Photo-induced toxicity of PAHs to *Hyalella azteca* and *Chironomus tentans*: effects of mixtures and behavior

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Abstract

In the aquatic environment, polycyclic aromatic hydrocarbon (PAH) contamination can result from several anthropogenic sources such as petroleum runoff, industrial processes, and petroleum spills. When ultraviolet light (UV) is present at sufficient intensity, the acute toxicity of some PAHs to aquatic biota is greatly enhanced. This photo-induced toxicity of PAHs is directly influenced by the amount of PAH and by the level of UV intensity present in the aquatic environment. Thus, behavioral responses and habits that affect an aquatic organism’s exposure to UV as well as exposure to PAHs can influence the extent to which damage due to photo-induced toxicity occurs. Experiments demonstrated the effects of photo-induced toxicity of anthracene and fluoranthene on the survival of two benthic macroinvertebrates, the midge *Chironomus tentans* and the freshwater amphipod *Hyalella azteca*. This study further investigated the survival and behavior of the test organisms in different substrates (no substrate, a sand monolayer, leaf discs, and sediment) with and without UV. The free-swimming, epibenthic *H. azteca* avoided the effects of photo-induced toxicity of PAHs to some extent by hiding in leaves when this substrate was available. Results emphasize the importance of organisms’ behavior in affecting the photo-induced toxicity of PAHs in the aquatic environment. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) can pollute aquatic ecosystems via urban runoff, oil spills, and point source pollution from steel and coking operations and from petroleum refining (Bowling et al., 1983; Landrum and Scavia, 1983; Landrum et al., 1987; Newsted and Giesy, 1987; Arfsten et al., 1996; Suedel and Rodgers, 1996). The toxicity of PAHs to aquatic life is greatly enhanced when biota are simultaneously exposed to ultraviolet light (UV), particularly UV-A (Bowling et al., 1983; Landrum and Scavia, 1983; Landrum et al., 1987; Newsted and Giesy, 1987; Oris et al., 1990). Toxicity occurs when UV excites the electrons in PAHs, resulting in the formation of toxic singlet oxygen as a by-product (US EPA, 1993). The toxic singlet oxygen can damage biological membranes (Ankley et al., 1995; Monson et al., 1995). Hydrophobic PAHs can accumulate in lipid tissue, and photo-induced toxicity occurs when bioaccumulated PAHs and UV light are present simultaneously (US EPA, 1993). Thus, in aquatic systems photo-induced toxicity of many PAHs to aquatic animals is likely to be due to internal mechanisms and not due to externally formed photoproducts.

In the aquatic environment, hydrophobic PAHs often sorb to particulate matter such as sediments (Ankley et al., 1994, 1995). Although fewer studies have focused on the photo-induced toxicity of PAH-contaminated sediments than on water contaminated with PAHs, these contaminated sediments are acutely toxic in the presence of UV light in laboratory and field exposures (Ankley et al., 1994; Monson et al., 1995; Ireland et al., 1996).

Recently, scientific interest in the environmental impacts of photo-induced toxicity of PAHs has increased. Several studies provide evidence that photo-induced toxicity is related directly to both dose of PAH...
Factors that influence either exposure to PAHs or exposure to UV will influence the impact of photo-induced toxicity on aquatic organisms. In the field, several natural environmental factors can interact to influence both PAH dose and UV intensity. Storm events result in the mobilization and resuspension of PAHs sorbed to sediments (Ireland et al., 1996). However, the increased turbidity associated with storm events can reduce the amount of UV that penetrates the water column, attenuating photo-induced toxicity (Ireland et al., 1996). Dissolved humic materials can also attenuate acute photo-induced toxicity in aquatic biota by reducing anthracene bioaccumulation and by reducing exposure to UV (Oris et al., 1990).

In addition to environmental factors, species-specific traits that protect organisms from sunlight, such as pigmentation or burrowing behavior, may also protect organisms from the effects of photo-induced toxicity (Boese et al., 1997). For example, sediment-dwelling invertebrates such as *Chironomus* spp. have been shown to increase the availability of sediment-bound benzo[a]pyrene by mobilizing sediment, while simultaneously increasing the turbidity of the water (Clements et al., 1994). Other researchers have suggested that burrowing in sediments could ameliorate some of the effects of photo-induced toxicity (Ankley et al., 1994). Thus the overall response to photo-induced toxicity is affected by the presence and intensity of UV light, the type of substrate available, and species-specific life history and behavior patterns (Landrum et al., 1987; Ankley et al., 1994; Arfsten et al., 1996).

Measurement of tissue residues of bioaccumulated PAHs has provided insight into the biological fate and effects of these compounds. Interspecific differences in the ability to metabolize compounds contribute to differences in the toxic response between species (Driscoll and Landrum, 1997; Driscoll et al., 1997). Species with greater lipid content may be less sensitive due to their ability to store PAHs (Driscoll and Landrum, 1997), and species with greater ability to metabolize the compound will be more sensitive to its effects (Driscoll et al., 1997). The use of tissue residues of individual PAHs provides for comparison of their toxic effects on the organism after considering possible differences in uptake of the compounds of interest (Ankley et al., 1995, 1997). These studies show that the PAHs anthracene and pyrene are several times more toxic than fluoranthene, and highlight the different potencies of individual PAHs (Ankley et al., 1997).

This study determined the single and combined effects of two PAHs that exhibit photo-induced toxicity, anthracene and fluoranthene, in spiked sediment and spiked water exposures to the midge *Chironomus tentans* and the freshwater amphipod *Hyalella azteca*. These two benthic macroinvertebrates are excellent biological indicators because they are in contact with sediment, they are tolerant of a wide range of physicochemical characteristics of sediment, and much information exists on their sensitivities to various toxicants (Ingersoll et al., 1995). Additional experiments focused on the influence of behavior in affecting organisms’ response to photo-induced toxicity of PAHs when different substrates were present. Refugia that enable organisms to avoid exposure to UV could influence the actual exposure to photo-induced toxicity experienced by organisms in the environment. In this study, survival and behavior in different substrates—a sand monolayer, sediment, leaves, and no substrate—were monitored in order to determine the effects of refugia in influencing the overall toxic response. Substrates were chosen to represent different aspects of the natural habitat that could be manipulated in the laboratory. In the mixture studies, sediment and water were dosed with the compounds of interest. In the sand and leaf substrate treatments, the compounds of interest were water-borne.

Previous studies have suggested that photo-induced toxicity of PAHs is additive in nature. Equitoxic mixtures of PAHs prevent marine infaunal amphipods from re-burying after exposure to PAH mixtures and subsequent exposure to UV for 1 h (Swartz et al., 1997). Two- and four-component mixtures of PAHs have a slightly less than additive effect under fluorescent lighting (without UV) (Munoz and Tarazona, 1993). However, the effects of species-specific behavior in attenuating photo-induced toxicity have not been demonstrated experimentally.

### 2. Materials and methods

#### 2.1. Test organisms and exposure conditions

Test organisms were obtained from laboratory cultures in the Aquatic Toxicology Laboratory at Wright State University, where experiments were conducted. *C. tentans* were 8–10 days old and *H. azteca* 7–14 days old at the beginning of the experiments (US EPA, 1994; Ingersoll et al., 1995). All tests, including sediment and water-only exposures and tests of behavior, lasted 10 days. All experiments used 250-ml borosilicate glass for exposure chambers. Growth of the test organisms was assessed by measuring the dry weight of a subsample of test organisms at test initiation and then measuring the dry weight of test organisms at test completion. Dry weight (to the nearest 0.01 mg) was measured at the completion of each test after drying organisms at 100°C for 24 h (US EPA, 1994).

For the single-compound tests, *C. tentans* and *H. azteca* were exposed in the same chambers and fed according to the following regime: ground Tetramin for *C. tentans* was fed on days 1, 5, and 8; ground rabbit
chow for *H. azteca* was fed on days 2, 6 and 9 (ASTM, 1993). Because mixture studies involved testing different combinations of anthracene and fluoranthene on the two test organisms, *C. tentans* and *H. azteca* were exposed in separate chambers for these tests. In the single-compound tests and in the mixture test, each concentration of PAH in water or sediment was replicated three to four times, with 10 individuals of each species exposed per replicate.

For the behavioral observation experiment, two replicates of 10 individuals were exposed to fluoranthene at levels of 0, 6.25, 12.5, and 25 µg/l. This range in test concentrations corresponded to concentrations at which sublethal effects were observed in the single-compound fluoranthene tests. Four types of substrate were used: no substrate, leaves only, a sand monolayer, and 2 cm sediment. In initial experiments, a sediment–leaf substrate was also employed; however, this treatment regime was not useful because animals burrowing or hiding under the leaves could not be accounted for without disturbing the test system. For the sand monolayer treatments, approximately 10 g of Ottawa silica sand (600–850 µm diameter; Fisher Scientific International, Inc.) was added to each exposure chamber. This amount of sand provided a single layer of sand across the bottom of the test chamber. For the leaf substrate, five 1-cm discs of red maple (*Acer rubrum*) leaves were placed in each exposure chamber. Leaves were collected dry shortly after abscission from an un-impacted area (Wright State Biological Preserve, Wright State University, Dayton, OH, USA). Leaves were soaked in de-ionized water for 3 weeks before use, changing the water once per week. All test treatments were replicated with and without UV.

### 2.2. Laboratory lighting

UV light in the laboratory approximately simulated the ratios of ambient levels of UV light expected (approximate ratio of 100 visible: 10 UV-A: 1 UV-B) (Henderson, 1977). Two standard fluorescent light bulbs, one UV-A bulb and one UV-B bulb (Wesco distributors, Dayton, OH, USA) were used, with the addition of cheesecloth and acetate screening materials to achieve final intensity levels of 60–68 W/cm² UV-A and 2–5 W/cm² UV-B as measured on the laboratory bench next to the exposure chambers. The UV-A bulb used had a spectral output ranging from 310 to 420 nm, peaking at 350 nm. The UV-B bulb used had a spectral output ranging from 250 to 400 nm, peaking at 290 nm. Acetate screened out wavelengths below 290 nm. In the non-UV exposures, only fluorescent light bulbs were used. UV light intensity was measured using a broadband radiometer (UV-103, Macam Photometrics, Livingston, Scotland, UK). UV-A and UV-B intensity levels were recorded daily during experiments.

All experiments were simultaneously conducted under fluorescent lighting only, without UV. UV intensity measured under these conditions was negligible (less than 0.1 µW/cm² UV-B and less than 2 µW/cm² UV-A).

### 2.3. Sediment characteristics and spiking

Sediment (35% sand, 30% silt, 20% pebbles, 15% clay) was collected from a stream in the Wright State University Biological using a grab sampler. Total organic carbon (TOC) was 0.39% (Belmonte Park Laboratories, Dayton, OH, USA). Sediment was stored in acid-washed airtight plastic containers at 4°C and tested within 8 weeks of collection (ASTM, 1994; Ingersoll et al., 1995). Sediment was sieved through a coarse sieve (No. 3 1/2) to remove large particles, dried at 100°C for 24 h, weighed and spiked with PAHs dissolved in acetone. The acetone was evaporated overnight and the following day sediment was mixed thoroughly by hand (3 min total mixing time), re-hydrated and distributed to the test chambers. Sediment was mixed after acetone had evaporated, and again when re-hydrated in exposure water before distribution to the test chambers.

### 2.4. Test compounds and concentrations

Anthracene (MW 178.23; Aldrich Chemical Co.; 98% pure) and fluoranthene (MW 202.26; Aldrich Chemical Co.; 98% pure) used in the experiments were dissolved in HPLC grade acetone to make a stock solution. Water was spiked with PAHs via the shell coating method (Newsted and Giesy, 1987). In sediment tests, 33% of the overlying water was replaced daily with diluted spring water; in water-only tests, 50% of the spiked water was renewed daily. In the behavior experiments, the no-substrate, sand monolayer, and leaf substrate treatments all used water-borne fluoranthene.

Single-compound exposures were first conducted to calculate LC₅₀ values for use in determining the concentrations to be used in the mixture exposures. Nominal concentrations used to calculate LC₅₀ for photo-induced toxicity in water-only exposures were as follows: anthracene, 0, 2.20, 4.40, 8.75, 17.50, and 35.00 µg/l; fluoranthene, 0, 6.25, 12.50, 25.00, 50.00, and 100.00 µg/l. Sediment was spiked for testing at the following concentrations for anthracene and fluoranthene individually: 0, 100, 500, 1000, 2500, and 5000 µg/kg sediment.

LC₅₀ values from the UV studies were used to determine the mixture values, conducted with UV. Concentrations used in mixture studies were determined based on these LC₅₀ results and the concept of Toxic Units (Newman, 1995). Following the methods outlined in Newman (1995), mixtures were as follows for each organism in each sediment and in water: mix A = 0.25
2.5. Water quality

Spring water (from a spring located at Howell’s Orchard in Dayton, OH, USA) diluted with Nanopure® water to a hardness of approximately 165–175 mg CaCO₃/l was used in all tests. Water quality in the exposures was determined as described in Standard Methods (APHA, 1995). Dissolved oxygen (DO) and temperature were recorded daily. Conductivity, pH, alkalinity, and hardness were measured at test initiation and completion. DO was measured with a YSI Model 57 probe, and conductivity was measured with a YSI Model 335-C-T probe. Phenolphthalein alkalinity was measured by titration with sulfuric acid, and hardness was measured by titration with EDTA (APHA, 1995).

2.6. Chemical analysis

Water and sediment samples were prepared for chemical analysis by gas chromatography with flame ionization detection (GC-FID) (Carlo-Erba 6000 Vega series; Italy). Water samples (250 ml) spiked with PAH were extracted using packed C-18 columns (J.T. Baker, Paris, KY, USA). The columns were then eluted with 2 ml HPLC grade methanol. Samples were extracted within 24 h of collection and the extracts were stored for no longer than 1 month prior to gas chromatography (GC) analysis. Immediately prior to GC analysis, extracts were filtered through a 0.45 µm syringe and an internal standard (2-fluorobiphenyl) was added to the sample. Sediment was extracted in a Soxhlet apparatus using a 3:1 mixture of acetone and hexane. The sample was then dried and concentrated prior to analysis (Ireland et al., 1996). Concentrations were measured in overlying water at the beginning and at the termination of the sediment tests. The detection limit obtained using these methods is one part per billion fluoranthene or anthracene.

Samples prepared for GC analysis as described above were measured using GC-FID via splitless injection and using a nonpolar SE*-54 column (Ireland et al., 1996). Concentrations were calculated via linear regression using a standard curve consisting of five concentrations. Samples were analyzed at test initiation and termination. The highest and lowest concentrations were analyzed. Blank water samples were extracted and analyzed at least monthly during analysis. Blank water samples controlled for interfering substances in the exposure water and sediment. Each sample was injected twice, and the average peak ratio of the duplicate injections was used in calculating the concentration.

2.7. Behavioral observations

During the experiments that monitored behavior, behavioral observations were recorded three times per day. Behavioral observations were limited to the following: *H. azteca*, *C. tentans* in no substrate: clumped or not clumped; *H. azteca*, *C. tentans* in leaf substrate: under leaves or not under leaves; *H. azteca* in sand, hiding in sand or not; *C. tentans* in sand, making a case in sand or not; *H. azteca*, *C. tentans* in sediment: burrowing or visible on top. At each observation time, the number of organisms behaving in a particular way was recorded for each replicate of each treatment. For each type of behavior, the number of test organisms per beaker (replicate) that exhibited that behavior was counted and recorded. Clumping was defined as being grouped with the other test organisms (for *C. tentans*, in an intertwined fashion; for *H. azteca*, in an aggregated fashion), and all organisms so distributed were considered to be clumped. For simplicity, we will use the term ‘clumping’ throughout the discussion of our results to refer to aggregating in a group, whether exhibited by *H. azteca* or *C. tentans*.

2.8. Statistical analysis

We obtained LC₅₀s for anthracene and fluoranthene in sediment and water for *H. azteca* and *C. tentans* using Probit analysis (Probit version 1.5, US EPA, Cincinnati, OH, USA). Analysis of variance (ANOVA) procedures were used to test for any significant differences in survival among the mixture treatments or among the non-UV treatments in both the single-compound and mixture experiments (Toxstat version 3.0, University of Wyoming, Laramie, WY, USA). ANOVA procedures were also used to test for any significant differences in growth among treatment groups in both the single-compound and mixture experiments. In the event that data did not meet the assumptions of normality and homogeneity of variance, non-parametric tests were used to test for significant effects among the treatment groups. ANOVA procedures were also used to compare the UV to the non-UV control in all exposures. Statistical significance was established at \( p < 0.05 \).

Prior to ANOVA, the Shapiro–Wilks test was used to check that data were normally distributed. Bartlett’s test for homogeneity of variance was applied to data sets that met the assumption of normality. When data met the assumptions of normality and homogeneity of variance, ANOVA followed by the Dunnet’s, Bonferroni’s, or Tukey’s tests were used to determine which treatments were significantly different from the others. To test for significant effects when data did not meet the assumptions of normality or homogeneity of variance, the William’s test or the Wilcoxon rank-sum test was used.
3. Results

3.1. Water quality

During the experiments, water temperature ranged from 21 to 24°C, and did not vary more than 2°C in any given 10-day experiment. Dissolved oxygen was maintained at or above 80% saturation by aeration when necessary. UV-A intensity ranged from 30 to 65 μW/cm², and UV-B ranged from 0.5 to 5 μW/cm². UV-A intensity varied no more than 14 μW/cm² in any one experiment. pH ranged from 7.79 to 8.88; hardness ranged from 140 to 170 mg CaCO₃/l in water-only tests and from 166 to 203 mg CaCO₃/l in sediment tests. Alkalinity ranged from 108 to 168 mg CaCO₃/l in water-only tests and from 108 to 210 mg CaCO₃/l in sediment tests. Conductivity ranged from 240 to 360 μmhos/cm.

3.2. Chemical analysis

Water samples analyzed resulted in 80–100% recovery of nominal levels of PAHs. Sediment analysis recovered 40–100% of the nominal value. Concentrations of PAHs in overlying water of the spiked sediment tests ranged from non-detectable to 5–7 μg/l in the anthracene 500 and 1000 μg/kg treatments to 12–14 μg/l in the C. tentans sediment mixture exposures. Blank sediment samples indicated no interfering substances in the control sediment or in the exposure water.

3.3. Survival

LC₅₀ values for H. azteca in water and sediment exposures for both anthracene and fluoranthene, and LC₅₀ values for C. tentans in water exposures for both PAHs are presented in Table 1. We used nominal concentration values to calculate the LC₅₀ values. LC₅₀ values for C. tentans sediment exposures were not calculable because sufficient mortality did not occur in these exposures. H. azteca thus appeared to be more resistant to toxic effects in the sediment than C. tentans. In the water-only exposures, LC₅₀ values of C. tentans were higher than for H. azteca (Table 1). In the H. azteca water-only exposures, the LC₅₀ for fluoranthene was slightly higher than the LC₅₀ for anthracene. In the H. azteca sediment exposures, the LC₅₀ for the two test compounds were similar.

Survival of H. azteca in the water exposures with UV was the only statistically significant endpoint in the mixture exposures (Fig. 1). H. azteca survival differed significantly from the controls in all three mixtures exposed to UV (Dunnett’s test). The survival of H. azteca in the water exposures with UV also suggested additivity of the photo-induced toxicity of anthracene and fluoranthene; survival in the theoretically equitoxic mixtures was approximately 50%. In the C. tentans water-only exposure under UV, mixtures B and C were significantly different from the control, while mixture A was not statistically different from the control (Dunnett’s test) (Fig. 2). Survival in all PAH treatments differed significantly from the control in all but the C. tentans sediment mixtures.

Although not statistically significant, H. azteca mortality was observed in the two highest fluoranthene treatments (50 and 100 μg/l) without UV. This result was similar to the results of no-UV exposures of aquatic invertebrates to fluoranthene. In the development of water quality criteria for fluoranthene, the US EPA uses a genus mean acute value for amphipods of approximately 60 μg fluoranthene/l (US EPA, 1993). H. azteca survival was reduced to 60% (±5%) at 50 μg fluoranthene/l without UV and reduced to 40% (±8.2%) at 100 μg/l without UV. In comparison, in the UV treatments, mortality of H. azteca at 50 and 100 μg fluoranthene/l was 100% by day 4 of the 10-day study.
Fig. 1. *Hyalella azteca* survival in mixtures of anthracene and fluoranthene in water and sediment. Error bars represent standard deviation. *Treatments statistically significantly different from the control treatment. Mix A = 0.25 (LC$_{50}$ anthracene) + 0.75 (LC$_{50}$ fluoranthene); Mix B = 0.50 (LC$_{50}$ anthracene) + 0.50 (LC$_{50}$ fluoranthene); Mix C = 0.75 (LC$_{50}$ anthracene) + 0.25 (LC$_{50}$ fluoranthene). See text for further explanation of mixtures.

Fig. 2. *Chironomus tentans* survival in mixtures of anthracene and fluoranthene in water and sediment. Error bars represent standard deviation. *Treatments statistically significantly different from the control treatment. Mix A = 0.25 (LC$_{50}$ anthracene) + 0.75 (LC$_{50}$ fluoranthene); Mix B = 0.50 (LC$_{50}$ anthracene) + 0.50 (LC$_{50}$ fluoranthene); Mix C = 0.75 (LC$_{50}$ anthracene) + 0.25 (LC$_{50}$ fluoranthene). See text for further explanation of mixtures.
3.4. Growth

Growth did not provide a statistically significant indication of toxicity in these experiments. Although not statistically significant, a trend was observed in that *H. azteca* growth in spiked sediment exposures was less in all UV exposures than in the non-UV exposures. *H. azteca* growth ranged from 0.03 to 0.21 mg per individual per 10-day exposure (SD = 0.13). *C. tentans* growth ranged from 0.05 to 0.38 mg per individual per 10-day exposure period (SD = 0.18).

3.5. Behavioral observations

Observations of behavior in treatments with different substrates illustrate the possible effect of behavior on survival, particularly in the case of *H. azteca* (Figs. 3–6). Overall, behavior did not differ over time, or from day to day. No significant behavioral responses were observed in sediment or sand monolayer treatments for either of the test organisms.

The behavior and survival of *H. azteca* were influenced by the different UV, fluoranthene, and substrate treatments (Figs. 3–6). *H. azteca* spent more time under leaves than not under leaves at concentrations of 6, 12.5, and 25 μg fluoranthene/l under UV, in comparison to the controls and in comparison to no-UV exposures (Dunnet’s test) (Figs. 5, 6). *H. azteca* clumping in UV treatments (6.25, 12.5, 25 μg/l) differed from non-UV treatments and from UV controls; no individuals clumped in the no-UV treatments, and very few individuals (5%) clumped in the UV control (Dunnet’s test) (Figs. 5, 6).

There was no significant difference in *H. azteca* survival in the UV and non-UV controls with leaves; similarly, there was no difference in survival in no-substrate controls compared to leaf substrate control treatments. Survival in the no-UV treatments did not differ significantly between the different substrate treatments. Survival in the UV treatments 6.25 μg fluoranthene/l—no substrate, 25 μg fluoranthene/l—no substrate, and 25 μg fluoranthene/l with leaves was significantly less than the control treatment (Bonferroni’s test).

Analysis of the *C. tentans* behavioral data in treatments without substrate revealed no difference between the UV and non-UV controls in survival or behavior. Comparing *C. tentans* behavior revealed that animals in the 12.5 μg fluoranthene/l and 25 μg fluoranthene/l with UV treatments clumped together significantly more than animals in control and in no-UV treatments (Tukey’s test). When comparing *C. tentans* survival in the fluoranthene treatments to survival of the control, the 12 μg fluoranthene/l without UV and 25 μg fluoranthene/l with UV were significantly different (Bonferroni’s test).

4. Discussion

Low doses of both anthracene and fluoranthene resulted in greatly decreased survival when UV was simultaneously present. *C. tentans* revealed no effects in
Fig. 4. *Hyalella azteca* survival when exposed to flouranthene without UV light with no substrate or leaves as substrate. Error bars represent standard deviation.

Fig. 5. Behavioral responses of *Hyalella azteca* exposed to flouranthene and UV light. Error bars represent standard deviation. *Treatments statistically significantly different from the control.*
the non-UV treatments, while *H. azteca* demonstrated slightly reduced survival in only the two highest concentrations in water exposures without UV. *H. azteca* appeared to be more sensitive than *C. tentans* to the phototoxic effects of anthracene and fluoranthene than *C. tentans*; the LC$_{50}$ for *H. azteca* was consistently lower than the LC$_{50}$ for *C. tentans*. Observations of the behavior of these test organisms suggest that *H. azteca* may modify its behavior in response to the presence of phototoxic PAHs and UV light. Our study can not rule out other factors that may contribute to the different responses of the two test species, such as differences in metabolism between the two species, resistant integument or pigment, or differences in bioaccumulation of the compounds of interest (Landrum and Scavia, 1983; Ankley et al., 1994). Tissue residue data from each assay would have enhanced the predictive value and relationships of this study. Although we did not have the resources for such analysis at the time of these experiments, this approach could be useful in future work.

Results of the water-only exposures were similar to previously published studies. Suedel and Rodgers (1996) reported a 10-day LC$_{50}$ for fluoranthene in water to *H. azteca*, without UV, to be 30.3 µg/l. For *C. tentans*, a similar value was reported to be 37.8 µg/l (Suedel and Rodgers, 1996). These LC$_{50}$ values are lower than our observations of fluoranthene toxicity without UV; we found reduced *H. azteca* survival at 50 and 100 µg/l, but no effects on *C. tentans* without UV. Suedel and Rodgers (1996) found *C. tentans* survival to be the most sensitive endpoint in the sediment test, with a 10-day LC$_{50}$ of 23.6 µg fluoranthene/l in overlying water without UV. A similar value for *H. azteca* was 60.6 µg/l in overlying water without UV (Suedel and Rodgers, 1996). Another 10-day exposure of *H. azteca* and *C. tentans* to fluoranthene-amended sediment without UV resulted in EC$_{50}$ of approximately 500–1480 mg/kg OC for *H. azteca*, and 682–1740 mg/kg OC for *C. tentans*, depending on the source of the sediment (Suedel and Rodgers, 1993). In comparison, our results suggest an LC$_{50}$ for *H. azteca* of approximately 829 µg/g OC for fluoranthene with UV light (Table 1).

Although not statistically significant, the PAH mixture that was higher in anthracene than in fluoranthene concentration (Mix C; 0.75 LC$_{50}$ of anthracene + 0.25 LC$_{50}$ of fluoranthene) was slightly more toxic than the other two mixtures tested (Figs. 1, 2). This effect might be explained by the higher bioconcentration factor of anthracene in the organism in comparison to fluoranthene (Newsted and Giesy, 1987). The difference in bioconcentration factor could have resulted in a higher molar concentration of anthracene within the organism in comparison to fluoranthene (Newsted and Giesy, 1987).

Sediment exposures did not provide definitive evidence of additivity of the compounds of interest. The 10-day LC$_{50}$ for *H. azteca* exposed in sediment spiked with anthracene was 3332 µg/kg and the LC$_{50}$ for exposure to sediment spiked with fluoranthene was 3248 µg fluoranthene/kg sediment. These estimates are less than previous studies that have investigated fluoranthene-spiked sediment without UV, but similar to the US EPA, which reported an LC$_{50}$ for fluoranthene to the freshwater amphipod to be approximately 3000 µg/kg dry sediment (US EPA, 1994). Other studies...
have reported significant effects on Chironomus riparius emergence at sediment concentrations of 80 mg/kg, without UV (Stewart and Thompson, 1995). Our low recovery in sediment exposures may confound our conclusions, suggesting that our spiking procedure may not have been adequate. However, other studies suggest that spiking method does not affect availability of fluoranthene (Stewart and Thompson, 1995).

Growth was not a sensitive indicator of effects in our study. This contrasts with other studies in which growth was a more sensitive indicator of toxic effects than survival (Ingersoll et al., 1995; Burton et al., 1996). The lack of significant effects on growth could be partially due to the need for greater replicates in order to detect significant effects; eight replicates per treatment in toxicity tests has been recommended as a standard number (Ingersoll et al., 1995). Growth rates of H. azteca and C. tentans were less than those reported in some other studies of feeding behavior and growth, perhaps due to differences in the type or amount of food provided (Liber et al., 1996; Moore and Farrar, 1996). Other studies similarly found no significant effects on growth of test organisms exposed to photo-induced toxicity of PAHs in sediments (Ankley et al., 1994).

Results suggest that in the presence of refugia the effects of photo-induced toxicity on the test organism H. azteca were significantly attenuated (Figs. 3, 4). H. azteca exhibited a behavioral response specific to its epibenthic life habits. When leaves were provided, survival of H. azteca was significantly increased in comparison to treatments in which no refuge from UV light was provided (Figs. 5, 6). Similarly, when refuge was provided H. azteca spent more time hiding in the UV-PAH treatments (Figs. 5, 6). It is possible that the presence of organic matter (i.e. the leaves or the sediment) could have affected the PAH availability. However, measurements of the PAH levels in the water were similar in all treatments. Further, measurements of behavior indicated that H. azteca spent more time seeking refuge under UV in the higher concentrations of fluoranthene than in the lower concentrations of fluoranthene. The different substrate treatments did not significantly affect growth of the test organisms.

The clumping behavior of H. azteca and C. tentans in the UV-no substrate treatments may be a reaction to stress rather than a behavioral adaptation. Clumping is a response exhibited by C. tentans in response to stress. Other than this clumping, C. tentans did not exhibit any significant behavioral adaptation to the stress of photo-induced toxicity of fluoranthene. Baker and Ball (1995) studied the effect of light on C. tentans predator avoidance behavior and found that light level did not affect the positioning of C. tentans that began experimental exposure with tubes; however, when organisms began exposure without tubes, they chose dark areas to avoid predation. In this study, the only behavioral response that was significant for C. tentans to avoid predation was hiding in tubes (Baker and Ball, 1995). However, Baker and Ball (1995) were interested in comparing light and dark effects on C. tentans, while the goal in our experiments was to compare UV treatments with treatments under ambient fluorescent laboratory lighting. Other researchers working with cadmium-contaminated sediments and Chironomus spp. concluded that avoidance behavior did not affect toxicity; organisms did not actively avoid contaminated sediments (Hare and Shooner, 1995).

Our observations suggest that the photo-induced toxicity of anthracene and fluoranthene is due to sensitization of PAHs occurring within biological tissue. Toxicity occurs when UV, PAH and organism are exposed simultaneously (Bowling et al., 1983; Landrum et al., 1987). Thus, attenuation of the photo-induced toxicity of fluoranthene to H. azteca occurred when refuge was available. Overall, our study demonstrates quantitatively that substrate as refugia will attenuate the photo-induced toxicity of PAHs for some epibenthic organisms such as amphipods. These behavioral effects combine with other factors, such as turbidity, to affect exposure in the field (Bowling et al., 1983; Oris et al., 1990; Ireland et al., 1996).

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References


