantified using a Macam Photometrics (Livingstone, Scotland) Model UV-202 radiometer. Light measurements were taken at least once daily during all experiments. Using this instrument, a UV-A intensity of 4,038 $\mu W/cm^2$ was measured under clear (sunny) conditions in Commerce, Texas, USA (33°15′N, 95°54′W) on July 14, 1999, at 1300 h Central Daylight Time.

Toxicokinetic study

Mature glochidia were harvested from three adult mussels, pooled together, and immediately exposed to a constant, sublethal concentration of fluoranthene (nominal concentration = 10.0 μg/L) for the uptake phase, then transferred to fluoranthene-free water for the depuration phase. Exposure and depuration occurred under ambient laboratory lighting (UV-A = <2 $\mu W/cm^2$) in flow-through dosing chambers (modified 2-L beakers). Dosing chambers held 1,800 ml of test solution and ~2,000 individuals. Carbon filtered, dechlorinated tap water (temperature, 19.5°C; dissolved oxygen, 6.46 mg/L; pH, 8.07; total hardness, 200 mg $CaCO_3$/L; alkalinity, 120 mg $CaCO_3$/L; conductivity, 230 μS/cm; ammonia, <0.1 mg NH_3/L) was used in this experiment.

The addition of glochidia into the dosing chamber represented time 0 for the uptake phase. Glochidia (~200 individuals) were removed at times 0, 0.5, 1, 2, 4, 8, and 16 h, split into five samples (~30 glochidia each) per sampling time, and analyzed for fluoranthene tissue residues as described below. After 16 h, the remaining glochidia were transferred to the fluoranthene-free beaker. This represented time 0 for the depuration phase. At times 0.5, 1, 2, 4, 8, and 16 h, glochidia (~200 individuals) were removed (as described in the uptake phase) and analyzed for the fluoranthene tissue residues.

Acute toxicity tests

Sensitivity of glochidia to photoactivated fluoranthene was evaluated using 24-h static renewal toxicity tests [12]. Moderately hard, reconstituted water was used as the diluent. This was prepared by adding $MgSO_4$, KCl, $NaHCO_3$, and $CaSO_4$ to deionized water following U.S. Environmental Protection Agency (U.S. EPA) methods [28]. The quality of water in these tests included temperature, 24.5 ± 1.0°C; dissolved oxygen, 7.74 ± 0.36 mg/L; pH, 8.26 ± 0.03; total hardness, 80.3 ± 6.1 mg $CaCO_3$/L; alkalinity, 69.7 ± 5.9 mg $CaCO_3$/L; conductivity, 325.5 ± 4.64 μS/cm; ammonia, <0.1 mg NH_3/L. Mature glochidia were harvested from three adult mussels, pooled together, and immediately distributed to test chambers (50-ml glass beakers) containing ~30 ml of test solution. For each experiment, three sets of three replicates were used in

each of four test concentrations and the control to enumerate viable glochidia. An additional set of four replicates was used for each test concentration and control for determining fluoranthene tissue residues. All test chambers (total = 65/experiment) contained between 20 and 30 glochidia. Glochidia were given an initial 4-h fluoranthene uptake period under ambient laboratory lighting in order to achieve a steady-state internal dose of fluoranthene. Following this 4-h uptake period, glochidia in the additional set of four replicates were sampled for fluoranthene tissue residues ($n = 4$), while the glochidia in the other three replicates were simultaneously exposed to continuous UV radiation and fluoranthene. Test solutions were renewed every 8 h by gently pouring off ~20 ml of solution from the test chamber and replacing it with fresh solution. A static renewal design was necessary because fluoranthene is significantly degraded in static dosing chambers in the presence of UV radiation.

Endpoint determination

Viability of glochidia (endpoint for LC50, LD50, and LT50) was determined based on the ability of the glochidia to close immediately when a noxious stimulus (NaCl) was added to the test chamber [10,13]. The ability to close tightly is necessary for the glochidia to attach to the fish host on which they develop and metamorphose. Individuals unable to demonstrate this response because they are too weak or already closed would be unable to attach to a fish host and complete development.

Viable and nonviable glochidia were enumerated by adding a few grains of NaCl directly to the test chamber. This was sufficient to cause irritation and immediate closure of healthy glochidia. The number of glochidia open before and after the application of NaCl was determined under a dissecting microscope. Glochidia open prior to addition of NaCl and closed after its addition were considered viable. Following enumeration, glochidia were discarded and were no longer used in the experiment.

LC50/LD50 determinations

Sensitivity of glochidia to photoactivated fluoranthene was evaluated using a series of three replicate 24-h static renewal toxicity tests (described above). Intensity of UV-A in these three experiments averaged 69.0 ± 1.0 μW/cm^2. Sets of replicate test chambers were evaluated at 8, 16, and 24 h in order to determine median lethal concentrations (LC50) and median lethal doses (LD50). A fourth experiment was conducted using identical techniques to those described for the other three experiments with the exception that the glochidia were exposed to ambient laboratory lighting at a higher series of fluoranthene treatments.

Effect of varying UV light intensity

The relationship between the rate of mortality and UV intensity was evaluated in a series of four 24-h static renewal toxicity tests conducted at four different levels of UV radiation (UV-A = 15, 31, 50, and 68 μW/cm^2). Toxicity tests conducted with the lowest UV intensities, 15 and 31 μW/cm^2, were extended to 56 and 40 h, respectively, due to the low mortality that occurred within the lowest fluoranthene treatments during the initial 24-h period. Experience indicated that the initial adverse response affecting the viability of glochidia in PAH phototoxicity tests was valve closure. Thus, observations of valve closure were made throughout the test period; however, actual counts using the addition of NaCl to enumerate viable glochidia were restricted to three occasions. These counts were then used to determine median lethal times (LT50) for each fluoranthene treatment.

Analytical

Bioaccumulation of fluoranthene in glochidia was determined at all sampling times in the toxicokinetic study and for all fluoranthene treatments in the acute toxicity tests following the 4-h uptake period. Glochidia (~30 individuals/sample) were placed on aluminum foil, rinsed with deionized water, allowed to air dry at room temperature in the dark for 2 h, and weighed. Fluoranthene was immediately extracted from glochidia by grinding them in a Ten Broeck tissue homogenizer (Wheaton, Millville, NJ, USA) with 1 ml cyclohexane:acetone (2:1) and quantifying using reverse phase high-performance liquid chromatography. Fifty microliters of sample or standard solution were injected directly onto a Waters 3.9 mm × 15 cm μBondapak C18 column (Waters Scientific, Fairchild, OH, USA). An isocratic elution was performed with acetonitrile: water (9:1) at 1.0 ml/min. A Waters 474 scanning fluorescence detector was used at an excitation wavelength of 360 nm with emission detection set at 460 nm. Peaks were recorded and quantified using Waters Millennium^{32} Chromatography Manager software. The limit of detection for fluoranthene was 0.1 μg/L, or 5.0 pg total mass. The recovery of fluoranthene in spiked tissue samples was 95.4 ± 9.1% ($n = 14$). All fluoranthene tissue residues are reported on a dry weight basis.

Fluoranthene concentrations in water samples were analyzed directly with no extraction or concentration using reverse phase high-performance liquid chromatography in a manner similar to that described above. In the toxicokinetic study, concentrations were determined at 0, 8, and 16 h of both the uptake and depuration phases. In the acute toxicity tests, concentrations were determined at the beginning of each toxicity test and at least once more for initial and 8-h test solution concentrations during the test period. Fluoranthene water concentrations are reported as the geometric mean between the measured concentrations in the initial and 8-h solutions.

Statistical analysis

All statistics were performed using SAS® for Windows (Version 6.12, Cary, NC, USA). Rate constants for K_u and K_d of fluoranthene in glochidia were determined based on the assumptions of first-order kinetics using two different methods. First, rate constants were determined based on the use of a two-compartment model [29]. Rate constants K_u and K_d were estimated simultaneously from the uptake portion of this study using the method of nonlinear least squares regression with the SAS NLIN procedure. These estimated rate constants were also used to calculate bioconcentration factors (BCFs) using the equation $BCF = K_u/K_d$.

Second, K_u and K_d were estimated directly from the data generated during uptake and depuration phases of this study, respectively [29]. The K_u was determined using the initial rates estimate method, in which the initial linear tissue concentration values during the uptake phase of the study (between 0 and 1 h) were used to determine the slope of the time–concentration relationship. The depuration data was fit to a two-compartment elimination model [29] because of apparent differences in the slope between the beginning and end of depuration. Depuration rate constants for the fast (K_{d1}) and slow (K_{d2}) compartments were determined using the slope of the relationship between

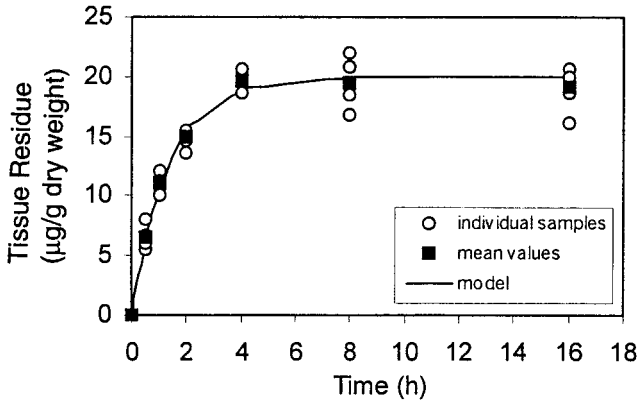

Fig. 1. Accumulation of fluoranthene into the tissues of glochidia over the 16-h uptake phase of the toxicokinetic experiment. Solid line represents the expected tissue residues based on the two-compartment model. Model: tissue residue = 1,394 ml/g/h/(0.769/h) × 0.011 µg fluoranthene/L(1 − $\exp^{-0.769/h\times time}$). n = 5 at each sampling time.

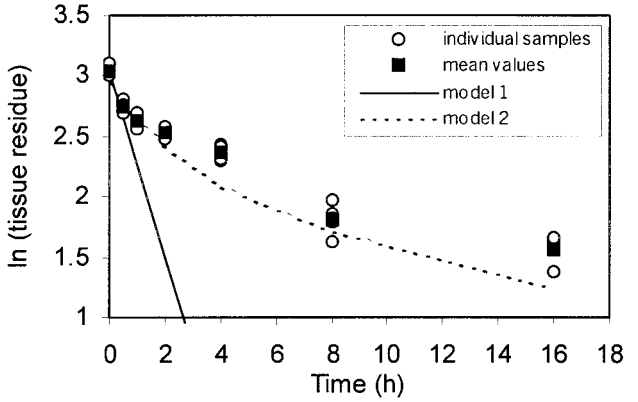

Fig. 2. Natural log transformed data from the 16-h depuration phase of the toxicokinetic experiment. Solid line (model 1) represents the expected tissue residues based on the K_d simultaneously estimated with K_u in the two-compartment model. Model 1: ln(tissue residue) = 3.04 − 0.769/h × time. Dashed line (model 2) represents the expected tissue residues based on the K_{d1} and K_{d2} derived from the two-compartment elimination model. Model 2: ln(tissue residue) = $0.87\exp^{(-0.290/h\times time)} + 2.06\exp^{(-0.031/h\times time)}$. n = 5 at each sampling time.

the natural log transformed tissue concentration data and time between 0 and 1 h and 8 and 16 h, respectively.

Probit analysis was used to determine LC50s, LD50s, LT50s, and their associated 95% confidence intervals for the acute toxicity tests. The dependence of the LT50 values on the product of the initial fluoranthene tissue residues and the UV intensity to which the glochidia were exposed was modeled using general linear models. Bioconcentration factors during the toxicity tests were calculated by dividing the 4-h fluoranthene tissue residues (µg/g dry weight) by the initial fluoranthene water concentrations (µg/ml). All values are reported as mean ± SD unless otherwise noted.

RESULTS

Toxicokinetics study

Concentrations of fluoranthene in water during the uptake phase of this study averaged 11.0 ± 0.9 µg/L. Fluoranthene was rapidly accumulated within glochidia (weight = 3.2 ± 0.6 µg/ind dry wt) in a hyperbolic fashion, reaching an apparent steady state at 19.7 ± 0.8 µg/g following 4 h of exposure (Fig. 1). Tissue concentrations remained at steady state thereafter for the duration of the uptake experiment. The relationship between tissue residue and time was fit to a two-compartment model using nonlinear regression to estimate K_u and K_d simultaneously. K_u was estimated to be 1,394 ± 98 ml/g/h (mean ± asymptotic standard error [SE]), and K_d was estimated to be 0.769 ± 0.061/h (mean ± asymptotic SE). Based on these estimated rate constants, the BCF was 1,813. Using the initial rates estimate, K_u was estimated to be 1,191 ± 77 ml/g/h (mean ± SE). The experimental BCF, as measured at hour 16 of the uptake phase, was 1,735 ± 162.

Glochidia were also shown to eliminate fluoranthene over the 16-h period (Fig. 2). Concentrations of fluoranthene in water during the depuration phase of this study were always below the limit of detection. Following 16 h, fluoranthene tissue residues were 4.8 ± 0.5 µg/g, or 22.8% of the initial fluoranthene tissue residue. Natural log transformation failed to linearize the elimination data (Fig. 2), suggesting that elimination was occurring through multiple compartments. Based on a two-compartment elimination model, K_{d1} and K_{d2} were estimated to be 0.290 ± 0.040/h and 0.031 ± 0.009/h (mean ± SE), respectively.

LC50/LD50 determinations

In the three replicate experiments designed to determine the overall sensitivity of glochidia to photoactivated fluoranthene, five concentrations of fluoranthene (0, 1.2, 2.0, 3.4, and 6.1 µg/L) were achieved. These experiments were all conducted in the presence of similar intensities of UV light (UV-A = 69.0 ± 1.0 µW/cm^2), which roughly corresponds to ~1.7% of that found in full sunlight in Texas during July at solar noon. Mortality in these toxicity tests was <7.5% in all treatments prior to UV exposure and in all 0-µg fluoranthene/L treatments under UV.

Glochidia bioaccumulated fluoranthene in direct proportion to the water concentrations to which they were exposed. Initial tissue residues of fluoranthene in glochidia were below the limit of detection, 4.77 ± 1.77, 6.22 ± 2.09, 9.17 ± 1.11, and 15.30 ± 3.49 µg/g in the 0-, 1.2-, 2.0-, 3.4-, and 6.1-µg/L treatments, respectively. Mean BCF across all treatments was 2,152 ± 847.

In all three replicate experiments, mortality was dependent on fluoranthene exposure. Both LC50 and LD50 values decreased in a time-dependent manner from 8 to 24 h (Table 1).

In the fourth experiment, which was conducted under ambient laboratory lighting, five concentrations of fluoranthene were achieved (0, 24.1, 36.8, 66.3, and 110.5 µg/L). Mortality in the 0-µg fluoranthene/L treatment was <6.5% at all sampling times. Little toxicity was evident in all fluoranthene treatments at 8, 16, and 24 h. Following 24 h of exposure, 10.5, 5.8, 3.3, and 2.9% mortality were observed in the 24.1-, 36.8-, 66.3-, and 110.5-µg fluoranthene/L treatments, respectively. Due to the lack of toxicity in this experiment, LC50 and LD50 values are reported as >110.5 µg fluoranthene/L and >257.5 µg fluoranthene/g, respectively (Table 1).

Effect of varying UV intensity

In the four experiments designed to determine the effect of varying UV intensity on the phototoxicity of fluoranthene to glochidia, five concentrations of fluoranthene (0, 5.4, 7.8, 11.9, and 17.2 µg/L) were achieved. Mortality in these toxicity tests was <6.5% in all treatments prior to UV exposure and in all 0-µg fluoranthene/L treatments under UV. As in the previous

Table 1. Results of experiments designed to determine the overall sensitivity of glochidia to photoactivated fluoranthene. Lethal concentration (LC50) values (μg fluoranthene/L), LD50 values (μg fluoranthene/g glochidia dry wt), and associated 95% confidence intervals are reported for 8, 16, and 24 h. Trials 1–3 were conducted in the presence of ultraviolet (UV) (UV-A = 69.0 ± 1.0 μW/cm²). Mean LC50 and LD50 values (± standard deviation [SD]) are reported for trials 1–3. The no UV trial was conducted under ambient laboratory lighting (UV-A = <2 μW/cm²)

Experiment		8 h	16 h	24 h
Trial 1	LC50	5.62 (5.22–6.16)	3.44 (2.34–8.56)	2.90 (2.68–3.16)
	LD50	12.69 (12.03–13.54)	9.21 (7.71–13.02)	8.35 (6.64–17.18)
Trial 2	LC50	6.16 (4.40–13.74)	4.52 (4.22–4.84)	2.44 (2.26–2.64)
	LD50	14.59 (13.67–15.70)	11.02 (10.40–11.73)	6.87 (6.52–7.23)
Trial 3	LC50	4.98 (4.65–5.36)	4.31 (3.93–4.66)	2.00 (1.39–2.42)
	LD50	17.01 (15.84–18.41)	14.74 (13.41–16.00)	5.73 (3.43–7.33)
Mean (SD)	LC50	5.59 (0.59)	4.09 (0.57)	2.45 (0.45)
	LD50	14.76 (2.17)	11.66 (2.82)	6.98 (1.31)
No UV	LC50	>110.48	>110.48	>110.48
	LD50	>257.50	>257.50	>257.50

set of experiments, glochidia bioaccumulated fluoranthene in a concentration-dependent manner. Initial tissue residues of fluoranthene in glochidia were below the limit of detection, 14.37 ± 3.30, 17.13 ± 4.31, 28.45 ± 5.76, and 33.65 ± 10.43 μg/g in the 0-, 5.4-, 7.8-, 11.9-, and 17.2-μg/L treatments, respectively. Mean BCF across all treatments was 1,709 ± 514.

The rate of mortality (in terms of median lethal times) was a function of both the initial fluoranthene tissue residues and light treatment (Fig. 3). At the highest light intensity (68 μW/cm²), LT50s ranged from 3.1 h in those glochidia with a mean fluoranthene tissue residue of 35.98 ± 2.98 μg/g to 11.3 h in those glochidia with a mean fluoranthene tissue residue of 16.24 ± 1.40 μg/g. In contrast, at the lowest light intensity (15 μW/cm²), LT50s ranged from 16.9 h in those glochidia with a mean fluoranthene tissue residue of 23.93 ± 1.56 μg/g to 47.2 h in those glochidia with a mean fluoranthene tissue residue of 10.33 ± 1.75 μg/g.

A logarithmic plot of the product of initial tissue residues and UV-A intensity versus median lethal time produced a significant linear model ($p < 0.0001$), with median lethal times decreasing as the product of tissue residue and light intensity increased (Fig. 4). This relationship did not deviate from linearity across all data points. Regression analysis revealed that the product of tissue residue and light intensity could explain 88% of the variability in the median lethal time data.

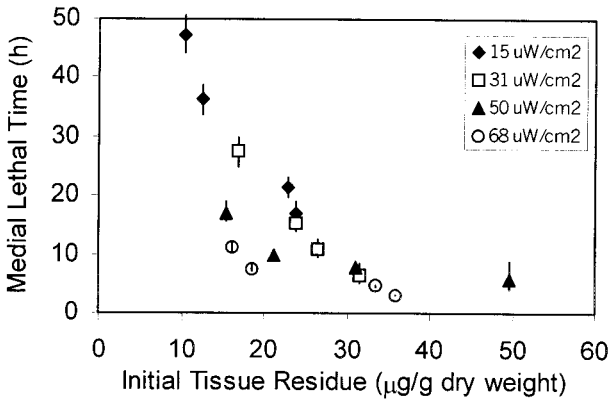

Fig. 3. Relationship between median lethal times and initial fluoranthene tissue residues. Estimated median lethal times (± 95% confidence limits) are at ultraviolet (UV)-A intensities of 15 (♦), 31 (■), 50 (▲), and 68 (○) μW/cm².

DISCUSSION

Unlike the adult mussel, which is protected from environmental hazards by the ability to open and close their thick valves, the small planktonic glochidia must keep their valves open to attach to a fish host. This could allow for both the rapid uptake of environmental contaminants into the tissues of glochidia and exposure to environmentally relevant levels of UV radiation, suggesting that larval glochidia could be especially vulnerable to the effects of photoinduced toxicity of PAH. The results of the present study involving fluoranthene confirm this. Glochidia of *U. imbecillis* rapidly accumulated fluoranthene within their tissues, achieving an apparent steady state following only 4 h of exposure. Then, in the presence of low intensities of UV, glochidia were >45 times more sensitive to fluoranthene than in the absence of UV. Thus, the data presented here are in agreement with previous reports concerning the enhanced toxicity of fluoranthene in the presence of UV radiation [19,20,26,30] and demonstrate that at least one life stage of freshwater mussels could be adversely impacted by the presence of photoactivated PAHs in the environment.

The uptake rate constants (K_u) for fluoranthene in glochidia were similar using both the two-compartment model and the initial rates estimate. These estimates are considerably higher than those reported in a variety of other organisms. For ex-

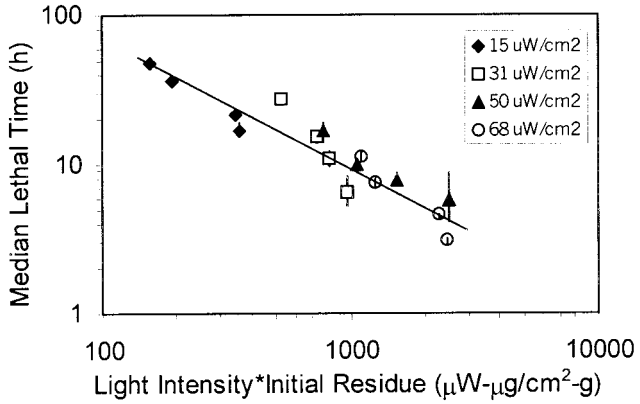

Fig. 4. Relationship between median lethal time and the product of initial fluoranthene tissue residues and intensity of ultraviolet (UV)-A. Regression formula, log(LT50) = 3.51 − 0.84log(product); r^2 = 0.883. LT50, median lethal time (h); product = initial tissue residues (μg/g dry wt) × UV-A intensity (μW/cm²). Error bars represent 95% confidence limits.

ample, under exposure conditions similar to those reported in the present study, freshwater amphipods, *Hyalella azteca* and *Diporeia* spp., had relatively lower conditional rate constants, ranging from 210 to 439 ml/g/h [31]. Lower conditional rate constants have also been reported in juvenile fathead minnows, *Pimephales promelas,* (297 ml/g/h) and in larval bullfrogs, *Rana catesbeiana* (45 ml/g/h) [32,33]. The high conditional rate of uptake reported here for glochidia is probably a result of their shell morphology. The shell valves are thin and highly porous, facilitating the exchange of nutrients and gases [34], and probably contaminants as well. In addition, the shell valves are normally open in anticipation of an encounter with a host fish, exposing the entire organism to the water column. Thus, the uptake of nonpolar organic compounds, like fluoranthene, directly from water probably occurs over the entire surface of soft tissues and through the shell valves. In contrast, uptake of nonpolar organics in amphipods and larger organisms is probably limited to respiratory surfaces, although it has been suggested that these compounds may also pass to a limited extent through the chitinous exoskeleton [31]. In addition to the above anatomical considerations, the high surface-to-volume ratio of glochidia probably contributes to the high rate of uptake of these compounds compared with that of larger organisms. The overall BCF achieved in the present study, 1,943 ± 295, was within the range reported for several different species of aquatic vertebrates and invertebrates [35].

The depuration rate constant (K_d) for fluoranthene was 0.769/h when simultaneously estimated with K_u using nonlinear regression. However, inspection of the natural log transformed depuration data suggests that this model was inadequate in describing fluoranthene depuration kinetics in glochidia. The initial slope of the time–concentration relationship (between 0 and 1 h) appears to be considerably steeper than the slope at the end of the depuration phase (between 8 and 16 h). This suggests that the glochidia possess a saturable compartment that does not depurate very quickly. Based on a two-compartment elimination model, the fast compartment had a depuration rate constant of 0.290/h, while the slow compartment had a depuration rate constant of 0.031/h. Further studies are currently being conducted to better delineate the multiple compartment nature of fluoranthene elimination in glochidia.

The results of the present study demonstrate that the glochidia of *U. imbecillis* are sensitive to photoactivated fluoranthene when compared with the larvae other species. In the present study, *U. imbecillis* glochidia had a 24-h LC50 of 2.45 μg fluoranthene/L when exposed to a UV-A intensity of 69 μW/cm^2. Using intensities of UV-A similar to those used in the present study, larvae of the fathead minnow (*P. promelas*) had a 96-h LC50 of 9.0 μg fluoranthene/L [32] and larvae of the bullfrog (*R. catesbeiana*) had a 96-h LC50 of 111.3 μg fluoranthene/L [33]. In fact, these results, and those of previous studies, suggest that the developmental stages of bivalved mollusks are among the most sensitive organisms tested to date to photoactivated fluoranthene. Embryos of a marine bivalve, the coot clam (*Mulina lateralis*) had a 48-h EC50 of 1.09 μg fluoranthene/L when exposed to a UV-A intensity of 397 μW/cm^2 [30]. In contrast, juvenile mysid shrimp (*Mysidopsis bahia*) exposed to the same intensity of UV-A were considerably less sensitive (48-h LC50 = 5.32 μg fluoranthene/L) [30]. Using UV-A intensities as high as 724 μW/cm^2, Spehar et al. [26] found that the most sensitive species in a diverse group of both freshwater and saltwater taxa were the juvenile winter flounder, *Pleuronectes americanus,* (96-h LC50 = 0.1 μg fluoranthene/L) and the oligochaete, *L. variegatus* (96-h LC50 = 1.2 μg fluoranthene/L). It is interesting to note that both these species are naturally afforded protection from UV radiation by sediments due to their benthic existence, whereas the developmental stages of bivalved mollusks are pelagic and lack this type of behavioral protection.

Observations such as those mentioned above have led to the suggestion that test organism sensitivity to photoactivated fluoranthene is inversely related to their potential for exposure to sunlight in nature [20,26]. Organisms commonly exposed to sunlight have evolved biochemical and morphological means of protecting themselves from the damaging effects of UV radiation. Biochemically, organisms have evolved pigmentation to reflect or absorb UV radiation or efficient DNA repair mechanisms [36]. Morphologically, organisms, such as the adult bivalved mollusk, have evolved protection in the form of a thick integument or shell. By decreasing the exposure of their underlying soft tissues to UV radiation, these organisms are also afforded some protection from photoactivated PAHs. Unlike adult bivalves, the larvae are not enclosed by thick valves, and they are found in the water column, where they can be exposed to biologically significant levels of UV radiation. Based on their pelagic existence, it may seem logical to presume that the larvae of at least some species have evolved some form of protection against the damaging effects of UV radiation. However, the results of the current study suggest that these mechanisms have not evolved to any great extent in *U. imbecillis* glochidia. Certainly, the extent to which the glochidia of other species have evolved mechanisms of protection from UV radiation, and its influence on PAH phototoxicity, is one area warranting further research.

Predictive models describing the influence of light intensity on PAH phototoxicity have recently been described [19,37]. These models are based on the Bunsen–Roscoe photochemical law of reciprocity, which states that, in the absence of biological defense and repair mechanisms, phototoxic potency should be inversely proportional to both the internal concentration of PAH and the intensity of light to which the organism is exposed. Based on this relationship, the time-dependent mortality of the oligochaete *L. variegatus* was accurately described for fluoranthene, anthracene, and pyrene [19,37]. One of the objectives of the current study was to determine whether this model would be of general utility with respect to accurately describing the phototoxic potency of fluoranthene in glochidia across several different light intensities. A plot of the product of initial tissue concentration and light intensity versus median lethal time was linear across all data points generated in these studies, suggesting that this model does adequately describe this relationship in *U. imbecillis* glochidia. The estimated slope of the regression line for glochidia generated in this study (−0.84 ± 0.08) (mean ± SE) is reasonably similar to the predicted slope of −1 based on reciprocity [19]. Previous studies involving the oligochaete *L. variegatus* [19] and larvae of the northern leopard frog, *Rana pipiens* [21], have also reported similar findings. This provides further evidence that the underlying mechanism of fluoranthene phototoxicity, believed to be photosensitization resulting from the generation of reactive oxygen species, is similar across different taxa.

Freshwater mussels typically inhabit shallow streams and rivers, which are common recipients of stormwater runoff. Although water concentrations of fluoranthene in these streams are generally low (in the ng/L range), incoming stormwater

runoff can have very high concentrations. Published concentrations of fluoranthene in urban stormwater runoff generally range from <0.1 to 4.0 μg/L [38,39]. Parking-lot runoff can have especially high concentrations of fluoranthene. Pitt et al. [40] reported a mean fluoranthene concentration of 37.0 μg/L, with individual samples having concentrations as high as 110.0 μg/L. In light of the data presented here, it seems reasonable to conclude that photoactivated fluoranthene does pose a significant hazard to glochidia and that photoactivated PAHs, in general, could be responsible for some of the observed declines in population recruitment. Further studies, especially those involving threatened and endangered species and those conducted in situ, are necessary to further characterize this risk.

CONCLUSIONS

The results of the present study have demonstrated that fluoranthene poses a hazard to the glochidial larvae of at least one species of freshwater mussel. Glochidia rapidly bioaccumulated waterborne fluoranthene, achieving steady state in only 4 h. In the presence of environmentally relevant levels of UV radiation, fluoranthene was acutely toxic to glochidia. In fact, these tests demonstrated that glochidia are among the most sensitive organisms tested to date. As in other species, the rate of mortality was inversely related to the product of fluoranthene dose, as measured within the tissues of glochidia, and the intensity of UV-A to which the glochidia were exposed, suggesting that this relationship can be used to accurately predict the time-dependent mortality of glochidia. The results of the present study confirm the necessity of considering photoactivation as a key process in determining the overall risk of PAHs to at least one stage in the life cycle of freshwater mussels.

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