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Toxicity of Fluoranthene to *Daphnia magna, Hyalella azteca, Chironomus tentans,* and *Stylaria lacustris* in Water-Only and Whole Sediment Exposures

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Fluoranthene is a polycyclic aromatic hydrocarbon (PAH) with a hydrophobic nature (water solubility = 265 µg/L; U.S. EPA 1980) and a propensity to sorb to sediments. Fluoranthene has a K_{OC} of 4.65, an intermediate value for PAHs (range 2.95 to 5.92; Pavlou 1987). Fluoranthene can be toxic to some aquatic organisms at concentrations lower than its aqueous solubility (Suede1 and Rodgers 1993; Swartz 1990). Therefore, desorption from sediments could produce aqueous concentrations that are harmful to aquatic organisms. Very few studies (e.g., Suede1 and Rodgers 1993) have examined the toxicity of fluoranthene to freshwater organisms. Data for other PAHs show that crustaceans are the most sensitive species, followed by polychaete worms and fish (Neff 1979). Effects of fluoranthene-amended sediments on selected marine benthic organisms were examined by Swartz et al. (1988; 1990).

The objectives of this research were to 1) determine the relative sensitivities of *Daphnia magna* Straus, *Hyalella azteca* Saussure, *Chironomus tentans* Fabricius, and *Stylaria lacustris* Linnaeus in 48-hr and 10-d aqueous phase exposures to fluoranthene; and 2) determine the relative responses of these organisms in 10-d fluoranthene-amended sediment exposures.

MATERIALS AND METHODS

The amphipod *Hyalella azteca,* the midge *Chironomus tentans,* the cladoceran *Daphnia magna,* and the naidid *worm Stylaria lacustris* were selected for testing as they occupy different niches in sediments. All test organisms were cultured at the University of Mississippi Biological Field Station (UMBFS) according to the methods described in Suede1 and Rodgers (1993).

Three sets of experiments were conducted: 48-hr and 10-d static water only tests, and 10 d whole sediment exposures. All toxicity tests were conducted once in light and temperature controlled incubators at 2O±l°C under a 16 h light/ 8 h dark photoperiod. All test organisms were exposed to fluoranthene separately, with 10 *D. magna,* 10 *H. azteca,* 10 *S. lacustris,* and 6 *C. tentans* per replicate beaker, with three replicates per fluoranthene concentration in all tests. There were 6 fluoranthene treatments plus a control in all tests except

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for *S. lacustris*, which had 5 treatments plus a control. Water-only tests were conducted in 250 mL borosilicate glass beakers with 200 mL UMBFS pond water, except for *S. lacustris* tests, which were conducted in 50 mL beakers with 40 mL UMBFS pond water. UMBFS pond water was used as a control, except for *D. magna* tests, where adjusted UMBFS pond water was used. Hardness and alkalinity of adjusted water were increased with (0.1 g/L) $NaHCO_3$ and $CaCl_2$ to a total hardness of 80 mg/L as and alkalinity $CaCO_3$ of 60 mg/L as $CaCO_3$. Glass beads (150-212 µm, Sigma Chemical Co., St. Louis, Missouri) were used as a substrate in *C. tentans* water-only tests. Test organisms were not fed during 48-hr exposures.

Procedures for 10-d water-only tests followed those of 48-hr tests, except that pond water from the University of North Texas Biological Field Station (UNT) was used. Feeding regimes for each organism were as follows: *D. magna* - 0.2 mL synthetic diet (yeast, catfish chow, alfalfa) daily; *H. azteca* - ground rabbit chow suspension on Day 0 and Day *5; C. tentans* - 0.2 mL Tetra® conditioning food suspension every other day. Water chemistry analyses for 48-hr and 10-d water-only tests are presented in Table 1.

Table 1. Water chemistry analyses from 48-hr and 10-d water-only tests.

	UNT	UMBFS
pH	8.1 - 8.4	6.5 - 7.3
D.O.	6.0 - 8.6 mg/L	6.0 - 8.6 mg/L
Conductivity	280 - 360 µmhos/cm	20 - 30 µmhos/cm
Alkalinity	150 - 175 mg/L	5 - 25 mg/L
Hardness	100 - 130 mg/L	4 - 18 mg/L
Fluoranthene Solubility	150 µg/L	250 µg/L

Sediment was collected from the UMBFS and sieved through a 2.0 mm mesh stainless steel sieve prior to testing to remove coarse debris. Sediments were amended with a fluoranthene stock solution dissolved in HPLC grade acetone. Appropriate quantities of the fluoranthene stock solution were added to 250 mL Qorpak glass bottles and the acetone allowed to completely evaporate, leaving behind a crystalline fluoranthene residue on the glass. Wet sediment was then added to the glass bottles and mixed on a rolling mill at 5-6 rpm for 24-hr following the method of Ditsworth et al. (1990). After the mixing period, sediments were transferred directly into test beakers. Sediment storage, characterization, and handling followed the procedures of Plumb (1981). Sediment characteristics and corresponding analyses are listed in Table 2.

In sediment tests, each 200-mL beaker contained 40 mL of sediment and 160 mL of UMBFS pond water (1:4 sediment to water ratio). *S. lacustris* was exposed to sediment in 50 mL beakers with 8 mL sediment and 32 mL

Table 2. Properties of University of Mississippi Biological Field Station (UMBFS) sediment and corresponding analysis.

Parameter	UMBFS Sediment	Analytical Method	Analysis
Solids (%)	72.95	Drying at 104°C	Black 1986
Organic Matter (%)	2.4	Ashing at 550°C	Black 1986
Organic Carbon (%)	0.38	Induction Furnace (LECO CR-12)	Black 1986
Cation Exchange Capacity (me/lOOg)	1.50	Displacement After Washing	Plumb 1981
Eh (mv)	+57	Orion Redox Probe	Plumb 1981
pH	6.0	Orion pH Probe	Plumb 1981
%Coarse Sand	8.4	Dry Sieving	Gee and Bauder 1986
%Medium Sand	31.7	Dry Sieving	Gee and Bauder 1986
%Fine Sand	35.8	Dry Sieving	Gee and Bauder 1986
%Total Sand	75.9	Dry Sieving	Gee and Bauder 1986
%Silt	21.4	Hydrometric	Gee and Bauder 1986
%Clay	2.7	Hydrometric	Gee and Bauder 1986

UMBFS pond water. Sediment/water mixtures were allowed to equilibrate 18-24 hr before adding organisms. Unaltered UMBFS pond water and UMBFS sediment were used as controls in all sediment tests. Water chemistry analyses for 10-d sediment tests were as follows: dissolved oxygen, 5.8-7.2 mg/L; pH, 6.4-7.2; conductivity, 200-240 umhos/cm; hardness, 72-80 mg/L as $CaCO_3$; alkalinity, 65-75 mg/L as $CaCO_3$.

Water and sediment samples were extracted with hexane and analyzed using a Perkin-Elmer model LS-5B luminescence spectrometer to determine dissolved fluoranthene concentrations. Fluoranthene excitation and emission wavelengths using this method were 354 nm and 464 nm, respectively. Fluoranthene recovery in water samples ranged from 90 to 100% and in sediments ranged from 70 to 80%. Detection limits were 0.5 µg/L in water and 1.0 µg/kg in sediment.

Median lethal concentrations (LC_{50}s) for toxicity tests were calculated using a probit procedure (Stephan 1977). Analysis of variance (ANOVA) was used to detect differences between treatment means and Dunnett's Multiple Range Test was used to determine the No Observed Effects Concentration (NOEC, based

on survival) for each organism (Gulley et al. 1989). The 5% alpha level was used in all tests.

RESULTS AND DISCUSSION

D. magna and *H. azteca* responded similarly to fluoranthene in 48-hr water-only exposures with 48-hr LC_{50} values of 105.7 and 92.2 µg/L, respectively (Table *3). C. tentans* and *S. lacustris* were not as sensitive to fluoranthene, with 48-hr LC_{50} values above the aqueous solubility for fluoranthene in UMBFS pond water (220-250 µg/L). The 48-hr NOEC for *D. magna* was 85 µg/L, greater than the 48-hr NOEC for *H. azteca (* < 74 µg/L). NOECs for *C. tentans* and *S. lacustris* in 48-l-n tests were greater than the aqueous solubility for fluoranthene (Table 3). Results from 48-hr water-only tests indicated that 48-hr exposures of *C. tentans* and *S. lacustris* to fluoranthene may not be sufficient to detect fluoranthene toxicity.

In 10-d water-only tests, *H. azteca* and C. *tentans* were the most sensitive when exposed to fluoranthene, with 10-d LC_{50} values of 30.3 and 37.8 µg/L, respectively (Table *3). D. magna* was less sensitive, with a 10-d LC_{50} value of 102.6 µg/L. The 10-d fluoranthene water-only NOEC for *D. magna* was 90 µg/L, 3 to 4 times greater than the NOEC for *H. azteca* (18 µg/L) and C. *tentans* (30 µg/L).

Measured sediment concentrations (excluding controls) in the 10-d sediment tests ranged from 2.7-52.3 mg/kg (13-107 µg/L in overlying water) in the *D. magna* test, 9.3-33.8 mg/kg (24-85 µg/L in overlying water) in the *H. azteca* test, 1.0-30.7 mg/kg (2.3-45 µg/L in overlying water) in the *C. tentans* test, and 9.5-56.8 mg/kg (27-137 µg/L in overlying water) in the *S. lacustris* test.

C. tentans was the most sensitive of the species examined when exposed for 10 days to fluoranthene-amended sediments, with 10-d LC_{50} values of 23.6 µg/L based on overlying concentrations (Table *3). H. azteca* and *D. magna* were somewhat less sensitive (10-d water-only LC_{50}s = 60.6 and 110.5 µg/L, respectively). *S. lucustris* was less sensitive to fluoranthene than the other organisms tested, with a 10-d LC_{50} of > 137 µg/L in overlying water. These trends were also evident based on 10-d NOEC values in order of increased sensitivity to fluoranthene: *C. tentans = H. azteca > D. magna > S. lacustris.*

Comparisons of 48-hr and 10-d water-only fluoranthene exposures indicate that considerable differences exist in sensitivities of the test species after various exposure durations. The LC_{50} and NOEC values were similar for *D. magna* exposed to fluoranthene in 48-hr and 10-d water-only tests. However, *H. azteca* and *C. tentuns* 10-d LC_{50} and NOEC values were considerably lower than their 48 h values, indicating that an exposure duration of 48-hr is not sufficient to elicit the toxic effects of fluoranthene to these organisms.

In 10-d whole sediment tests, C. *tentans* was the most sensitive test organism examined, as measured by LC_{50} and NOEC values (Table 3). C. *tentans* was 4 times more sensitive than *D. magna* and twice as sensitive as *H. azteca* in 10-d whole sediment exposures.

Table 3. LC_{50} and NOEC values (95 % C.I.) based on measured concentrations for *D. magna, H. azteca, C. tentans* and S. *lacustris* in aqueous phase and whole sediment fluoranthene exposures.

	D. magna	***H. azteca***	***C. tentans***	***S. lacustris***
Aqueous Tests (µg/L)				
48-hr LC_{50}	105.7 (81.7-142.2)	92.2 (775102.3)	> 250	> 220
48-hr NOEC	85	<74	> 250	> 220
10-d LC_{50}	102.6 (92.3-116.7)	30.3 (11.1-62.0)	37.8 (30.1-46.2)	[a]
10-d NOEC	90	18	30	[a]
Sediment Tests				
10-d LC_{50} (µg/L water)	110.5 (91.9-149.4)	60.6 (46.5-80.8)	23.6 (16.4-35.9)	> 137
10-d NOEC (µg/L water)	75	<24	20	115
10-d NOEC (mg/kg dry sediment)	13.1	< 12.9	13.9	26.8

[a]Not determined.

H. azteca and *C. tentans* were two to three times more sensitive than *D. magna* in 10-d water-only exposures but were equal to or less sensitive than *D. magna* in 48-l-n exposures. Conducting 48-hr or 96-hr exposures using *H. azteca* and *C. tentans* could lead to erroneously concluding water or sediment contaminated with fluoranthene is not toxic. *D. magna* was more sensitive to fluoranthene in 48-hr exposures, while *H. azteca* and *C. tentans* were more sensitive to fluoranthene in 10-d exposures. *H. azteca* and *C. tentans* may

also be similarly affected in experiments using other neutral organic compounds that have a similar mode of action as fluoranthene. These results indicate the importance of test duration in determining adverse effects of neutral organic compounds such as fluoranthene to aquatic organisms.

At present, the U.S. EPA criterion for fluoranthene in freshwater (U.S. EPA 1980) is based on unmeasured values for *D. magna* (48-hr LC_{50} = 325,000 µg/L) and the bluegill sunfish, *Lepomis macrochirus* (96-hr LC_{50} = 3,980 µg/L) which are well in excess of the aqueous solubility of fluoranthene (265 µg/L). Results of this study, based on measured fluoranthene concentrations, indicate a 48-hr LC_{50} of 106 µg/L and a 10-d LC_{50} of 103 µg/L for *D. magna* in water-only tests, which are more realistic estimates of fluoranthene toxicity to *D. magna* than those reported by U.S. EPA (1980). However, 10-d LC_{50} and NOEC values for *D. magna* in water-only and whole sediment tests would not be protective of benthic species such as *H. azteca* and *C. tentans.* Based on 10-d water-only and whole sediment exposures to fluoranthene in this study, the 10-d NOEC for *H. azteca* of 20 µg/L would also be protective of *D. magna, C. tentans,* and *S. lacustris.*

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