



ACADEMIC  
PRESS

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SCIENCE @ DIRECT®

Ecotoxicology and Environmental Safety 54 (2003) 105–117

Ecotoxicology  
and  
Environmental  
Safety

<http://www.elsevier.com/locate/ecoenv>

# The toxicity of fluoranthene to *Hyaella azteca* in sediment and water-only exposures under varying light spectra<sup>☆</sup>

Steven E. Wilcoxon,<sup>a</sup> Peter G. Meier,<sup>a</sup> and Peter F. Landrum<sup>b,\*</sup>

<sup>a</sup>Department of Environmental and Industrial Health, University of Michigan, Ann Arbor, MI 48109, USA

<sup>b</sup>National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, 2205 Commonwealth Boulevard, Ann Arbor, MI 48105, USA

Received 31 December 2001; accepted 7 June 2002

## Abstract

In the US Environmental Protection Agency methods for sediment toxicity testing, the light regimen is specified as a 16:8 light dark cycle with 500–1000 lx. The potential for photoinduced toxic effects from this requirement is evaluated. *Hyaella azteca* were exposed to fluoranthene in both water only and sediment to examine the impact of light spectra on the toxicity of fluoranthene. The light sources included gold fluorescent light ( $\lambda > 500$  nm), cool white fluorescent light, and UV-enhanced fluorescent light. Toxicity was determined as mortality after 10 days of exposure. The extent of mortality was determined both as LC<sub>50</sub> and LR<sub>50</sub> (median lethal body residue). In water-only exposures, the toxicity of fluoranthene was greatest under the UV-enhanced spectra, followed by fluorescent light, and least toxic under the gold light. Both the LC<sub>50</sub> and LR<sub>50</sub> values exhibited the same pattern. The toxicity under gold light gave an LR<sub>50</sub> of 0.81 mmol kg<sup>-1</sup> (0.82–0.79, 95% CI) similar to values expected for the acute toxicity of nonpolar narcotic (anesthetic) compounds. The LR<sub>50</sub> values under the other two light sources were substantially lower, 4 and 58 times lower for the fluorescent and UV-enhanced exposures, respectively. In sediment, toxicity was not significantly affected by the light source. Toxicity occurred only when the body residue concentration approached that of the LR<sub>50</sub> under gold light from the water-only exposures. Thus, *H. azteca* were significantly protected from the light by burrowing into the sediment.

© 2002 Elsevier Science (USA). All rights reserved.

**Keywords:** *Hyaella azteca*; Fluoranthene; Light; Sediment; Phototoxicity

## 1. Introduction

The US Environmental Protection Agency (USEPA) formalized methods for freshwater sediment bioassays in 1994 (USEPA, 1994), as a result of consensus discussions on the existing state-of-the-art methods (USEPA, 1992). The EPA method called for a 16:8 h light:dark photoperiod at about 500–1000 lx for culturing and testing *Hyaella azteca* (USEPA, 1994; Norberg-King, 1992); this requirement was not changed in the recently updated freshwater methods (USEPA, 2000). Although a consensus photoperiod was selected, no rationale was given for choosing the 16:8 h cycle nor was a specific light spectrum specified. The USEPA Method (USEPA, 1994, 2000) states that the lights

should be “wide-spectrum fluorescent lights,” about 500–1000 lx, for the 16:8 h light:dark photoperiod (USEPA, 1994, 2000). Although these light conditions are standardized, they are a matter of convention and have not been studied to determine their impact on the sediment test results.

In the draft Sediment Quality Criteria for the Protection of Benthic Organisms: Fluoranthene (USEPA, 1993), USEPA acknowledged the phototoxic potential of fluoranthene, as well as other polycyclic aromatic hydrocarbons (PAHs), under ultraviolet (UV) light exposures, and that there were insufficient toxicity data under UV light to calculate final acute and chronic values for freshwater or saltwater organisms individually. However, when the available data were combined, a final acute value with UV exposure, FAV<sub>UV</sub>, was 66 times lower than the freshwater FAV<sub>dark</sub> and 32 times lower than the saltwater FAV<sub>dark</sub>. In the draft sediment quality criteria (SQC) for fluoranthene, USEPA

<sup>☆</sup>GLERL Contribution 1251.

\*Corresponding author. Fax: +1-734-741-2055.

E-mail address: [peter.landrum@noaa.gov](mailto:peter.landrum@noaa.gov) (P.F. Landrum).

reasoned that organisms that remain buried or organisms in the shade could survive PAH concentrations that would be lethal if they emerged from sediment or shade into the sunlight (USEPA, 1993). The development of standardized conditions for sediment toxicity tests did not address this possibility (USEPA, 1994). Thus, if *H. azteca*, an epibenthic species, are tested with sediment-associated PAH, the light regimen may impact the results.

The phototoxic potential of PAHs was recognized as early as 1938 (Mottram and Doniach, 1938) but was lost to environmental science until 1983 (Bowling et al., 1983). Since then, the phenomenon has been studied in *Drosophila melanogaster*, Ciliata, Choretha larvae, yeast cells, tissue cultures (Landrum et al., 1984), bluegill sunfish (Oris and Giesy, 1986), *Daphnia magna* (Newsted and Giesy, 1987; Davenport and Spacie, 1991), duckweed (*Lemna gibba*) (Huang et al., 1993), the green alga *Selenastrum capricornutum* (Gala and Giesy, 1994), *H. azteca* (Ankley et al., 1994), *Chironomus tentans* (Ankley et al., 1994), *Lumbriculus variegatus* (Ankley et al., 1994, 1995; Monson et al., 1995), and salamander eggs (Blaustein et al., 1995). Early studies focused primarily on demonstrating that UV exposures induce phototoxicity of the PAH in question to the species of concern. More recent studies have examined the effects of light intensity, photoperiod, and spectral quality on the degree of toxicity exhibited by PAHs (Oris and Giesy, 1986; Huang et al., 1993; Monson et al., 1995).

In the previous studies, aquatic organisms were examined in liquid-phase exposures. Recently, evaluations were made exposing benthic macroinvertebrates to PAH-contaminated sediments in the presence of UV radiation. In the first published account, 10-day toxicity tests with three polluted sediments on three benthic macroinvertebrate species (*H. azteca*, *C. tentans*, and *L. variegatus*) under fluorescent and UV-supplemented light demonstrated that *H. azteca* exposed to sediments with higher PAH levels had significantly decreased survival with UV exposure compared with the same sediments under fluorescent light, and compared with control and low PAH sediments under both light regimens (Monson et al., 1995). In the same study, *L. variegatus* were more sensitive than *H. azteca* to the UV exposures both in sediment and in subsequent water exposure, while *C. tentans* were largely unaffected by UV light. Each of the above studies ignored the potential UV component of fluorescent light; thus, the impact of the required light regimen for sediment toxicity testing was not evaluated.

This study examines the phototoxicity of sediment-associated PAHs to *H. azteca*. Two basic hypotheses were addressed: *H. azteca* exposed to water and sediments spiked with increasing doses of fluoranthene will show decreased fluoranthene median lethal concen-

tration ( $LC_{50}$ ) and median lethal body residue concentration ( $LR_{50}$ ) with increased UV content ( $\lambda > 300$  nm). Also, *H. azteca* exposed to fluoranthene and UV light will exhibit behavioral changes to minimize UV exposure and the combined toxic response.

## 2. Materials and methods

### 2.1. Chemicals

Unlabeled fluoranthene was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). [ $^{14}C$ ] Fluoranthene (specific activity of 45 Ci mol $^{-1}$ ) was obtained from Sigma Chemical Company (St. Louis, MO, USA). Prior to use, the purity of [ $^{14}C$ ] fluoranthene was determined by thin-layer chromatography on silica plates (Alltech Associates, Deerfield, IL, USA) using hexane:benzene (8:2, v/v) and liquid scintillation counting (LSC), and found to be 96–98% pure. This stock was used without further purification.

### 2.2. Water

All water was taken from the Huron River, upstream of Hudson Mills Metropark, Dexter, Michigan, and stored at 4°C. Prior to use, the water was passed through 1- and 0.2- $\mu$ m filters (Fin-L-Filter, Cole-Palmer Instrument Co., Vernon Hills, IL, USA) and allowed to equilibrate to 23°C. Water characteristics over the course of the studies were: mean pH 8.2 $\pm$ 0.18; mean alkalinity 174 $\pm$ 13 mg L $^{-1}$  CaCO $_3$ ; mean hardness 259 $\pm$ 23 mg L $^{-1}$  as CaCO $_3$ .

### 2.3. Organisms

*H. azteca* were cultured at the Great Lakes Environmental Research Laboratory (GLERL), NOAA, Ann Arbor, Michigan. Amphipods were sorted by size to yield organisms of a mean length corresponding to 6-day-old amphipods, specifically organisms that passed through a 500- $\mu$ m sieve yet were retained on a 355- $\mu$ m sieve (USEPA, 1994, 2000). These animals were held in a separate container under experimental conditions for 3 days prior to the start of each experiment.

### 2.4. Lights

Two 4-ft shop lights (4 bulbs total) were mounted in a wooden frame 18 in above the laboratory bench. Three test spectra were achieved using three different combinations of bulbs (Table 1).

Animals and sediments were manipulated under ambient gold light, which consisted of ceiling-mounted fluorescent lights covered with gold filters (Deep Orange No. 158, Lee Filters, Andover, England) resulting in low

Table 1  
Light sources for toxicity testing

Spectra	Bulb type and quantity	Manufacturer
Gold	4 gold (F40/GO)	General Electric, Cleveland, OH, USA
Ambient gold	F40/CW filtered by Deep Orange No. 158 Filters	Lee Filters, Andover, England
Fluorescent	4 cool white fluorescent (F40/CW)	Sylvania, Cleveland, OH, USA
UV enhanced	3 cool white fluorescent (F40/CW) 1 UVA-340	Sylvania, Cleveland, OH, USA Q-Panel Lab Products, Inc., Cleveland, OH, USA

intensity light, 92 lx,  $\lambda > 500$  nm. Spectra for the light sources were determined using a 550-1 Radiometer/Photometer fitted with the Model 555-61 monochromator, Model 555-65 Wavelength Drive Assembly, and Model 555-64 Filter Wheel Drive Assembly (EG&G, Salem, MA, USA). Integrated intensity measurements were made at bench level with an International Light (Newburyport, MA, USA) IL1700 Research Radiometer/Photometer with a SUD005 wideband UV sensor (250–400 nm). The total UV extinction coefficient was determined for Huron River water under experimental conditions by measuring the change in UV intensity with water depth.

### 2.5. Water spiking

Separate working stock solutions were prepared for each water concentration by combining appropriate amounts of a [ $^{12}\text{C}$ ] fluoranthene stock solution (2.5 mg ml $^{-1}$  in acetone), [ $^{14}\text{C}$ ] fluoranthene, and acetone. The control stock contained only acetone. Aliquots of working stocks were diluted with hexane to confirm concentrations by gas chromatography/mass spectrometry (GC/MS) as previously described (Harkey et al., 1995). Triplicate 5- $\mu\text{L}$  aliquots of each working stock were added to 12-mL Complete Counting Cocktail 3a70B (Research Products International Corp., Mount Prospect, IL, USA) and analyzed by LSC on a Tri-Carb Liquid Scintillation Analyzer (Model 2500TR, Packard Instruments Co., Meriden, CT, USA) for [ $^{14}\text{C}$ ] fluoranthene activities. After background was subtracted, samples were corrected for quench by the external standards ratio method. Working stocks (490- $\mu\text{L}$ ) were added to 3.5 L of filtered (0.2  $\mu\text{m}$ ) Huron River water, mixed, and allowed to equilibrate over-

night. Prior to use, the [ $^{14}\text{C}$ ] fluoranthene concentration in each test solution was measured in triplicate (1 mL) by LSC. The new specific activity (mCi [ $^{14}\text{C}$ ] fluoranthene as determined by LSC divided by mmol total fluoranthene as determined by GC/MS) for each stock solution was used to calculate the total amount of fluoranthene in each sample.

### 2.6. Sediment spiking

Sediment was collected by Ponar grab from a 45-m-deep station (43.03°N, 86.37°W) in Lake Michigan. Sediments from this station were previously shown to contain low concentrations of PAHs (Eadie et al., 1982). The sediment was sieved at 1 mm and stored at 4°C until spiked.

Sediment was spiked using the rolling jar method (USEPA, 1994; Ditsworth et al., 1990). Separate working stock solutions were prepared in the same manner as for the water exposures and analyzed by GC/MS and LSC as above to determine specific activity. Stock solutions were evaporated on the sides of 3.8-L glass jars. Sieved sediment (3600 g wet wt) and filtered (0.2- $\mu\text{m}$ ) Huron River water (150 mL) were added to each jar. The slurry was rolled for 4 h at 23°C, stored at 4°C for 2 days, rolled for an additional 8 h at 23°C, and subsequently stored at 4°C for 18 months prior to use.

In each experiment, [ $^{14}\text{C}$ ] fluoranthene was measured on Exposure Days 2, 5, and 10 by extracting sediment samples (100–150 mg) with scintillation cocktail (3a70b, Research Products International, Mt. Prospect, IL, USA) overnight, without sonication, prior to LSC. The xylene base of the scintillation cocktail serves as the extracting solvent for the fluoranthene. Direct comparisons showed no significant difference in activity before and after sonication with a Tekmar (Cincinnati, OH, USA) high-intensity probe-sonicator.

### 2.7. Water-only exposures

Ten-day water-only bioassays were conducted with *H. azteca* under three different spectra: Gold ( $\lambda > 500$  nm), fluorescent, and UV enhanced. *H. azteca* were exposed to control water and five fluoranthene concentrations. Each concentration required a total of nine replicate beakers: five for mortality determination and four for tissue concentration analysis (one each on Days 1, 2, 5, and 10). Each replicate beaker (400 mL) contained 20 organisms, 200 mL of spiked water, and a 1-cm square of cotton gauze for substrate that was presoaked for 48 h in filtered Huron River water. *H. azteca* were fed 0.5 mL yeast-cerophyl-trout chow (YCT) every other day (USEPA, 1994). All tests were conducted at 23°C and 16:8 h light:dark photoperiod. Two-thirds of the water in each beaker was renewed daily. Water chemistry parameters (DO, temperature, pH, hardness,

and alkalinity) were determined prior to renewal on Day 5. Fluoranthene concentrations were determined daily, before and after water renewal, by LSC of triplicate samples (1 mL). The range of fluoranthene concentrations was different for each spectrum, and time-weighted average values are given in Table 3. These fluoranthene concentrations represent total Fluoranthene equivalents, because fluoranthene is expected to photodegrade under the fluorescent and UV-enhanced spectra.

On Days 1, 2, 5, and 10, mortality was determined nondestructively in each of the five beakers at each concentration. Organisms were considered dead if they did not move their gnathopods on gentle prodding. Narcotized animals (those unable to maintain an upright body orientation, yet still able to move) were counted as survivors.

On Days 1, 2, 5, and 10, surviving organisms from a separate beaker at each concentration were blotted dry, weighed, and held in scintillation cocktail for 24 h for extraction. Radioactivity was then determined by LSC. Tissue concentrations also represent total fluoranthene equivalents due both to photodegradation and to the ability of *H. azteca* to metabolize fluoranthene (Kane Driscoll et al., 1997b).

The lipid composition was determined by a colorimetric assay (Van Handel, 1985) for organisms from culture on Day 0 and on surviving organisms at Day 10.

### 2.8. Sediment exposures

Ten-day sediment bioassays were conducted with *H. azteca* under two spectra: fluorescent and UV enhanced. Previous experiments had determined the toxicokinetics and toxicity of fluoranthene in sediment bioassays with *H. azteca* under ambient gold light (Kane Driscoll et al., 1997b).

*H. azteca* were exposed to control and [<sup>14</sup>C] fluoranthene-spiked sediments (0.2, 102, 193, 389, and 867 nmol g<sup>-1</sup> dry wt). Each treatment consisted of 12 beakers. Each beaker contained 10 organisms, 100 g (wet wt) sediment, and 175 mL overlying Huron River water. *H. azteca* were fed 0.5 mL YCT every other day (USEPA, 1994). All tests were conducted at 23°C and 16:8 h light:dark photoperiod. Approximately one-third of the overlying water in each beaker was renewed twice daily. Water chemistry parameters (DO, temperature, pH, hardness, and alkalinity) were determined periodically throughout the experiment.

On Days 1, 5, and 10, four beakers from each treatment were removed. Surviving organisms were counted, weighed, and held in scintillation cocktail overnight. Subsurface sediment samples (approximately 100 mg) were sonicated in scintillation cocktail. Fluoranthene equivalents were then determined by LSC. Concentrations in sediment are expressed throughout as nanomoles of fluoranthene equivalents per gram dry

weight. Lipids were determined using the same procedures as described above in the water-only section.

### 2.9. Statistics and calculations

Mortality data for water exposures were analyzed by the probit method using *Toxcalc Version 5.0* (Tidepool Scientific Software, McKinleyville, CA, USA). Mortality data for sediment exposures were analyzed with the same software using the trimmed Spearman–Kärber method. Data was adjusted for mortality in the control using Abbott's formula (Abbott, 1925): adjusted  $p_i = (p_i - p_0)/(1 - p_0)$ , where  $p_i$  is the observed proportion mortality for concentration  $I$ , and  $p_0$  is the observed proportion mortality for the control. Means were compared by one-way ANOVA with subsequent multiple pairwise Bonferroni  $t$  tests using *Sigmastat V2.0* (SPSS, Chicago, IL, USA).

## 3. Results

### 3.1. Light intensities

The UV extinction coefficient measured for the bandwidth of 250–400 nm was calculated as the slope of the regression of the natural log of the intensity versus depth. The extinction coefficient was 1% per cm in Huron River water. However, the total UV intensity measured at bench top in air was approximately equal to that under 10 cm Huron River water. It was thought that the experimental setup allowed additional light to reach the sensor surface under water due to refraction of light striking the sides of the beaker by the overlying water column. Thus, light measured at bench level in air was essentially the same as that at the sediment surface under water in this experimental design.

Light intensities varied and were always less than 1% of the incident sunlight in Ann Arbor, Michigan, on a clear day ( $3.98 \times 10^{-3}$  W cm<sup>-2</sup> measured on 4/5/96 at 12:00 P.M.). The spectral characteristics of the experimental light sources and sunlight were determined in air at bench or ground level (Fig. 1).

The proportion of light in each spectral region varied with the light source. The visible:UVA:UVB ratio was calculated for each source and sunlight, with the visible component for each source set to 100% (Table 2). Sunlight in Ann Arbor, Michigan, on 9/30/99 had a visible:UVA:UVB ratio of 100:6.6:0.15. The fluorescent, gold, and ambient gold lights (used to manipulate samples and in previous experiments (Kane Driscoll et al., 1997a, b) had lower proportions of UVA than sunlight, (3.21%, 0.05%, and 1.66% of visible, respectively) while the UV-enhanced light had a substantially larger proportion of UVA (35.29% of visible) (Table 2). The proportions of the UVB component for the

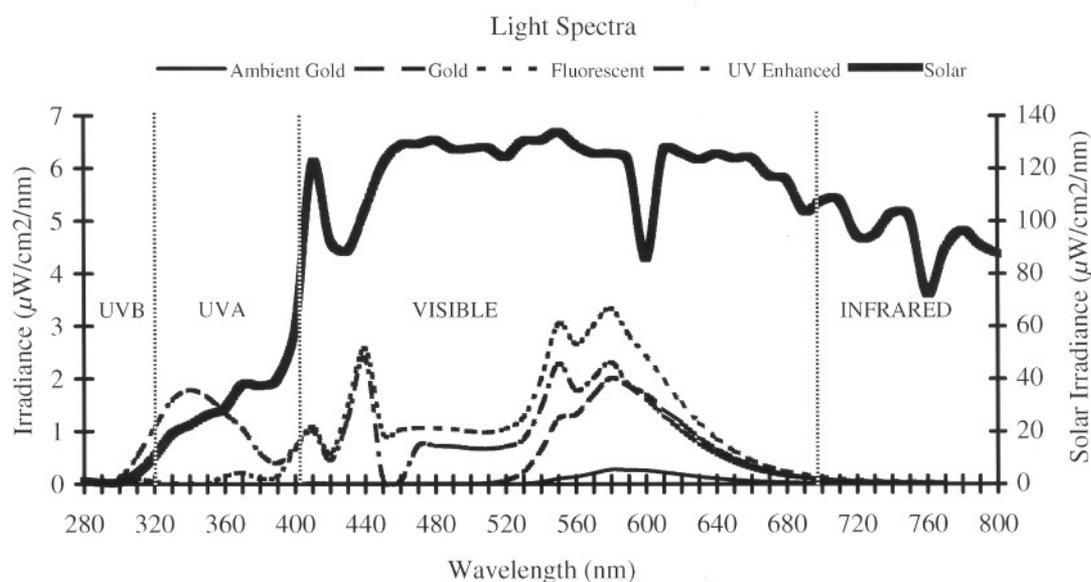


Fig. 1. Irradiance versus wavelength for four experimental light sources (left axis) and solar radiation (right axis).

Table 2  
Intensity of light sources by spectral region

Spectral region	Light source				
	Gold	Fluorescent	UV	Ambient	Sunlight
	$\langle \mu W cm^{-2} \rangle$				
UVB	0.17	1.32	7.54	0.71	64.76
UVA	0.09	13.65	102.08	0.38	3822.87
Visible	167.72	424.69	289.24	23.09	42,681.79
	$\langle \% \text{ of visible component of respective source} \rangle$				
UVB	0.10	0.31	2.61	3.09	0.15
UVA	0.05	3.21	35.29	1.66	6.61
Visible	100.00	100.00	100.00	100.00	100.00
	$\langle \% \text{ of respective spectral region in sunlight} \rangle$				
UVB	0.26	2.04	11.65	1.10	100.00
UVA	0.003	0.48	3.62	0.01	100.00
Visible	0.39	1.00	0.68	0.05	100.00

fluorescent and gold lights (0.31% and 0.10% of visible, respectively) were comparable to that of sunlight, while the UV enhanced and ambient gold lights had relatively larger proportions of UVB (2.61% and 3.09% of visible, respectively).

When the measured intensity of sunlight for each spectral region is set to 100%, only the fluorescent UVB component (2.04%) and the UV-enhanced UVB and UVA components (11.65% and 3.62%, respectively) are significantly greater than 1% of the sunlight intensities (Table 2). While the UVA intensities of gold light (0.003% of sunlight) and ambient gold light (0.01% of sunlight) are extremely low, the UVB intensities of these two sources, 0.26% and 1.10% of sunlight, respectively, were surprising, because gold light is often described as having no intensity below 500 nm.

### 3.2. Water-only exposures

#### 3.2.1. Water concentrations

The water concentrations in these exposures declined prior to renewal by an average of 36.5% day<sup>-1</sup> (SD = 7.6%,  $n = 140$ ) across all exposures. The concentrations reported (Table 3) are 10-day time-weighted averages. At the highest concentration reported for the gold light exposure, the water concentration is a 5-day time-weighted average, because 100% mortality was achieved at that time and the dose was terminated.

#### 3.2.2. Mortality

Under gold light, survival on Day 10 ranged from 81% at 78 nmol L<sup>-1</sup> to 0% at 935 nmol L<sup>-1</sup>. Although mean control survival, 77%, was below 80%, survival in

Table 3

Measured fluoranthene water concentrations, apparent 10-day bioconcentration factors, median lethal concentrations, and median lethal residues

Light source	Measured water concentration (nmol L <sup>-1</sup> ), n = 20		Apparent 10-day BCF			Median lethal concentration			Median lethal residue		
	Mean	SD	Mean	SD	n	nmol L <sup>-1</sup>	95% CI	Slope	mmol kg <sup>-1</sup>	95% CI	Slope
Gold	78	24	2491	188	3	411	361–454	7.6	1.37 <sup>a</sup>	ND <sup>b</sup>	2
	165	44	2705	639	3				0.81 <sup>c</sup>	0.79–0.82	39
	324	87	2362	463	3						
	653	173	1341 <sup>c</sup>								
	935	248	1397 <sup>c</sup>								
Fluorescent	14	3	1625	151	4	68.0	62–73	5.4	0.20	0.17–0.23	2.5
	26	7	1629	373	4						
	52	14	2140	172	4						
	78	22	3729	757	4						
	102	28	4741	277	4						
UV enhanced	0.7	0.2	986	171	4	11.0	9.5–13	4.0	0.014	0.011–0.019	2.5
	1.6	0.4	880	122	4						
	5	1	986	100	4						
	11	3	1203	246	4						
	32	8	2639	547	2						

<sup>a</sup>This LR<sub>50</sub> is an estimate based on three concentrations due to high mortality at the two highest concentrations.

<sup>b</sup>Not determined.

<sup>c</sup>These values are based on the assumption that the Day 10 tissue residues are equal to the means of the tissue residues for the prior three time points for the two highest concentrations.

three of the five control replicate beakers was >80% and the mean survival in the trace concentration was >80%; thus, the overall health of the organisms was good. The data for LC<sub>50</sub> and LR<sub>50</sub> determinations were adjusted for control mortality. Under fluorescent light, survival on Day 10 ranged from 95% in the controls to 18% at 102 nmol L<sup>-1</sup>. Under UV-enhanced light, Day 10 survival ranged from 87% at 1.6 nmol L<sup>-1</sup> to 3% at 32 nmol L<sup>-1</sup>.

Ten-day LC<sub>50</sub> values determined under the three spectra followed the expected relationship of UV enhanced < fluorescent < gold (Table 3). The LC<sub>50</sub> under gold light, 411 nmol L<sup>-1</sup> (95% CI = 361–454 nmol L<sup>-1</sup>), was 6-fold larger than under fluorescent light and 37-fold larger than under UV-enhanced light than for gold light (Table 3). Further the LC<sub>50</sub> under UV-enhanced light was 6-fold lower than under fluorescent light. The slopes of the dose-response curves also followed the relationship of UV enhanced (4.0) < fluorescent (5.4) < gold (7.6) (Table 3).

### 3.2.3. Body burden

In most concentrations under all three light spectra, *H. azteca* achieved steady-state tissue residues between 2 and 5 days (Fig. 2). The notable exceptions are the two highest concentrations under fluorescent light, where the tissue residues increased at each time point. Day 10 tissue residues were not measured for the two highest concentrations under gold light because of high mortality, 95% and 100%, respectively. However, since tissue residues at Days 1, 2, and 5 were relatively constant for

these two concentrations, Day 10 tissue residues were estimated as the means of the three prior time points, 0.88 and 1.31 mmol kg<sup>-1</sup>, respectively, for calculating LR<sub>50</sub>.

Apparent 10-day bioconcentration factors (BCFs) were determined for each exposure based on the time-weighted average water concentrations and the mean tissue residues (Table 3). The relationship between water concentration and apparent 10-day BCF for fluorescent and UV enhanced was approximately constant below the LC<sub>50</sub> and increased at higher doses (Table 3). The BCF at the lower doses also seemed to decline with increasing amounts of UV light.

Ten-day LR<sub>50</sub> values determined for the three spectra followed the same pattern as the 10-day LC<sub>50</sub> values: UV enhanced < fluorescent < gold (Table 3). Using the measured tissue residues, the 10-day LR<sub>50</sub> under gold light, 1.37 mmol kg<sup>-1</sup> (95% CI was not determined), was estimated from the three lower concentrations. If the Day 10 tissue residues are assumed to be the means of the prior three time points for the two highest concentrations, then the 10-day LR<sub>50</sub> under gold light would be 0.81 mmol kg<sup>-1</sup> wet wt (95% CI = 0.79–0.82 mmol kg<sup>-1</sup>). Since the two highest concentrations resulted in high mortality at Day 5, they would also cause high mortality at Day 10. These estimated 10-day LR<sub>50</sub> values under gold light are 4- to 7-fold larger than for fluorescent light, 0.20 mmol kg<sup>-1</sup> (95% CI = 0.17–0.23 mmol kg<sup>-1</sup>), and approximately 58- to 100-fold larger than for UV-enhanced light, 0.014 mmol kg<sup>-1</sup> (95% CI = 0.011–0.019 mmol kg<sup>-1</sup>).

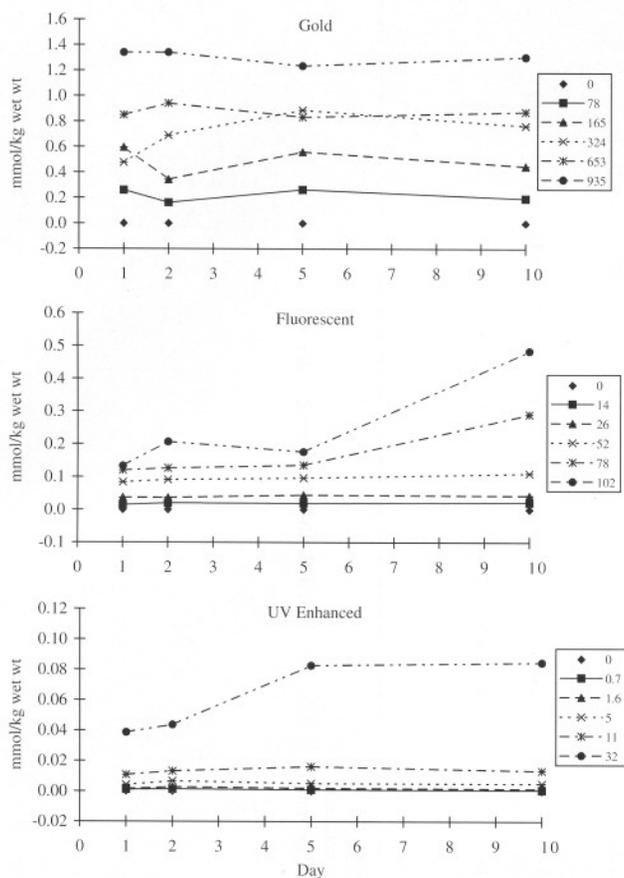


Fig. 2. *H. azteca* mean measured tissue residue versus time in water-only exposures under gold, fluorescent, and UV-enhanced light. Legend values are fluoranthene water concentrations in  $\text{nmol L}^{-1}$ . Under gold light, Day 10 values for 634 and 935  $\text{nmol L}^{-1}$  are estimates based on the means of the three prior time points.

Although the differences between the 10-day  $\text{LR}_{50}$  values for the three spectra were greater than those between the  $\text{LC}_{50}$  values, the slopes of the dose–response curves were more consistent (gold = 2, fluorescent = 2.5, and UV-enhanced estimate = 2.5) (Table 3). However, if the assumed Day 10 tissue residues for the two highest concentrations under gold light (see above) are included, the dose–response curve becomes much steeper (slope = 39, 95% CI = 27.9–50.4).

### 3.3. Sediment exposures

#### 3.3.1. Sediment concentrations

All three sediment exposures used subsamples from a single set of spiked sediments. Throughout the exposures, the subsurface fluoranthene concentrations (Table 4) showed no significant decline over time.

#### 3.3.2. Mortality

*H. azteca* survival was greater than 80% in nearly all sediment concentrations under both light spectra. Under fluorescent light, only the trace concentration,

0.2  $\text{nmol g}^{-1}$ , and the highest concentration, 933  $\text{nmol g}^{-1}$ , resulted in 10-day survival below 80% (78% and 33%, respectively). In the first UV-enhanced exposure, only the two highest concentrations, 398 and 645  $\text{nmol g}^{-1}$ , resulted in less than 80% survival (10% and 0%, respectively). In the second UV-enhanced exposure only the highest concentration, 965  $\text{nmol g}^{-1}$ , resulted in less than 80% survival (0%). Under UV-enhanced light, the highest survival was at intermediate concentrations in both experiments.

The trimmed Spearman–Kärber method provided estimates of the  $\text{LC}_{50}$  under fluorescent light of 720  $\text{nmol g}^{-1}$  dry wt sediment (95% CI = 608–852  $\text{nmol g}^{-1}$ ) (Table 4). In the first UV-enhanced exposure, the  $\text{LC}_{50}$  of 302  $\text{nmol g}^{-1}$  (95% CI = 279–328  $\text{nmol g}^{-1}$ ) was less than half that under fluorescent light. The second UV-enhanced exposure resulted in an intermediate  $\text{LC}_{50}$  of 535  $\text{nmol g}^{-1}$  (95% CI = 455–628  $\text{nmol g}^{-1}$ ) only 26% less than that under fluorescent light. Based on the 95% CIs, the first UV-enhanced  $\text{LC}_{50}$  is significantly different from the other two. To determine whether the lower  $\text{LC}_{50}$  in the first UV-enhanced exposure was an artifact of the lower measured concentration for the highest dose in this exposure (645  $\text{nmol g}^{-1}$  versus 933  $\text{nmol g}^{-1}$  and 965  $\text{nmol g}^{-1}$  for the fluorescent and the second UV-enhanced exposures, respectively), the mean measured sediment concentrations across all three experiments also were used to calculate  $\text{LC}_{50}$  values (Table 4). This is a reasonable approach since sediments for all three experiments were taken from the same set of spiked sediments. This  $\text{LC}_{50}$  for the first UV-enhanced exposure, 281  $\text{nmol g}^{-1}$  (95% CI = 256–309  $\text{nmol g}^{-1}$ ), was also significantly lower than the corresponding  $\text{LC}_{50}$  values for the fluorescent exposure, 666  $\text{nmol g}^{-1}$  (95% CI = 570–779  $\text{nmol g}^{-1}$ ), and the second UV-enhanced exposure, 516  $\text{nmol g}^{-1}$  (95% CI = 438–609  $\text{nmol g}^{-1}$ ). The lower  $\text{LC}_{50}$  values in the first UV-enhanced exposure is primarily the result of high mortality in the second highest dose not seen in the fluorescent or second UV-enhanced experiments.

#### 3.3.3. Body burden

Unlike in the water-only exposures, *H. azteca* fluoranthene tissue residues in sediment exposures increased at each time point until the end of the experiment (Fig. 3). Tissue residues were not determined for the highest concentration in UV-enhanced light exposures because of 100% mortality on Day 10.

*H. azteca* exposed to spiked sediments accumulated similar amounts of fluoranthene under fluorescent and UV-enhanced light. Under fluorescent light, *H. azteca* accumulated up to 1.05  $\text{mmol fluoranthene kg}^{-1}$  (SD = 0.24  $\text{mmol kg}^{-1}$ ). In the first UV-enhanced light exposure, *H. azteca* achieved tissue residues as high as 0.32  $\text{mmol kg}^{-1}$  (SD = 0.02  $\text{mmol kg}^{-1}$ ) in the second

Table 4  
Measured fluoranthene sediment concentrations, BSAFs, median lethal concentrations, and median lethal residues

Light source	Measured sediment concn (nmol g <sup>-1</sup> dry wt), n = 12		Apparent Day 10 BSAF, n = 4		Median lethal concentration				Median lethal residue	
	Mean	SD	Mean	SD	nmol g <sup>-1</sup> dry wt	95% CI	μg <sup>-1</sup> g OC <sup>a</sup>	95% CI	mmol <sup>-1</sup> kg	95% CI
Fluorescent	0	0	ND	ND	720 <sup>b</sup>	608–852	32,362	27,328–38,295	0.955	0.875–1.04
	0.2	0.03	0.020	0.007						
	127	104	0.173	0.060	666 <sup>c</sup>	570–779	29,934	25,620–35,013		
	174	57	0.105	0.022						
	403	165	0.053	0.037						
	933	356	0.029	0.006						
UV enhanced No. 1	0	0	ND	ND	302 <sup>b</sup>	279–328	13,574	12,540–14,743	0.323	0.3228–0.3231
	0.2	0.02	0.018	0.005						
	90	14	0.147	0.018	281 <sup>c</sup>	256–309	12,630	11,506–13,889		
	232	107	0.093	0.051						
	398	158	0.075	0.021						
	645	369	ND	ND						
UV enhanced No. 2	0	0	ND	ND	535 <sup>b</sup>	455–628	24,046	20,451–28,227	ND <sup>d</sup>	ND
	0.2	0.01	0.025	0.003						
	90	11	0.173	0.073	516 <sup>c</sup>	438–609	23,192	19,687–27,373		
	173	41	0.143	0.051						
	367	84	0.100	0.042						
	965	396	ND	ND						

<sup>a</sup> Based on nmol g<sup>-1</sup> dry wt. determination normalized to 0.45% OC for Lake Michigan sediments used.

<sup>b</sup> LC<sub>50</sub> and 95% CI calculated from the measured sediment concentrations from the respective experiment.

<sup>c</sup> LC<sub>50</sub> and 95% CI calculated from the mean measured sediment concentrations across all three experiments: 0, 0.22, 102, 193, 389, and 848 nmol g<sup>-1</sup> dry wt.

<sup>d</sup> Not determined.

highest concentration, while in the second UV-enhanced light exposure, levels as high as 0.61 mmol fluoranthene  $\text{kg}^{-1}$  (SD = 0.16 mmol  $\text{kg}^{-1}$ ) were achieved in the second highest concentration. These levels are similar to the 0.68 mmol  $\text{kg}^{-1}$  (SD = 0.20 mmol  $\text{kg}^{-1}$ ) for the second highest concentration under fluorescent light.

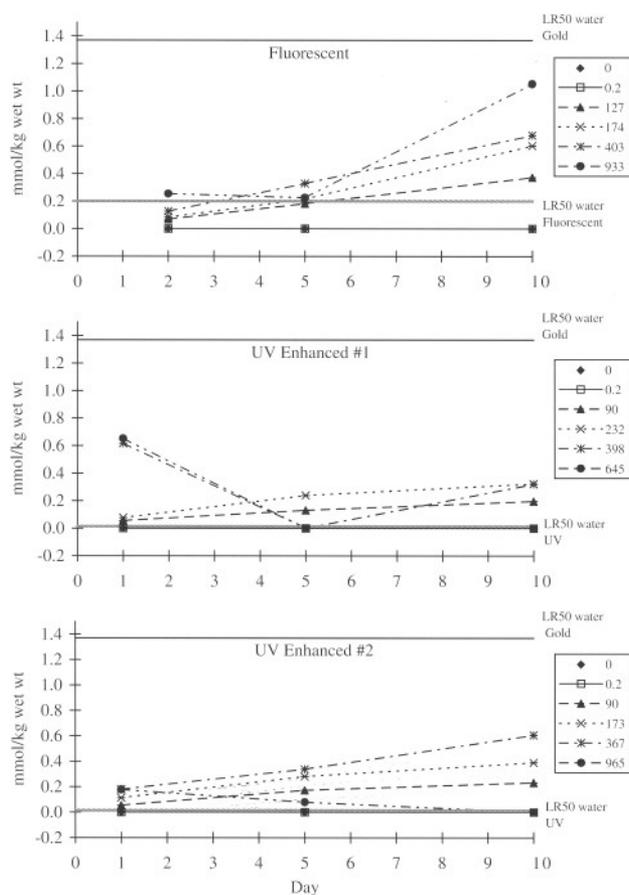


Fig. 3. *H. azteca* mean measured tissue residue versus time in sediment exposures under fluorescent and UV-enhanced light. Legend values are fluoranthene sediment concentrations in  $\text{nmol g}^{-1}$  dry wt. LR<sub>50</sub> values from water-only exposures are indicated on the right.

The LR<sub>50</sub> determined for the fluorescent exposure, 0.955 mmol  $\text{kg}^{-1}$  (95% CI = 0.875–1.04 mmol  $\text{kg}^{-1}$ ), was nearly three times higher than that for the first UV-enhanced exposure, 0.323 mmol  $\text{kg}^{-1}$  (95% CI = 0.3228–0.3231 mmol  $\text{kg}^{-1}$ ). In the second UV-enhanced test, an LR<sub>50</sub> could not be determined using the trimmed Spearman–Karber method, because survival was near 90% in all concentrations except the highest, which resulted in 100% mortality.

The tissue concentrations and LR<sub>50</sub> values determined in sediment exposures exceed the LR<sub>50</sub> values determined in water-only tests under their respective light sources (Tables 3 and 4). In all sediment tests, the only significant toxicity observed was where the *H. azteca* concentrations approached the estimated LR<sub>50</sub> value found in the water-only test under Gold light.

Biota–sediment accumulation factors (BSAFs), normalized for *H. azteca* lipid content and sediment organic carbon (OC) content, reveal similar patterns for all three sediment exposures (Table 4, Fig. 4). In all three exposures, animals accumulated little fluoranthene in the lowest concentration. BSAFs peaked at about 100  $\text{nmol g}^{-1}$  dry wt, then declined slightly with increased sediment concentration.

## 4. Discussion

### 4.1. Light exposure

The light spectra used here were included for different reasons. The gold light was included because no photodegradation of fluoranthene is expected above 500 nm and for comparison to previous studies that used the ambient gold light. The fluorescent light meets the guidelines for the EPA testing methods. The UV-enhanced light was intended to mimic the spectral quality of sunlight that can penetrate to relevant depths in natural freshwater. Comparing light sources with sunlight has often involved the proportional distribution

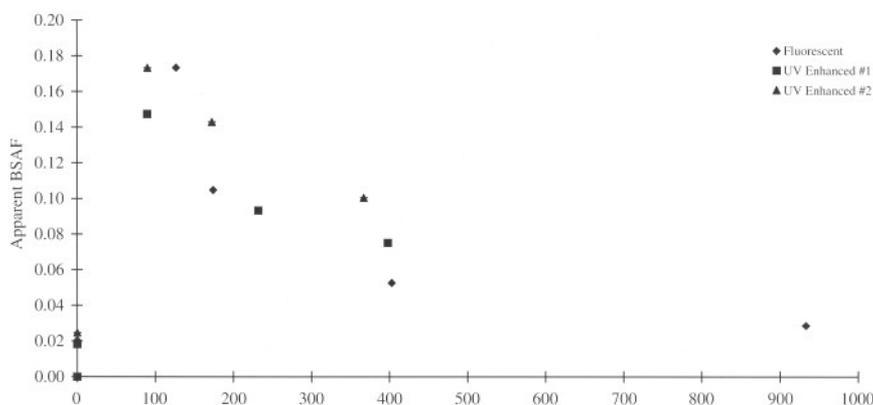


Fig. 4. Sediment fluoranthene concentration versus 10-day apparent BSAF.

in light categories, i.e., visible:UVA:UVB ratio. However, this can be misleading. The visible:UVA:UVB ratio measured for UV-enhanced light, 100:35:2.6, seems higher in UVA and UVB than that measured for sunlight, 100:6.6:0.15 (this study), and previously reported values, 100:10:1 (Huang et al., 1993). When the visible component is ignored, the UVA:UVB ratio for UV-enhanced light becomes approximately 13:1, which is lower than that measured for sunlight, 44:1, yet comparable to previously reported values of 8:1 (Holst and Giesy, 1989) and 9:1 for sunlight (Monson et al., 1995; Boese et al., 1997). To further confuse the issue, UVA:UVB ratios as high as 37:1 and 43:1 were measured in natural waters at depths of 10–17 cm (Monson et al., 1995). In short, the proportional ratios of visible, UVA, and UVB light vary with natural conditions (season, time of day, air versus water, depth, turbidity, filtering chromophores) as well as metric limitations (detector bandwidth, radiometer sensitivity, definition of categorical limits), so that their utility is extremely limited without standardization.

The pertinent question is whether the light intensities tested are biologically relevant in natural waters and at wavelengths relevant to the chromophore tested. Because of the experimental design, the organisms were exposed to the same intensities as measured at bench top level. Using our broadband UV extinction coefficient, the UV intensities in Huron River water would decline by 50% at 0.7 m. However, this river is not very deep so UV could easily reach the bottom in many locations. The fluorescent light intensity resulted in UV intensities equivalent to sunlight at 3.9-m depth in Huron River water. Using a UVB extinction coefficient of  $0.575 \text{ m}^{-1}$  determined for offshore Lake Michigan water (Landrum et al., 1984) the UVB component of the lights tested represents the fraction of UVB from sunlight that would be found between 3.7 m (UV enhanced) and 10.3 m (gold) in Lake Michigan. Similarly, using the UVA extinction coefficients of  $0.45 \text{ m}^{-1}$  for Lake Michigan and  $1.32 \text{ m}^{-1}$  for a small eutrophic impoundment (Fink's Pond, Ingham County, MI) (Oris and Giesy, 1986), the UVA component of the lights tested represents the fraction of UVA component of sunlight that would be found from 7.4 m (UV enhanced) to 23.0 m (gold) in Lake Michigan and from 2.5 m (UV enhanced) to 7.9 m (gold) in the eutrophic system. Thus, UV penetration in natural systems would be expected to augment the toxicity of PAHs.

Gold light was included in this study because no photodegradation of fluoranthene was expected above 500 nm. This would provide a diurnal photoperiod for *H. azteca* while maintaining a "dark" exposure for the fluoranthene, which has absorbance maxima at 234, 274, 286, 339, and 357 nm (when dissolved in methanol) with negligible absorbance above 400 nm (Simons, 1979). It was surprising to discover that gold light does have a small UV component. The UVB intensity of the gold

light represents 0.26% of the UVB intensity of sunlight (Table 2), 12.9% of the UVB intensity of fluorescent light, and 2.3% of the UVB intensity of the UV-enhanced light. The UVA intensity of the gold light is considerably lower, representing 0.003% of sunlight UVA (Table 2), 0.7% of the fluorescent UVA, and 0.09% of the UV-enhanced UVA. Although the UV component of gold light is at a very low intensity, its contribution to the toxicity of fluoranthene in *H. azteca*, while expected to be essentially unimportant, cannot be completely ruled out in these 10-day exposures.

EPA recommends wide-spectrum fluorescent lights at "about 500–1000 lx" for toxicity and bioaccumulation tests of sediment-associated contaminants (USEPA, 1994, 2000). This recommendation is problematic in two ways. First, "wide-spectrum" is not well defined. Second, the intensity is reported in photometric units (normalized to an average human eye) which essentially ignores the relevance of the UV portion of the electromagnetic spectrum. The light sources used in this study conform to the EPA recommendation for test light intensity of "about 500–1000 lx" (gold = 744 lx, fluorescent = 1538 lx, UV enhanced = 1055 lx). However, their spectra are very different and lead to very different toxicities (see below).

## 4.2. Water-only exposures

### 4.2.1. Mortality

The toxicity of fluoranthene to *H. azteca* in water-only exposures as expected clearly depends on the amount of UV light present (Fig. 5). The 10-day  $\text{LC}_{50}$  under gold light,  $411 \text{ nmol L}^{-1}$ , is comparable to  $\text{LC}_{50}$  values from two previous experiments under ambient gold light,  $564 \text{ nmol L}^{-1}$  (SKD No. 1, 95% CI =  $524\text{--}603 \text{ nmol L}^{-1}$ ) and  $481 \text{ nmol L}^{-1}$  (SKD No. 2, 95% CI =  $448\text{--}516 \text{ nmol L}^{-1}$ ) (Kane Driscoll et al., 1997b). Both of these light sources have very low UV intensities.

Fluorescent light has a small UV component (Fig. 1, Table 2) that caused a 6-fold reduction in the 10-day  $\text{LC}_{50}$ ,  $68 \text{ nmol L}^{-1}$ . This is 3- to 4-fold lower than a previously determined 10-day  $\text{LC}_{50}$  for fluoranthene in *H. azteca*,  $221 \text{ nmol L}^{-1}$  (Suedel et al., 1993), and approximately 2-fold lower than the dark final acute value ( $\text{FAV}_{\text{dark}}$ ),  $166 \text{ nmol L}^{-1}$  (USEPA, 1993). The description of the light source for Suedel et al. (1993) simply states that tests were performed in a lighted incubator at 50–100-ft candles (538–1076 lx). This was presumably from a cool white fluorescent source. Similarly, EPA describes the light for the  $\text{FAV}_{\text{dark}}$  as "normal laboratory light" (USEPA, 1993). Again, this is presumably a cool white fluorescent source that conforms to the EPA recommendation of 500–1000 lx. If these presumptions are correct, then the lower fluorescent  $\text{LC}_{50}$  from the current study can be partially

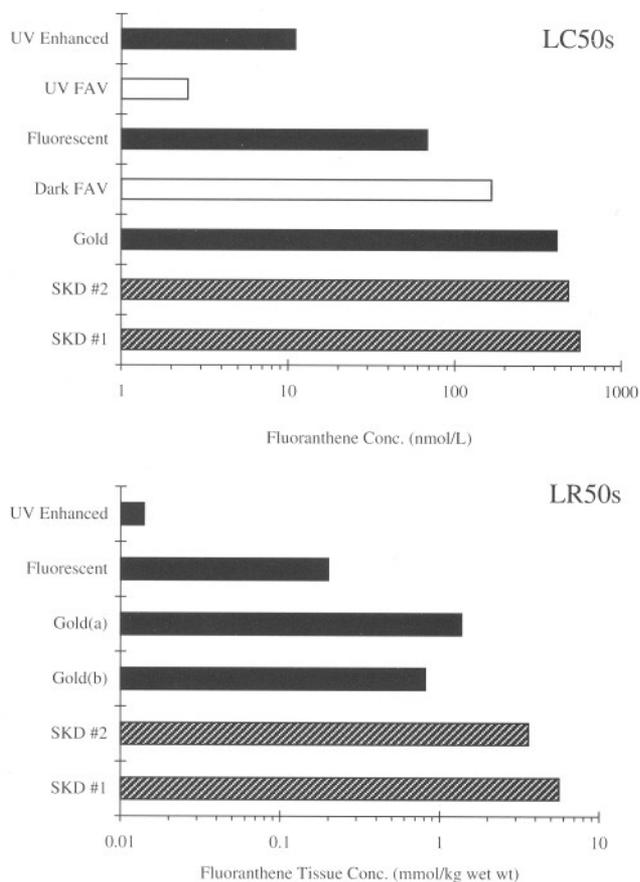


Fig. 5. Water-only 10-day LC<sub>50</sub> and LR<sub>50</sub> values. The black bars are data from this study. Open bars are final acute values from SQC for Fluoranthene (USEPA, 1993). Crosshatched bars are from a previous study using ambient gold light (Kane Driscoll et al., 1997b). Gold(a) includes only the measured tissue residues on Day 10. Gold(b) includes estimates for the Day 10 tissue residues for the two highest concentrations based on the means of the previous three time points.

explained by the higher light intensity, 1538 lx, and perhaps by more rigorous accountability of the water concentration using the time-weighted average value. The difference may also reflect differing sensitivities to fluoranthene of *H. azteca* and the species included in the FAV. Additionally, the lower value may also result from using the time-weighted average water concentrations compared with Suedel et al. (1993) in which no indication of correction for loss was indicated.

The greater UV component of the UV-enhanced light caused a 37-fold decrease in the 10-day LC<sub>50</sub> compared with gold light and a 6-fold decrease compared with fluorescent light. The UV-enhanced LC<sub>50</sub>, 11 nmol L<sup>-1</sup>, is more than 4-fold higher than the FAV<sub>UV</sub>, 2.5 nmol L<sup>-1</sup> (USEPA, 1993). Without a direct comparison of light intensities, spectra, and exposure duration used for the FAV<sub>UV</sub> tests, the sensitivity of *H. azteca* to fluoranthene/UV relative to the species included in the FAV<sub>UV</sub> cannot be determined.

#### 4.2.2. Body burden

As with the LC<sub>50</sub> values, the 10-day LR<sub>50</sub> values decreased with increased UV component. Estimates for the LR<sub>50</sub> under gold light, 1.37 and 0.81 mmol kg<sup>-1</sup>, are 2- to 7-fold lower than estimates from previous experiments in our laboratory under ambient gold light, 5.6 mmol kg<sup>-1</sup> (SKD No. 1, 95% CI = 0.47–7.2 mmol kg<sup>-1</sup>) and 3.6 mmol kg<sup>-1</sup> (SKD No. 2, 95% CI = 2.6–9.2 mmol kg<sup>-1</sup>) (Kane Driscoll et al., 1997b) (Fig. 5). This difference is not likely due to spectral differences, since the ambient gold light has slightly more UVA and UVB than the gold light (Table 2). The current estimates are based on the fact that organisms in all of the other concentrations reached steady-state tissue levels by Day 5, suggesting that organisms in the two highest concentrations also reached steady-state tissue residues at that time. Also, because these concentrations caused high mortality at Day 5, they would cause high mortality at Day 10 as well. There is no clear explanation for the differences in the results of the two studies. However, it is not unusual for LC<sub>50</sub> values to vary by a factor of 2 or so over time and with different researchers.

In the presence of a UV component, LR<sub>50</sub> values were significantly decreased with increased UV intensity. The fluorescent LR<sub>50</sub>, 0.20 mmol kg<sup>-1</sup>, is nearly an order of magnitude lower than the gold light estimates, while the UV-enhanced LR<sub>50</sub>, 0.014 mmol kg<sup>-1</sup>, is approximately two orders of magnitude lower than the gold light estimates. If the LR<sub>50</sub> estimates under gold light are indeed low, then the difference in toxicity due to UV light is even greater.

#### 4.3. Sediment exposures

##### 4.3.1. Mortality

The equilibrium partitioning (EqP) model (DiToro et al., 1991), the basis for EPA Sediment Quality Guidelines, states that sediment toxicity can be predicted from the effects determined in water-only exposures. The results of the water-only exposures predict the following sediment OC-normalized LC<sub>50</sub> values: 416 μg g<sup>-1</sup> OC under gold light; 68 μg g<sup>-1</sup> OC under fluorescent light; and 11 μg g<sup>-1</sup> OC under UV-enhanced light.

In contrast to the water-only exposures, 10-day survival was high in all but the highest sediment concentrations under fluorescent light and in the second UV-enhanced exposure. The first UV-enhanced sediment exposure had high survival in all but the two highest concentrations. Previous experiments in our laboratory exposing *H. azteca* to similarly spiked sediments under ambient gold light resulted in high survival at 10 days in all concentrations so that LC<sub>50</sub> values could not be determined (Kane Driscoll et al., 1997a).

Under fluorescent light, the measured organic carbon-normalized LC<sub>50</sub>, 32,362 µg g<sup>-1</sup> OC, is approximately 500-fold higher than the value predicted from the water-only exposures and approximately 20- to 60-fold higher than previously determined values for 10-day sediment LC<sub>50</sub> and EC<sub>50</sub> (immobility) for fluoranthene in *H. azteca*, 512–1645 µg g<sup>-1</sup> OC (EC<sub>50</sub>) (Suedel et al., 1993) and 3420 µg g<sup>-1</sup> OC (LC<sub>50</sub>) (DeWitt et al., 1989).

The measured OC-normalized LC<sub>50</sub> for the first UV-enhanced light exposure, 13,574 µg g<sup>-1</sup> OC, is significantly lower than values for both the fluorescent light exposure and the second UV-enhanced exposure, 24,046 µg g<sup>-1</sup> OC. The measured OC-normalized LC<sub>50</sub> for the second UV-enhanced light exposure is not significantly different from the value for the fluorescent light exposure. These measured values are more than a 1000 times greater than the EqP-predicted LC<sub>50</sub> values using the UV-enhanced water-only exposure, 11 µg g<sup>-1</sup> OC. The difference between the first and second UV-enhanced exposures might have resulted from behavioral effects. If *H. azteca* remained in the sediment more then the results would have been more similar to the fluorescent light exposure; however, if the organisms transited into the water the toxicity would be increased. Thus, both results are possible within the same sediment experimental regimen. Certainly, the presence of UV has been demonstrated to impact the toxicity in sediment exposures for *H. azteca* in the presence of UV light compared with fluorescent light (Monson et al., 1995).

#### 4.3.2. Body burden

In contrast to the previous sediment exposures done under ambient gold light where *Hyalella* remained in the water column avoiding the contaminated sediments (Kane Driscoll et al., 1997a), in all of the current sediment exposures, *H. azteca* spent little time in the water column, preferring to remain burrowed in the sediment. Thus, the sediment gave substantial shielding from the UV radiation. As a result of this prolonged contact with the contaminated sediments, *H. azteca* achieved tissue residues substantially greater than the LR<sub>50</sub> values determined under the respective light spectra in water-only exposures (Fig. 3). In fact, significant mortality was found only when the tissue residues approached the LR<sub>50</sub> value determined under gold light in water-only exposures.

In the previous ambient gold light sediment exposures, *H. azteca* did not, in general, accumulate more than 1 mmol total fluoranthene eq kg<sup>-1</sup> (Kane Driscoll et al., 1997a). Similar accumulation levels were seen under fluorescent light (up to 1.05 mmol fluoranthene eq kg<sup>-1</sup>) and UV-enhanced light (up to 0.61 mmol fluoranthene eq kg<sup>-1</sup>) in the current study. However, the accumulation data in the current tests differ from those in the ambient gold light exposures in two respects. First, under fluorescent and UV-enhanced

light, tissue residues continued to increase until Day 10 in most sediment concentrations (Fig. 3). Second, significant mortality was observed at the highest concentrations under both fluorescent and UV-enhanced light at body residue levels that were not toxic under ambient gold light. This suggests that despite the shielding by the sediment some photoinduced toxicity may have occurred.

While the *H. azteca* remain in the sediment, they will be afforded substantial protection from the influence of the UV light. However, as indicated above the difference between the two UV-enhanced exposures could well have resulted from differential behavior where one group of organisms was more exposed to UV than the other. Further, the literature demonstrates that under some conditions *H. azteca* in sediment do demonstrate enhanced toxicity when exposed to enhance UV light compared with fluorescent light (Monson et al., 1995). Fundamentally, the USEPA recommendation for wide-spectrum fluorescent light creates the possibility for photoinduced toxicity based on the water-only results and this effect may well occur in sediment exposures depending on the behavior of the organisms, e.g., whether or not they remain buried in the sediment during the light portion of the photocycle.

## 5. Conclusions

It is clear that more work is needed to standardize light conditions in water-only and sediment bioassays. Although USEPA methods formalized light conditions for bioassays, they are not well defined, making comparisons between studies difficult particularly where photoinduced toxicity is an issue. Also, they do not resemble realistic conditions under sunlight, making management and regulatory decisions difficult when consideration of phototoxic effects must be managed.

Simply specifying light levels for toxicity testing is not adequate to define the exposure conditions for photoactive contaminants. Both the UV intensity and or a wavelength cutoff should be specified. If wide-spectrum fluorescent lights are used, the light intensity particularly for the UV component should be measured as a part of the experimental information. It is clear from the data that even the UV from fluorescent lights can contribute significantly to the toxicity of PAHs to *H. azteca*.

Finally, there was significant shading from exposures in sediments in this study. The organisms did not exhibit toxicity until the concentrations were in the range that were toxic under gold light. However, there were some indications that at the highest dose toxicity was found at levels of 1 mmol kg<sup>-1</sup> which was not toxic from previous studies under the ambient gold light but produced toxicity under fluorescent and UV-enhanced lights, thus suggesting that some photoinduced toxicity

could be occurring in the sediment exposures. Certainly the potential exists for the fluorescent light source to induce the toxicity of PAHs if the *H. azteca* leave the sediment and become exposed to the light.

## References

- Abbott, W.S., 1925. A method of computing the effectiveness of insecticides. *J. Econ. Entomol.* 18, 265–267.
- Ankley, G.T., Collyard, S.A., Monson, P.D., Kosian, P.A., 1994. Influence of ultraviolet light on the toxicity of sediments contaminated with polycyclic aromatic hydrocarbons. *Environ. Toxicol. Chem.* 13, 1791–1796.
- Ankley, G.T., Erickson, R.J., Phipps, G.L., Mattson, V.R., Kosian, P.A., Sheedy, B.R., Cox, J.S., 1995. Effects of light intensity on the phototoxicity of fluoranthene to a benthic macroinvertebrate. *Environ. Sci. Technol.* 29, 2828–2833.
- Blaustein, A.R., Emond, B., Kiesecker, J.M., Beatty, J.J., Hokit, D.G., 1995. Ambient ultraviolet radiation causes mortality in salamander eggs. *Ecol. Appl.* 5, 740–743.
- Boese, B.L., Lamberson, J.O., Swartz, R.C., Ozretich, R.J., 1997. Photoinduced toxicity of fluoranthene to seven marine benthic crustaceans. *Arch. Environ. Contam. Toxicol.* 32, 389–393.
- Bowling, J.W., Leverssee, G.J., Landrum, P.F., Giesy, J.P., 1983. Acute mortality of anthracene-contaminated fish exposed to sunlight. *Aquat. Toxicol.* 3, 79–90.
- Davenport, R., Spacie, A., 1991. Acute phototoxicity of harbor and tributary sediments from lower Lake Michigan. *J. Great Lakes Res.* 17, 51–56.
- DeWitt, T.H., Swartz, R.C., Lamberson, J.O., 1989. Measuring the acute toxicity of estuarine sediments. *Environ. Toxicol. Chem.* 8, 1035–1048.
- Di Toro, D.M., Zarba, C.S., Hansen, D.J., Berry, W.J., Swartz, R.C., Pavlou, S.P., Allen, H.E., Thomas, N.A., Paquin, P.R., 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ. Toxicol. Chem.* 10, 1541–1583.
- Ditsworth, G.R., Schults, D.W., Jones, J.K.P., 1990. Preparation of benthic substrates for sediment toxicity testing. *Environ. Toxicol. Chem.* 9, 1523–1529.
- Eadie, B.J., Landrum, P.F., Faust, W., 1982. Polycyclic aromatic hydrocarbons in sediments, porewater, and the amphipod *Pontoporeia hoyi* from Lake Michigan. *Chemosphere.* 9, 847–858.
- Gala, W.R., Giesy, J.P., 1994. Flow cytometric determination of the photoinduced toxicity of anthracene to the green alga *Selenastrum capricornutum*. *Environ. Toxicol. Chem.* 13, 831–840.
- Harkey, G.A., Van Hoof, P.L., Landrum, P.F., 1995. Bioavailability of polycyclic aromatic hydrocarbons from a historically contaminated sediment core. *Environ. Toxicol. Chem.* 14, 1551–1560.
- Holst, L.L., Giesy, J.P., 1989. Chronic effects of the photoenhanced toxicity of anthracene on *Daphnia magna* reproduction. *Environ. Toxicol. Chem.* 8, 933–942.
- Huang, X-D., Dixon, D.G., Greenberg, B.M., 1993. Impacts of UV radiation and photomodification on the toxicity of PAHs to the higher plant *Lemna gibba* (duckweed). *Environ. Toxicol. Chem.* 12, 1067–1077.
- Kane Driscoll, S., Harkey, G.A., Landrum, P.F., 1997a. Accumulation and toxicokinetics of fluoranthene in sediment bioassays with freshwater amphipods. *Environ. Toxicol. Chem.* 16, 742–753.
- Kane Driscoll, S., Landrum, P.F., Tigue, E., 1997b. Accumulation and toxicokinetics of fluoranthene in water-only exposures with freshwater amphipods. *Environ. Toxicol. Chem.* 16, 754–761.
- Landrum, P.F., Giesy, J.P., Oris, J.T., Allred, P.M., 1984. Photo-induced toxicity of polycyclic aromatic hydrocarbons to aquatic organisms. In: Vandermeulen, J.H., Hrudey, H.E. (Eds.), *Oil in Freshwater: Chemistry, Biology, Countermeasure Technology*. Pergamon Press, New York, pp. 304–318.
- Monson, P.D., Ankley, G.T., Kosian, P.A., 1995. Phototoxic response of *Lumbriculus variegatus* to sediments contaminated by polycyclic aromatic hydrocarbons. *Environ. Toxicol. Chem.* 14, 891–894.
- Mottram, J.C., Doniach, I., 1938. The photodynamic action of carcinogenic agents. *Lancet* 234, 1156–1159.
- Newsted, J.L., Giesy, J.P., 1987. Predictive models for photoinduced acute toxicity of polycyclic aromatic hydrocarbons to *Daphnia magna*, Strauss (Cladocera, Crustacea). *Environ. Toxicol. Chem.* 6, 445–461.
- Norberg-King, T., 1992. Development of a standard protocol for testing *Hyalella azteca*. Proceedings: Tiered Testing Issues for Freshwater and Marine Sediments. Office of Water, Office of Science and Technology, and Office of Research and Development, Washington, DC, pp. 361–386.
- Oris, J.T., Giesy, J.P., 1986. Photoinduced toxicity of anthracene to juvenile bluegill sunfish (*Lepomis macrochirus* Rafinesque): photo-period effects and predictive hazard evaluation. *Environ. Toxicol. Chem.* 5, 761–768.
- Simons, W.W., 1979. *The Sadtler Handbook of Ultraviolet Spectra*. Sadtler Research Laboratories, Philadelphia.
- Suedel, B.C., Rodgers, J.H., Clifford, P.A., 1993. Bioavailability of fluoranthene in freshwater sediment toxicity tests. *Environ. Toxicol. Chem.* 12, 155–165.
- US Environmental Protection Agency, (USEPA), 1992. Proceedings: Tiered Testing Issues for Freshwater and Marine Sediments. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, and Office of Research and Development, Washington, DC.
- US Environmental Protection Agency, (USEPA), 1993. Sediment quality criteria for the protection of benthic organisms: fluoranthene. Technical Report, EPA-822-R-93-012. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, and Office of Research and Development, Washington, DC.
- US Environmental Protection Agency, (USEPA), 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. Technical Report, EPA 600/R-94/024. U.S. Environmental Protection Agency, Office of Research and Development, Duluth, MN.
- US Environmental Protection Agency, (USEPA), 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, 2nd edition. Technical Report, EPA-823-F-00-02. U.S. Environmental Protection Agency, Office of Water, Washington, DC.
- Van Handel, E., 1985. Rapid determination of total lipids in mosquitoes. *J. Am. Mosq. Contr. Assoc.* 1, 302–304.