Weinstein 2002

ELSEVIER

Aquatic Toxicology 59 (2002) 153–161

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Photoperiod effects on the UV-induced toxicity of fluoranthene to freshwater mussel glochidia: absence of repair during dark periods

John E. Weinstein *

Department of Biology, The Citadel, Charleston, SC 29409, USA

Received 18 June 2001; received in revised form 19 September 2001; accepted 24 September 2001

Abstract

The effect of photoperiod on the ultraviolet radiation (UV)-induced toxicity of fluoranthene to glochidia of the freshwater mussel, *Utterbackia imbecillis*, was evaluated using a series of static renewal toxicity tests conducted using one of four different photoperiods (24 h light, 16 h light:8 h dark, 12 h light:12 h dark, 8 h light:16 h dark). Rates of acute mortality were dependent both upon fluoranthene dose and the photoperiod. Median lethal time (LT50) values calculated on the basis of accumulated UV exposure time (UV-LT50) were compared with LT50 values calculated from real time of exposure (R-LT50) to determine relative rates of photoactivated fluoranthene damage versus physiologic repair during periods of darkness. UV-LT50 values were only dependent on fluoranthene dose and not on photoperiod. The fact that UV-LT50 values did not increase with decreasing light cycle length indicates that physiologic repair during dark periods was not an important process in these experiments. These findings suggest that (1) species-specific and/or life history-specific factors, in part, determine the ability of an organism to repair photoactivated polycyclic aromatic hydrocarbon (PAH) damage during dark periods; and (2) predictions of the UV-induced toxic response of PAH in glochidia need only be based upon total UV dose (dose rate and duration) and PAH dose.

Keywords: Photoinduced toxicity; Freshwater mussels; Glochidia; Polycyclic aromatic hydrocarbons; Ultraviolet radiation

1. Introduction

Native freshwater mussels (Bivalvia; Unionidae) are among the most imperiled fauna in North America (Bogan, 1993; Williams et al., 1993). During the past 40 years, numbers of both individuals and species diversity of native mussels have precipitously declined. The natural habitats of freshwater mussels, including rivers and streams, are common recipients of stormwater runoff and municipal/industrial wastewaters. Both runoff and wastewater can contain a variety of potentially toxic inorganic and organic contaminants, which could be contributing to the decline of mussels. One such group of potentially toxic organic contaminants are the polycyclic aromatic hydrocarbons (PAHs). PAHs represent a major

* Tel.: +1-843-953-7796; fax: +1-843-953-7264
E-mail address: john.weinstein@citadel.edu (J.E. Weinstein).

0166-445X/02/$ - see front matter
PII: S0166-445X(01)00247-8

class of environmental contaminant that are common components of petroleum products and combustion products of fossil fuels. Although the majority of PAHs are relatively non-toxic in acute toxicity tests (Veith et al., 1983), an important subset of PAHs, including fluoranthene, are several orders of magnitude more toxic in the presence of ultraviolet (UV) wavelengths of natural sunlight. This UV-induced PAH toxicity occurs in a wide variety of aquatic organisms, including fish (Oris and Giesy, 1985, 1987), amphibians (Walker et al., 1998; Monson et al., 1999), oligochaetes (Ankley et al., 1995; Erickson et al., 1999), and cladocerans (Newsted and Giesy, 1987; Mekenyan et al., 1994). Recent studies involving the larval stages (glochidia) of freshwater mussels have revealed that they are among the most sensitive organisms tested to date to UV-induced PAH toxicity (Weinstein, 2001; Weinstein and Polk, 2001). In the absence of UV, PAHs at their water solubility limits were not acutely toxic to glochidia in these studies.

The UV-induced toxicity of PAHs has been attributed to the formation of photoactivated PAH molecules in biological tissue (Bowling et al., 1983; Allred and Giesy, 1985). Photoactivated PAH molecules, in the form of excited singlet- and triplet-state molecules, are thought to be the result of the ability of the parent molecule to absorb energy in the UV spectrum of natural and simulated sunlight (Newsted and Giesy, 1987). The energy associated with these photoactivated molecules is then transferred, through non-radiative pathways, to oxygen, generating reactive oxygen species (ROS), such as singlet oxygen and superoxide. ROS are potent oxidizing agents, and their generation in biological tissue is known to cause oxidative stress and subsequent toxicity (Ito, 1978). Recent studies involving fish gills (McCloskey and Oris, 1993; Weinstein et al., 1997) and PLHC-1 cells (Choi and Oris, 2000a) coexposed to PAH and UV radiation have supported the notion that photoactivated PAH generate ROS resulting in oxidative stress. These studies have also demonstrated that the mode of toxic action is primarily cell membrane disruption via lipid peroxidation.

Assessment of the hazard of photoactivated PAHs to aquatic organisms has generally been based upon the realization that this toxicity is controlled by two interacting factors, chemical dose of the PAH and the UV 'dose' (i.e. time-dependent photon intensity) available to interact with the PAH (Landrum et al., 1985; Oris and Giesy, 1985; Ankley et al., 1995). According to the Bunsen–Roscoe photochemical law of reciprocity, phototoxic potential of a PAH should be directly proportional to both the factors in the absence of biological defense and repair mechanisms (Dworkin, 1958). This relationship has been used to form the basis for the development of predictive models describing the time-dependent mortality in several species, including the oligochaete (*Lumbriculus variegates*) (Ankley et al., 1995) and tadpoles of the northern leopard frog (*Rana pipiens*) (Monson et al., 1999). However, these models may not have accurately predicted the hazard of these compounds in the environment since they are based upon experiments using continuous (24-h) UV exposures. Overall responses of an organism to photoactivated PAHs are not only determined by the rate of damage caused by the photoactivated PAH during UV exposures, but also by the rate at which the organism repairs the damage during dark periods. Results from experiments with fish using more environmentally-relevant photoperiods (e.g. 12 h light:12 h dark) have demonstrated that significant repair occurs during the dark periods, resulting in a delay of, and in some cases, an absence of overt toxic responses from the organism (Oris and Giesy, 1986). Due to the fact that these previously derived models did not account for repair processes occurring during dark periods, they may have overestimated the hazard of UV-induced PAH toxicity to these organisms.

Predictive models for the UV-induced toxicity of fluoranthene, anthracene, and pyrene have recently been developed for glochidia of the freshwater mussel, *Utterbackia imbecillis* (Weinstein, 2001; Weinstein and Polk, 2001). These models have also been based upon the Bunsen–Roscoe law of reciprocity using experiments with continuous 24-h UV exposures, and, therefore, they too may have overestimated the hazard of these com-

pounds. In order to assess the importance of physiologic repair during dark periods and refine these previously developed models for glochidia, the effect of daily light cycle duration (photoperiod) on the UV-induced toxicity of fluoranthene was examined. The approach taken in this study was to examine two types of median lethal times (LT50). One LT50 value was derived from real time exposures (R-LT50), which included the time of exposure in both periods of UV exposure and darkness. The other LT50 value was derived from the time of exposure to UV only (UV-LT50).

2. Materials and methods

2.1. Test organism

The freshwater mussel used in these experiments was the pond papershell, *U. imbecillis*. This species has a widespread distribution throughout the eastern United States, and its distribution and abundance are currently considered stable (Williams et al., 1993). Adult mussels were obtained from wild populations located in a pond in rural Scatter Branch, Hunt County, TX, USA. This pond receives no urban stormwater runoff, and presumably, the only source of PAHs into the pond is atmospheric deposition. Mussels were collected by hand, transported to a wet laboratory at Texas A&M University-Commerce (Commerce, TX), and maintained in a flow-through system with a water temperature of 20 ± 2 °C and a photoperiod of 14 h light:10 h dark.

Techniques for the collection of mature *U. imbecillis* glochidia have previously been reported (Johnson et al., 1993). Briefly, the maturity of the glochidia within the gills of brooding females was deduced by color. Gills containing mature glochidia are enlarged and brown in color, whereas enlarged beige or white gills contain immature glochidia. Mature glochidia were collected by excising the gills containing glochidia and manually shaking them in water. Glochidia were then allowed to settle to the bottom of the container, and the supernatant was removed. Mature glochidia settled to the bottom, whereas the immature ones remain in supernatant. This washing process was repeated at least three times to remove debris and immature glochidia. The remaining mature glochidia were then used immediately in the experiments.

2.2. Test compound

Saturated aqueous solutions of fluoranthene (molecular weight [MW] = 202.26, 98% purity, Aldrich Chemical Company, Milwaukee, WI, USA) were obtained by introducing 0.4 g of the neat chemical to 4 l of moderately hard reconstituted water (Lewis et al., 1994) in a separate amber glass container and mixing it at room temperature until steady state conditions were achieved. Fluoranthene-contaminated water was removed from their respective containers for testing by filtration through glass wool twice, sampled for HPLC analysis, then diluted to the appropriate concentrations prior to being used in the experiments. Aqueous solubility of fluoranthene at 22 °C was 135.6 ± 9.1 μg/l. Based upon the previously determined 24-h LC50 of 2.45 ± 0.45 μg fluoranthene per liter for glochidia exposed to constant UV exposure (Weinstein, 2001), the following nominal concentrations were chosen for the present study, 0, 1.5, 3, 6, and 12 μg/l.

2.3. Lights and light measurement

Ultraviolet and visible light was provided in the laboratory using a bank of fluorescent bulbs designed to simulate natural sunlight (Vita-Lite, Duro-test, Bloomfield, NJ, USA). These lamps have a spectral distribution very similar to that of natural sunlight (Weinstein and Oris, 1999). UV-A (320–400 nm) was quantified using a Macam Photometrics (Livingstone, Scotland) Model UV-202 radiometer. Light measurements were taken at least once daily during all experiments. Using this instrument, a UV-A intensity of 4,038 μW/cm^2 was measured under clear (sunny) conditions in Commerce, TX, USA (33°15′N, 95°54′W) on July 14, 1999 at solar noon (13:00 h Central Daylight Time).

2.4. Acute toxicity tests

Effect of photoperiod on fluoranthene phototoxicity to glochidia was evaluated using a series of four 96-h static renewal toxicity tests conducted at four different photoperiods (24 h light, 16 h light:8 h dark, 12 h light:12 h dark, 8 h light:16 h dark). Toxicity tests involving the 12 h light:12 h dark and 8 h light:16 h dark photoperiods were extended to 130 and 160 h, respectively, due to the low mortality that occurred during the initial 96-h period. Intensity of UV-A in these experiments averaged 49.5 ± 1.0 $\mu W/cm^2$ during light periods and less than 2.0 $\mu W/cm^2$ during dark periods. These light period intensities are similar to a summertime depth of approximately 1 m in a typical north-temperate eutrophic system (Oris and Giesy, 1985). Moderately hard, reconstituted water was used as the diluent. This was prepared by adding $MgSO_4$, KCl, $NaHCO_3$, and $CaSO_4$ to deionized water following US Environmental Protection Agency methods (Lewis et al., 1994). The quality of water in these tests was as follows, temperature, 23.0 ± 0.9 °C; dissolved oxygen, 8.60 ± 1.98 mg/l; pH 7.97 ± 0.03; total hardness, 70.5 ± 5.7 mg $CaCO_3$ per l; alkalinity, 52.5 ± 3.4 mg $CaCO_3$ per l; conductivity, 289.3 ± 10.3 $\mu S/cm$; ammonia, < 0.1 mg NH_3 per l.

Mature glochidia were harvested from three adult mussels, pooled together, and immediately distributed to test chambers (50-ml glass beakers) containing ~ 30 ml of test solution. For each experiment, three sets of three replicates were used in each of the four test concentrations and the control to enumerate viable glochidia. One set of replicates were used to enumerate viable glochidia at each time period, then discarded. An additional set of five replicates was used for each test concentration and control for determining fluoranthene tissue residues. All test chambers (total, 70 per experiment) contained between 20 and 30 glochidia. Glochidia were given an initial 4-h uptake period under ambient laboratory lighting (UV-A ≤ 2.0 $\mu W/cm^2$) in order to achieve a steady-state internal dose of PAH (Weinstein, 2001). Following this 4-h uptake period, glochidia in the additional set of five replicates were sampled and analyzed for tissue residues ($n = 5$).

Glochidia in the remaining sets of replicates were then exposed simultaneously to UV radiation and fluoranthene. Previous studies have demonstrated that the initial adverse response affecting the viability of glochidia in PAH phototoxicity tests is valve closure (Weinstein, 2001). Thus, observations of valve closure were made throughout the test period; however, actual counts using the addition of NaCl to enumerate viable glochidia were restricted to three occasions. These counts were then used to determine LT50 values for each treatment. Test solutions were renewed every 8 h by gently pouring off ~ 20 ml of solution from the test chamber and replacing it with fresh solution.

2.5. Endpoint determination

Viability of glochidia (endpoint for LT50) was determined based on the ability of the glochidia to close immediately when a noxious stimulus (NaCl) was added to the test chamber (Huebner and Pynnönen, 1992; Keller and Ruessler, 1997). The ability to close tightly is necessary for the glochidia to attach to the fish host on which they develop and metamorphose. Individuals unable to demonstrate this response because they were too weak or already closed would be unable to attach to a fish host and complete development.

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

2.6. Analytical

Bioaccumulation of fluoranthene in glochidia was determined for all fluoranthene treatments following the 4 h uptake period. Glochidia (~ 30 individuals per sample) were placed on aluminum

foil, rinsed with deionized water, allowed to air dry at room temperature in the dark for 2 h, and weighed. Fluoranthene was immediately extracted from glochidia by grinding them in a Ten Broeck tissue homogenizer with 1 ml cyclohexane:acetone (2:1) and quantified using reverse phase HPLC. Fifty microliters of sample or standard solution were injected directly onto a Waters 3.9 mm × 15 cm μBondapak C-18 column. An isocratic elution was performed with acetonitrile:water (9:1) at 1.0 ml/min. A Waters 474 Scanning Fluorescence Detector was used at an excitation wavelength of 360 nm with emission detection set at 460 nm. Peaks were recorded and quantified using Waters Millennium[32] Chromatography Manager software. The limit of detection for fluoranthene was 0.1 μg/l, or 5.0 pg total mass. The recovery of fluoranthene in spiked tissue samples was 118.1 ± 8.1% ($n = 4$). All fluoranthene tissue residues are reported on a dry weight basis.

Fluoranthene concentrations in water samples were analyzed directly with no extraction or concentration using reverse phase HPLC in a manner similar to that described above. Water concentrations were determined at the beginning of each toxicity test and at least once more for initial and 8 h test solution concentrations during the test period. Fluoranthene water concentrations are reported as the geometric mean between the measured concentrations in the initial and 8 h solutions.

2.7. Statistical analysis

Median lethal times (LT50) were calculated from the recorded time-mortality data. Two different types of LT50 estimates were derived for each fluoranthene treatment for each photoperiod (cf. Oris and Giesy, 1986). One LT50 value was calculated based upon real time exposure (R-LT50). This value included the time of fluoranthene exposure in both periods of UV exposure and periods of darkness. For each R-LT50 value, another LT50 value was calculated based solely upon accumulated UV exposure time (UV-LT50). For example, glochidia exposed to fluoranthene for 24 h in the 24 h light photoperiod accumulates 24 h of UV exposure, whereas glochidia exposed for 24 h in the 12 h light:12 dark photoperiod only accumulates 12 h of UV exposure. Probit analysis was used to determine both R-LT50 values and UV-LT50 values, and their associated 95% confidence intervals. These analyses were performed using SAS for Windows (version 6.12, Cary, NC, USA) (SAS Institute, 1990). Bioconcentration factors (BCFs) were calculated by dividing the 4-h fluoranthene tissue residues (μg/g dry weight) by the initial fluoranthene water concentrations (μg/ml). All values are reported as mean ± S.D., unless otherwise noted.

3. Results

Five concentrations of fluoranthene (0, 1.0, 2.1, 4.0, and 7.8 μg/l) were achieved. Mortality in these toxicity tests was < 7.5% in all treatments prior to UV exposure, and in all 0-μg fluoranthene per liter treatments under UV. Initial tissue residues of fluoranthene in glochidia were below the limit of detection, 2.06 ± 0.21, 3.92 ± 0.37, 6.82 ± 0.95, and 12.86 ± 3.13 μg/g dry weight in the 0, 1.0, 2.1, 4.0, and 7.8-μg/l treatments, respectively. The BCF across all treatments was 2147 ± 247.

The rate of mortality in terms of real time exposure was a function of both fluoranthene tissue residues and daily light cycle length (Fig. 1). In general, R-LT50 values increased with decreasing light cycle length. For example, at the longest

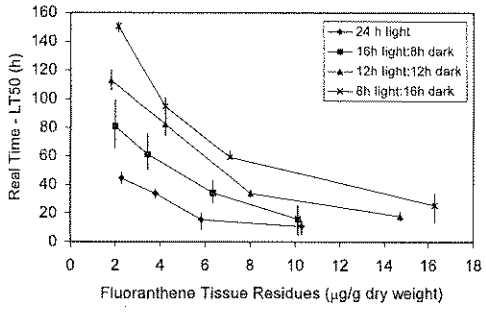

Fig. 1. Relationship between real-time median lethal time (R-LT50) and fluoranthene tissue residues for (hours light:hours dark) 24:0, 16:8, 12:12, 8:16 photoperiods. Error bars indicate 95% confidence limits.

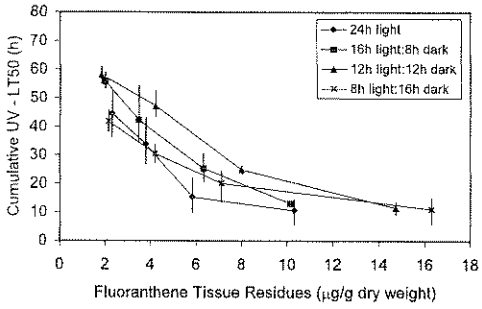

Fig. 2. Relationship between median lethal times calculated based on cumulative light exposures (UV-LT50) and fluoranthene tissue residues for (hours light:hours dark) 24:0, 16:8, 12:12, 8:16 photoperiods. Error bars indicate 95% confidence limits.

photoperiod (24 h light), R-LT50 values ranged from 44.5 h in those glochidia with a mean fluoranthene residue of 2.3 ± 0.6 μg/g to 10.6 h in those glochidia with a mean fluoranthene residue of 10.3 ± 1.7 μg/g. At the shortest photoperiod (8 h light:16 h darkness), R-LT50 values ranged from 150.4 h in those glochidia with a mean fluoranthene residue of 2.1 ± 0.3 μg/g to 25.5 h in those glochidia with a mean fluoranthene residue of 16.3 ± 1.2 μg/g.

In contrast, the rate of mortality in terms of accumulated UV exposure time was only dependent upon fluoranthene tissue residue and not on daily light cycle length (Fig. 2). For example, at the longest photoperiod (24 h light), UV-LT50 values ranged from 44.5 h in those glochidia with a mean fluoranthene residue of 2.3 ± 0.6 μg/g to 10.6 h in those glochidia with a mean fluoranthene residue of 10.3 ± 1.7 μg/g. At the shortest photoperiod (8 h light:16 h darkness), UV-LT50 values ranged from 41.7 h in those glochidia with a mean fluoranthene residue of 2.1 ± 0.3 μg/g to 11.1 h in those glochidia with a mean fluoranthene residue of 16.3 ± 1.2 μg/g.

4. Discussion

The major objective of this study was to determine the effect that photoperiod has on the UV-induced toxicity of fluoranthene in glochidia in order to assess the extent to which physiologic repair occurs during dark periods. Assessment of physiologic repair can be achieved by examination of the different LT50 types (cf. Oris and Giesy, 1986). R-LT50 can be considered to be an integrated measure of the difference between the rate of damage and the rate of repair. UV-LT50 can be considered to be a measure of the rate of damage for cumulative UV exposure, assuming that little or no net repair occurs during the UV exposure periods. If damage occurs only during the light cycle, and there is little or no repair during the dark cycle, then damage would be expected to accumulate as a direct function of the total amount of UV exposure, regardless of the duration of the dark periods. Therefore, if the damage is cumulative during the light cycles, then UV-LT50 values for a given fluoranthene dose should all be of the same magnitude, independent of photoperiod. In contrast, if significant levels of repair occur during dark periods, in the absence of little or no net cumulative damage during these same dark periods, then UV-LT50 values for a given fluoranthene dose should increase with increasing length of dark periods.

The results obtained in this study suggest that the UV-induced fluoranthene toxicity to glochidia involves only cumulative damage during the light periods. There was no evidence of repair in these studies during the dark periods. UV-LT50 values did not increase with decreasing light period duration, which would be expected if significant levels of repair were occurring during the dark periods. The fact that there was no evidence of repair in this study is surprising. In a similar study examining the effect of photoperiod on anthracene phototoxicity in bluegill sunfish (*Lepomis macrochirus*), Oris and Giesy (1986) found that the damage incurred during the light periods was partially repaired during the dark periods. At some anthracene concentrations, three-fold increases were observed in UV-LT50 values between the 24 h light photoperiod and the 18 h light:6 h dark photoperiod. The results of the current study suggest that the repair of damage resulting from photoactivated PAHs is not a universal phenomenon. Apparently, species-specific and life history-specific factors play an important role in determining the ability to repair this damage. Due

to the fact that the repair is a rate-limited process, it is possible that mature glochidia might be able to repair the damage resulting from lower levels of PAH and/or UV exposure. The ability to detect these subtle levels of repair would likely require comparisons of LC50s, as opposed to LT50s.

The damage incurred by photoactivated PAHs is believed to be the result of oxidative stress resulting from the generation of ROS, which produces toxic manifestations such as lipid peroxidation (Weinstein et al., 1997; Choi and Oris, 2000a,b). The normal repair of peroxidized lipids is a complex energy-requiring process that operates using a series of reductants, glutathione peroxidase, and phospholipase A_2 to preferentially hydrolyze the peroxidized fatty acids and replace them with normal fatty acids (Van Kuijk et al., 1987; Gregus and Klaassen, 1996). The absence of repair by the glochidia in the present study may be the result of one or more of the following reasons. First, the repair of lipid peroxidation is an energy requiring process, and it seems reasonable to assume that obligate parasites in their free-living stage, such as the glochidia used in the present study, have only limited energy stores. These limited energy stores could be rapidly depleted under conditions of toxicant-induced stress. For example, previous studies with multiple life history stages of grass shrimp (*Palaemonetes pugio*) have demonstrated the depletion of energy stores during exposure to environmentally-relevant sediment concentrations of the pyrethroid insecticide fenvalerate (McKenney et al., 1998). Second, glochidia may not possess adequate levels of those enzymes and/or substrates involved in the repair of lipid peroxidation, such as glutathione, glutathione peroxidase and phospholipase A_2. Previous studies have found that adult freshwater mussels possess levels of glutathione and glutathione peroxidase similar to other organisms (Cossu et al., 1997); however, no studies to date have investigated these levels in glochidia. Third, glochidia may only possess limited fatty acid stores to replace the peroxidized fatty acids. Anatomically, glochidia are very simple organisms with shell valves, a larval mantle, an adductor muscle, and a thread gland (Pekkarinen and Volovirta, 1996). They do not possess those tissues commonly associated with large fatty acid stores, such as adipose tissue and gonads. In the presence of a potent stressor, such as the ROS produced by photoactivated PAHs, any fatty acid stores that the glochidia do possess could be rapidly depleted. Certainly, further research is warranted concerning those reasons underlying the inability of glochidia to repair the damage produced by photoactivated PAHs.

Another objective of this study was to refine those previously developed time-dependent mortality models for glochidia (Weinstein, 2001; Weinstein and Polk, 2001) based upon experiments using environmentally-realistic photoperiods. These previously developed models were based upon experiments using continuous 24-h UV exposures, which did not allow for the incorporation of any repair processes that may have been occurring during the dark periods. The absence of repair in this study demonstrates that these previously developed models are valid. Therefore, in order to predict the UV-induced toxic response of PAH in glochidia, only total UV dose (dose rate and duration) and PAH dose is needed.

In a much broader context, the absence of repair in the current study indicates that glochidia may generally be more sensitive compared with other organisms to those pollutants producing oxidative stress. There is some evidence for this notion. Previous studies have found that the early life stages of freshwater mussels (glochidia and juvenile stage) are relatively sensitive to certain transition metals, such as copper, cadmium, and mercury (Keller and Zam, 1991; Pynnönen, 1995), which are capable of causing oxidative stress in animals (Stohs and Bagchi, 1995). In contrast, these early life stages are relatively tolerant to pollutants with other mechanisms of toxicity, such as the organophosphate insecticide malathion (Keller and Ruessler, 1997), the organochloride insecticides toxaphene and chlordane (Keller, 1993), the carbamate insecticide carbaryl, and the herbicide atrazine (Johnson et al., 1993). Collectively, these findings suggest that these early life stages will be relatively sensitive to other, as of yet untested, pollutants that are known to produce oxidative stress, such as the bipyridyl herbicide paraquat.

5. Conclusions

The results of the present study have demonstrated that the UV-induced toxicity of fluoranthene to glochidia involves cumulative damage during light periods with no evidence of repair during the dark periods. The absence of repair in this study suggests that this ability, in part, is a species-specific and/or life history-specific trait. Those organisms, such as glochidia, that lack the ability to repair this damage will be more susceptible to the UV-induced toxicity of PAHs in the environment than those organisms with this ability. The absence of repair also demonstrates that those previously developed predictive models for the time-dependent mortality in glochidia, which were based solely upon UV dose (exposure and duration) and PAH dose, are generally valid. Finally, the results of the present study underscore those results from previous studies suggesting that environmentally-relevant concentrations of fluoranthene do pose a significant hazard to the glochidia of at least one species of freshwater mussel.

Acknowledgements

The author is grateful to Kevin Polk and Waylan Metts at Texas A&M University-Commerce for their assistance in this research.

References

Allred, P.M., Giesy, J.P., 1985. Solar radiation-induced toxicity of anthracene to *Daphnia pulex*. Environ. Toxicol. Chem. 4, 219–226.

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.

Bogan, A.E., 1993. Freshwater bivalve extinctions (Mollusca: Unionidae): a search for clues. Am. Zool. 33, 599–609.

Bowling, J.W., Leversee, G.J., Landrum, P.F., Giesy, J.P., 1983. Acute mortality of anthracene contaminated fish exposed to sunlight. Aquat. Toxicol. 3, 79–90.

Choi, J., Oris, J.T., 2000a. Anthracene photoinduced toxicity to PLHC-1 Cell Line (*Poeciliopsis lucida*) and the role of lipid peroxidation in toxicity. Environ. Toxicol. Chem. 19, 2699–2706.

Choi, J., Oris, J.T., 2000b. Evidence of oxidative stress in bluegill sunfish (*Lepomis macrochirus*) liver microsomes simultaneously exposed to solar ultraviolet radiation and anthracene. Environ. Toxicol. Chem. 19, 1795–1799.

Cossu, C., Doyotte, A., Jacquin, M.C., Babut, M., Exinger, A., Vasseur, P., 1997. Glutathione reductase, selenium-dependent glutathione peroxidase, glutathione levels, and lipid peroxidation in freshwater bivalves, *Unio tumidus*, as biomarkers of aquatic contamination in field studies. Ecotoxicol. Environ. Saf. 38, 122–131.

Dworkin, M., 1958. Endogenous photosensitization in a carotenoidless mutant of *Rhodopseudonmonas spheroides*. J. Gen. Physiol. 41, 1099–1112.

Erickson, R.J., Ankley, G.T., DeFoe, D.L., Kosian, P.A., Makynen, E.A., 1999. Additive toxicity of binary mixtures of phototoxic polycyclic aromatic hydrocarbons to the oligochaete *Lumbriculus variegatus*. Toxicol. Appl. Pharmacol. 154, 97–105.

Gregus, Z., Klaassen, C.D., 1996. Mechanisms of toxicity. In: Klaassen, C.D. (Ed.), Casarett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill, New York, pp. 35–74.

Huebner, J.D., Pynnönen, K.S., 1992. Viability of glochidia of two species of *Anodonta* exposed to low pH and selected metals. Can. J. Zool. 70, 2348–2355.

Ito, T., 1978. Cellular and subcellular mechanisms of photodynamic action: the $^1O_2$ hypothesis as a driving force in recent research. Photochem. Photobiol. 28, 493–508.

Johnson, I.C., Keller, A.E., Zam, S.G., 1993. A method of conducting acute toxicity tests with the early life stages of freshwater mussels. In: Landis, W.G., Hughes, J.S., Lewis, M.A. (Eds.), Environmental Toxicology And Risk Assessment, STP 1179. American Society of Testing and Materials, Philadelphia, PA, pp. 381–396.

Keller, A.E., 1993. Acute toxicity of several pesticides, organic compounds, and a wastewater effluent to the freshwater mussel, *Anodonta imbecilis*, *Ceriodaphnia dubia*, and *Pimephales promelas*. Bull. Environ. Contam. Toxicol. 51, 696–702.

Keller, A.E., Ruessler, D.S., 1997. The toxicity of malathion to unionid mussels: relationship to expected environmental concentrations. Environ. Toxicol. Chem. 16, 1028–1033.

Keller, A.E., Zam, S.G., 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecilis*. Environ. Toxicol. Chem. 10, 539–546.

Landrum, P.F., Giesy, J.P., Oris, J.T., Allred, P.M., 1985. The photoinduced toxicity of polycyclic aromatic hydrocarbons to aquatic organisms. In: Vandermeulen, J.H., Hrudey, S. (Eds.), Oil in Freshwater: Chemistry, Biology, and Technology. Pergamon Press, New York, pp. 304–318.

Lewis, P.A., Klemm, D.J., Lazorchak, J.M., Norberg-King, T.J., Peltier, W.H., Heber, M., 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-91/002. US Environmental Protection Agency, Cincinnati, OH.

McCloskey, J.T., Oris, J.T., 1993. Effect of anthracene and solar ultraviolet radiation exposure on gill ATPase and selected hematologic measurements in the bluegill sunfish (*Lepomis macrochirus*). Aquat. Toxicol. 24, 207–218.

McKenney, C.L. Jr, Weber, D.E., Celestial, D.M., MacGregor, M.A., 1998. Altered growth and metabolism of an estuarine shrimp (*Palaemonetes pugio*) during and after metamorphosis onto fenvalerate-laden sediment. Arch. Environ. Contam. Toxicol. 35, 464–471.

Mekenyan, O.G., Ankley, G.T., Veith, G.D., Call, D.J., 1994. QSARs for photoinduced toxicity: I. Acute lethality of polycyclic aromatic hydrocarbons to *Daphnia magna*. Chemosphere 28, 567–582.

Monson, P.D., Call, D.J., Cox, D.A., Liber, K., Ankley, G.T., 1999. Photoinduced toxicity fluoranthene to northern leopard frogs (*Rana pipiens*). Environ. Toxicol. Chem. 18, 308–312.

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.

Oris, J.T., Giesy, J.P., 1985. The photoenhanced toxicity of anthracene to juvenile sunfish (*Lepomis* spp.). Aquat. Toxicol. 6, 133–146.

Oris, J.T., Giesy, J.P., 1986. Photoinduced toxicity of anthracene to juvenile bluegill sunfish (*Lepomis macrochirus* Rafinesquie): photoperiod effects and predictive hazard evaluation. Environ. Toxicol. Chem. 5, 761–768.

Oris, J.T., Giesy, J.P., 1987. The photo-induced toxicity of polycyclic aromatic hydrocarbons to larvae of the fathead minnow (*Pimephales promelas*). Chemosphere 16, 1395–1404.

Pekkarinen, M., Volovirta, I., 1996. Anatomy of the glochidia of the freshwater pearl mussel, *Margaritifera margaritifera* (L.). Arch. Hydrobiol. 137, 411–423.

Pynnönen, K.S., 1995. Effect of pH, hardness, and maternal pre-exposure on the toxicity of Cd, Cu, and Zn, to the glochidial larvae of a freshwater clam, *Anodonta cygnea*. Water Res. 29, 247–254.

SAS Institute, 1990. SAS®/STAT User's Guide, Ver 6, vol. 2. Cary, NC, USA.

Stohs, S.J., Bagchi, D., 1995. Oxidative mechanisms in the toxicity of metal ions. Free Radic. Biol. Med. 18, 321–326.

Van Kuijk, F.J.G.M., Sevanian, A., Handelman, G.J., Dratz, E.A., 1987. A new role for phospholipase A2: protection of membranes from lipid peroxidation damage. TIBS 12, 31–34.

Veith, G.D., Call, D.J., Brooke, L.T., 1983. Structure–toxicity relationships for the fathead minnow, *Pimephales promelas*: narcotic industrial chemicals. Can. J. Fish Aquat. Sci. 40, 737–748.

Walker, S.E., Taylor, D.H., Oris, J.T., 1998. Behavioral and histopathological effects of fluoranthene on bullfrog larvae (*Rana catesbeiana*). Environ. Toxicol. Chem. 17, 734–739.

Weinstein, J.E., 2001. Characterization of the acute toxicity of photoactivated fluoranthene to glochidia of the freshwater mussel, *Utterbackia imbecillis*. Environ. Toxicol. Chem. 20, 412–419.

Weinstein, J.E., Oris, J.T., 1999. Humic acids reduce the bioaccumulation and photoinduced toxicity of fluoranthene to fish. Environ. Toxicol. Chem. 18, 2087–2094.

Weinstein, J.E., Polk, K.D., 2001. Phototoxicity of anthracene and pyrene to glochidia of the freshwater mussel, *Utterbackia imbecillis*. Environ. Toxicol. Chem. 20, 2021–2028.

Weinstein, J.E., Oris, J.T., Taylor, D.H., 1997. An ultrastructural examination of the mode of UV-induced toxic action of fluoranthene in the fathead minnow, *Pimephales promelas*. Aquat. Toxicol. 29, 1–22.

Williams, J.D., Warren, M.L. Jr, Cummings, K.S., Harris, J.L., Neves, R.J., 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18, 6–22.