TOXICITY OF PHENANTHRENE TO SEVERAL FRESHWATER SPECIES

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INTRODUCTION

Phenanthrene is a by-product of fossil fuel combusion. It is a constituent of coal tars and has been detected in the stack gases of both oil- and coal-fired power and manufacturing plants. The purpose of this study was to determine the acute toxicities and chronic effects of phenanthrene to several freshwater organisms. The organisms used for acute exposures to phenanthrene were duckweed (Lemna minor), a coelenterate (Hydra sp.), an annelid (Lumbriculus variegatus (Muller)), a cladoceran (Daphnia magna), an amphipod (Gammarus pseudolimnaeus), rainbow trout (Salmo gairdneri) and bluegill (Lepomis macrochirus). Chronic exposures to phenanthrene were conducted with rainbow trout and Daphnia magna.

METHODS AND MATERIALS

Acute Toxicity Tests

The water supply for organism acclimation and testing was the municipal water supply for the City of Superior, WI. The water was dechlorinated by charcoal filtration and sodium sulfite addition. Water temperature was maintained at desired levels for the different test species by control of the dilution water at the headbox.

All acute toxicity tests were flow-through and conducted in a Benoit-type mini-diluter (Benoit et al., 1982) with duplicate exposure concentrations and

controls. The tests consisted of five toxicant concentrations with a dilution factor of 0.5.

Phenanthrene was added to the water by dissolution from phenanthrene-coated glass wool. Ten grams of the test compound was first dissolved in acetone, then added to a 22 x 500 mm glass tube filled with glass wool and air dried. Test water was pumped through the tubes (2-5 per test) prior to entering the dilution apparatus.

Test chambers were of varying sizes. Rainbow trout tests were conducted in 13.0 x 19.4 x 11.2 cm chambers containing 2.5 L of test solution. Bluegill and duckweed tests were conducted in 20 x 14 x 18.5 cm chambers containing 2.8 L of test solution. Bluegills and duckweed were added directly into these chambers, while in tests with cladocerans, annelids and Hydra, the organisms were placed into 250 mL beakers with screened holes on each side. These were then suspended in the larger chambers. Amphipods were placed into the larger chambers in stainless steel wire mesh cylinders (8-10 cm length x 3 cm diameter) closed at both ends with neoprene stoppers. Each cylinder also contained two leaf disks (Betula sp. and Populus sp.) 20 mm in diameter. The number of organisms per test chamber consisted of ten for all acute tests except for duckweed which had 40 fronds per chamber.

In the duckweed test, stock solutions of nutrients were prepared according to the recommendations in the ASTM (Draft No. 3) proposed practices. One mL of each of the three stock solutions was added simultaneously to each L of dilution water. The nutrient addition was done at the headbox with injection pumps enabling all chambers to receive the same concentrations of nutrients. Addition of

the nutrients resulted in slightly increased alkalinity and hardness values.

Dissolved oxygen was measured in the control, low, middle and high exposures at the initiation of each test and every 48 h thereafter (Table 1). Total hardness (EDTA), total alkalinity and pH were measured at least once during each test in control, low, middle and high exposures in accordance by accepted procedures (APHA 1980). Temperatures were recorded daily. Dissolved oxygen (mean percent saturation) ranged from 77.8 to 97.8 percent for the tests; mean total (EDTA) hardness ranged from 48.9 to 60.5 $mg.L^{-1}$ (as $CaCO_3$); mean total alkalinity ranged from 39.8 to 45.7 $mg.L^{-1}$ (as $CaCO_3$); and mean pH from 6.82 to 7.39 (Table 1).

Biomass loading requirements were met as stated by ASTM (1980). Tests were begun by placing the test organisms in the exposure chambers after several replacements of test solutions. The criteria for death were absence of respiratory movement or lack of reaction to gentle prodding. Death was not easily determined for cladocerans, annelids, Hydra or amphipods. The endpoint for cladocerans, annelids and amphipods was lack of reaction to gentle prodding. The Hydra EC50 was based on whether the body column and/or tentacles were shortened. Exposures were checked every 24 h for death and/or effect.

Test organisms were acquired from a variety of sources. Amphipods were collected from the Eau Claire River, Douglas County, WI; cladocerans, Hydra, duckweed and annelids were obtained from the University of Wisconsin-Superior culture units; rainbow trout and bluegills were obtained from the U.S. EPA Environmental Research Laboratory, Duluth, MN. Recommended procedures for care, handling and acclimation of test organisms were followed (ASTM 1980). The organisms were acclimated to the test water and exposure temperature for a minimum of two

Table 1. Water Characteristics for Acute and Chronic Exposures with Phenanthrene. Values are Means ± Standard Deviations, Ranges and Sample Sizes

Species	Dissolved Oxygen (%)	Total (EDTA) Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	Total Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	pH	Conductivity ($\mu mhos/cm$)	Temperature ^{o}C
Acute Tests						
Duckweed (*Lemna minor*)	90.8±4.4 (85.2-97.3) n=12	60.5±0.8 (59.9-61.7) n=4	45.7±0.5 (45.0-46.0) n=4	7.10±0.04 (7.06-7.14) n=4	161±1.2 (160-162) n=4	24.4±0.8 (22.2-26.1) n=60
Hydroid (*Hydra* sp.)	97.8±4.8 (87.5-106) n=12	48.9±1.9 (46.8-50.8) n=4	39.8±0.5 (39.4-40.1) n=4	7.39±0.06 (7.30-7.44) n=4	106±0 (106-106) n=4	20.0±0.4 (19.2-21.0) n=60
Annelid (*Lumbriculus variegatus*)	79.4±6.2 (67.1-85.8) n=12	53.0±1.5 (50.7-55.3) n=8	43.7±0.8 (42.4-44.8) n=8	7.00±0.02 (6.96-7.01) n=4	103±4 (97-108) n=8	16.4±0.8 (14.9-18.6) n=60
Cladoceran (*Daphnia magna*)	77.8±3.6 (72.3-81.5) n=8	53.1±0.9 (52.3-54.3) n=4	42.2±0.43 (41.8-42.8) n=4	7.22±0.03 (7.18-7.24) n=4	92±3 (90-95) n=4	18.4±0.4 (17.8-19.4) n=36
Amphipod (*Gammarus pseudolimnaeus*)	96.2±1.5 (93.7-98.8) n=11	50.5±1.0 (49.8-51.7) n=4	42.1±0.5 (41.6-42.8) n=4	6.82±0.03 (6.78-6.85) n=4	91±2 (88-93) n=4	16.1±0.6 (15.1-17.5) n=60
Rainbow Trout (*Salmo gairdneri*)	85.8±4.1 (79.8-91.0) n=12	50.6±1.4 (48.6-51.7) n=4	41.2±0.8 (40.0-41.8) n=4	7.20±0.04 (7.15-7.25) n=4	86.4±0.0 (86.4-86.4) n=4	12.1±0.3 (11.3-12.8) n=60
Bluegill (*Lepomis macrochirus*)	88.1±10.9 (69.8-101.4) n=12	52.8±1.7 (50.5-54.5) n=4	41.9±1.1 (40.4-43.0) n=4	7.14±0.08 (7.05-7.24) n=4	103.6±5.2 (98.1-109.0) n=4	19.8±1.9 (16.0-22.5) n=60

Table 1 Cont. Water Characteristics for Acute and Chronic Exposures with Phenanthrene. Values are Means ± Standard Deviations, Ranges and Sample Sizes

Species	Dissolved Oxygen (%)	Total (EDTA) Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	Total Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	pH	Conductivity (μmhos/cm)	Temperature ^{o}C
Chronic Tests						
Rainbow Trout	74.8±13.0 (61.8-87.8) n=72	50.4±7.5 (42.9-57.8) n=48	42.0±2.5 (39.5-44.4) n=48	6.94±0.10 (6.84-7.04) n=48	87±8 (78-108) n=48	10.2±0.1 (9.3-12.2) n=900
Daphnia magna	89.6±6.8 (82.9-100.1) n=5	50.6±3.3 (45.8-53.5) n=4	40.4±3.9 (34.6-42.8) n=4	7.26±0.05 (7.20-7.30) n=5	- -	20.5±0.1 (20.3-20.7) n=5

weeks prior to test initiation.

Rainbow Trout 90-Day Early Life-Stage Test

An early life-stage test was conducted in a modified Benoit mini-diluter (Benoit et al. 1982) using newly fertilized rainbow trout (Red Band Strain) embryos. These embryos were obtained from the U.S. Fish and Wildlife Service-National Fish Hatchery, Ennis, MT via the U.S. EPA Environmental Research Laboratory, Duluth, MN. The embryos were 3 d post-fertilization upon receipt. Fifty embryos were placed into each incubation cup, (one per test chamber), until eyed, then 15 embryos were transferred to a second cup and remained there until swim-up. The bottoms of the embryo cups were stainless steel mesh. The embryo cups were gently raised and lowered in the exposure water by a rocker arm assembly to facilitate water movement around the embryos. Fifteen fry were then released into the exposure chambers (19.4 x 13.0 x 11.2 cm) containing 2.5 L of solution. Organisms were continuously exposed for 90 d including incubation.

Observations were made daily for egg hatchability, survival of fry, developmental abnormalities, behavioral effects, and fish growth. Organisms were fed live brine shrimp (Artemia sp., EPA reference eggs) three times daily and trout starter pellets (Glencoe Mills, Glencoe, MN) once a day. Test water characteristics (Table 1) were monitored throughout the test according to accepted procedures (APHA 1980). Temperatures were monitored daily. The photo-period consisted of total darkness until swim-up, at which time it was adjusted to 8 h of darkness and 16 h of light (90.1 $\pm$ 8.2 ft candle).

Daphnia magna Flow-Through Chronic Test

Cladocerans were cultured for at least 14 d under static test conditions before use in the phenanthrene toxicity testing. Mass cultures contained 20 adults per 1600 mL water and were renewed three times per week. Individual adult cladocerans, from the stock culture, were transferred to 100 mL containers, with 80 mL of culture water, 24 h prior to test initiation. Neonates, from broods produced during this time, were pooled for the initiation of the experiment. A combination of trout chow suspension (5 $mg.L^{-1}$) and Selenastrum capricornutum (1 x 10^8 $cells.L^{-1}$) was fed to the mass culture and the individual containers.

Phenanthrene exposures were made in a Benoit style mini-diluter (Benoit et al. 1982) with replicate exposure tanks of 2.0 L each. Four 250 mL beakers, with screened sides, were suspended in each tank to contain eight individual cladocerans. Toxicant delivery was divided four ways in each tank to allow the addition of toxicant directly into each beaker. The highest toxicant volume in the tanks prior to drawdown offered 100 mL of exposure water per beaker. Each tank self-siphoned to renew the toxicant approximately every hour. Approximately 50 mL of exposure water was left for the test animal immediately after siphoning.

Chronic exposures were conducted with eight replicates for each phenanthrene concentration and control. All solutions were treated the same except for the phenanthrene addition. One cladoceran neonate, less than 24-h old, was added to each beaker by random selection from a common pool of organisms. Transfer of organisms was accomplished by a fire-polished, wide-mouth pipette. Each replicate was fed a combination of trout chow suspension and S. capricornutum twice a day. Food concentrations in the flow-through system were added at 5 $mg.L^{-1}$ trout

chow suspension and 1 x 10^8 cells.L^{-1} S. capricornutum but did vary depending on each tanks drawdown time. Exposure beakers were checked on Monday, Wednesday and Friday for deaths or reproduction. Culturing and testing were conducted at 20.0 $\pm$ 2^o C. Dissolved oxygen was maintained between 80 and 100 percent of saturation. Alkalinity, total (EDTA) hardness and pH values were consistent with the values measured for the other tests (Table 1).

Endpoints for the determination of a 21-d Maximum Acceptable Toxicant Concentrations (MATC) were: percent survival, mean number of young per starting female, and mean number of young per starting female per reproductive day. The MATC is the geometric mean of the "no observed effect concentration" (NOEC) and "lowest observed effect concentration" (LOEC). The number of young produced from a starting female are those young produced during the test regardless of whether or not the adult survived the test. The number of young per starting female per reproductive day is the number of young per female per day beginning with the day the first young were observed (usually day 12) in any exposure concentration.

Rejection criteria for the experiment were: greater than 25 percent mortality in the control treatment, less than an average of 40 young produced per adult in the control treatment and a temperature fluctuation in any of the treatments greater than $\pm$ 2^o C.

Statistical Treatment of Data

LC50 or EC50 with 95% confidence limits were calculated by the Trimmed Spearman-Karber method (Hamilton et al., 1977). No acute test had more than 10% mortality of the control organisms. Chronic exposure effects were determined by a

one-way analysis of variance (ANOVA) test. Specific concentrations causing significant reductions in reproduction were identified using Dunnett's one-tailed procedure (Steele and Torrie 1980).

Analytical Techniques

Exposure chambers were sampled at 24-h intervals for acute and twice weekly intervals for chronic tests. For all acute and rainbow trout chronic tests, water samples (50 or 100 mL) were pipetted into glass bottles with teflon-lined screw caps. With the Daphnia chronic test, composite samples (4-25 mL samples/exposure) were taken and treated as with the other tests. Hexane (5.0 mL) was then added, followed by vigorous stirring for 45 min on stir plates. A 1.0 mL aliquot of the hexane layer was pipetted into a vial and 10 uL of a 523 $ug.mL^{-1}$ solution of acenaphthene was added as an internal standard.

Samples were analyzed on a Hewlett-Packard 5794A gas chromatograph equipped with a flame ionization detector and using a 30M SE-54 capillary column (J & W Scientific, Folsom, CA). Ultra-high purity hydrogen was used as the carrier gas and pre-purified nitrogen used as make-up gas. The samples were run at 160 C isothermally (two injections/sample) with an injector temperature of 250 C and detector temperature of 300 C. The retention time of phenanthrene was 3.88 $\pm$ 0.02 min and 1.44 $\pm$ 0.02 min for the internal standard. The analytical stock solution was prepared by dissolving the compound (EPA Reference Standard, lot 1077, 98% purity) in acetone, and the analytical standards were prepared as dilutions of the stock solution in hexane. The detection limit for phenanthrene varied with each test and ranged from 0.001 to 0.010 $mg.L^{-1}$.

Spike and duplicate samples were taken at every sampling. Mean percentage spike recovery ranged from 94.8 ± 1.82% to 101 ± 2.31% with a grand mean recovery of 98.1% for all tests, while the mean percentage agreement between duplicate samples ranged from 89.7 ± 6.08% to 98.0 ± 1.73 % with a grand mean of 94.9%.

RESULTS

Acute Toxicity Tests

Duckweed was exposed to five concentrations (0.062 ± 0.015, 0.118 ± 0.027, 0.198 ± 0.042, 0.356 ± 0.060 and 0.658 ± 0.130 $mg.L^{-1}$) of phenanthrene, plus a control, in duplicate. Duckweed frond production was not affected by phenanthrene in a 96-h exposure. The reduction in fronds (percent effect) ranged from 1.0 ± 35.9 percent as compared to the controls (Table 2). Frond reduction revealed no pattern with phenanthrene concentration except that the highest exposure (0.658 $mg.L^{-1}$) had the greatest reduction at 35.9%. However, this was not significantly different from the controls at the 95% confidence level.

Adult Hydra were exposed to five concentrations (<0.006, 0.029 ± 0.010, 0.082 ± 0.010, 0.176 ± 0.038 and 0.416 ± 0.088 $mg.L^{-1}$) of phenanthrene, plus a control, in duplicate. Affected Hydra had shortened tentacles and body column. The 96-h EC50 estimate with its 95% confidence limits was 0.096 (0.080-0.114) $mg.L^{-1}$ (Table 3). No controls were affected.

Adult annelids were exposed to five concentrations (0.041 ± 0.010, 0.069 ± 0.019, 0.129 ± 0.036, 0.234 ± 0.032 and 0.419 ± 0.062 $mg.L^{-1}$) of phenanthrene,

Table 2. Effect of Phenanthrene on Duckweed (Lemna minor) Frond Production When Exposed to Various Concentrations for 96 h.

	Mean Exposure Concentration ± s.d. (mg/L)					
	Control <0.010	0.062±0.015	0.118±0.027	0.198±0.042	0.356±0.06	0.658±0.130
Initial frond count	80	80	80	80	80	80
Final mean frond count	183	187	182	158	176	146
Net difference	103	107	102	78	96	66
% Effect[1/]	-	3.9	1.0	24.3	6.8	35.9

1/ $\% \text{ Effect} = \frac{C-T}{C} \times 100$ Where: C = increase of fronds in control

T = increase of fronds in treatment

plus controls, in duplicate. No effect was noticed in any of the exposures. The 96-h EC50 was >0.419 $mg.L^{-1}$ (Table 3). No control organisms were affected.

Cladocerans (D. magna) were exposed to five concentrations (0.047 ± 0.007, 0.080 ± 0.010, 0.126 ± 0.010, 0.213 ± 0.028 and 0.352 ± 0.085 $mg.L^{-1}$) of phenanthrene, plus a control, in duplicate. Affected cladocerans were rendered motionless on the bottoms of the exposure chambers. The 48-h EC50 estimate with its 95% confidence limits was 0.117 (0.093-0.147) $mg.L^{-1}$ (Table 3). Ten percent of the control organisms were affected.

Adult amphipods (mean length 9 ± 2 mm; mean weight 0.027 ± 0.009 g) were exposed to five concentrations (<0.010, 0.025 ± 0.007, 0.085 ± 0.013, 0.206 ± 0.019 and 0.412 ± 0.029 $mg.L^{-1}$) of phenanthrene, plus a control, in duplicate. The animals were affected immediately after initial exposure with all dead in the highest exposure (0.412 $mg.L^{-1}$) at 72 h. Affected animals were rendered motionless on the bottoms of the exposure chambers. The 96-h EC50 estimate with its 95% confidence limits was 0.126 (0.113-0.139) $mg.L^{-1}$ (Table 3). Five percent of the controls were affected.

Rainbow trout (mean standard length 28 ± 2 mm; mean weight, 0.258 ± 0.070 g) were exposed to five concentrations (0.019 ± 0.004, 0.053 ± 0.009, 0.105 ± 0.008, 0.204 ± 0.062 and 0.375 ± 0.077 $mg.L^{-1}$) of phenanthrene, plus a control, in duplicate. Affected fish lost equilibrium immediately after initial exposure. The 96-h LC50 was 0.375 $mg.L^{-1}$ and the 96-h EC50 with its 95% confidence limits was 0.050 (0.040-0.062) $mg.L^{-1}$. Confidence limits could not be determined for the LC50 due to lack of partial mortalities at intermediate exposures (Table 3). No control fish died.

Table 3. LC50 and EC50 Estimates (95% Confidence Limits in Parentheses) for Phenanthrene in Acute Toxicity Tests with Several Aquatic Organisms

Test Species	Mean Weight ± s.d. (g)	LC50 ($mg \cdot L^{-1}$) 24 h	LC50 ($mg \cdot L^{-1}$) 48 h	LC50 ($mg \cdot L^{-1}$) 96 h	EC50 ($mg \cdot L^{-1}$) 24 h	EC50 ($mg \cdot L^{-1}$) 48 h	EC50 ($mg \cdot L^{-1}$) 96 h
Hydroid (*Hydra* sp.)	<0.001	- -	- -	- -	- -	0.120 (*)	0.096 (0.080-0.114)
Annelid (*Lumbriculus variegatus*)	0.004±0.002 n=10	- -	- -	- -	- -	- -	>0.419
Cladoceran (*Daphnia magna*)	- -	- -	- -	- -	- -	0.117 (0.093-0.147)	- -
Amphipod (*Gammarus pseudolimnaeus*)	0.027±0.009 n=10	- -	- -	- -	0.378 (0.269-0.530)	0.279 (0.175-0.446)	0.126 (0.113-0.139)
Rainbow trout (*Salmo gairdneri*)	0.258±0.070 n=10	- -	- -	0.375 (*)	0.146 (*)	0.055 (0.046-0.066)	0.050 (0.040-0.062)
Bluegill (*Lepomis macrochirus*)	0.046±0.031 n=20	- -	- -	0.234 (0.224-0.244)	0.056 (0.049-0.064)	0.051 (*)	0.049 (*)

*Ninety-five percent confidence limits were not determined due to a lack of partial mortalities and/or effect at any of the intermediate exposures.

Juvenile bluegills (mean standard length 13 ± 2 mm; mean weight, 0.046 ± 0.031 g) were exposed to five concentrations (0.037 ± 0.020, 0.074 ± 0.044, 0.100 ± 0.064, 0.176 ± 0.117 and 0.311 ± 0.185 g) of phenanthrene, plus a control, in duplicate. Affected fish lost equilibrium immediately after initial exposure and deaths began within 72 h. The 96-h LC50 with its respective 95% confidence limits was 0.234 (0.224-0.244) $mg.L^{-1}$. The 96-h EC50 was 0.049 $mg.L^{-1}$. No confidence limits could be determined for the EC50 due to a lack of partial effects at any of the intermediate exposures. No control fish died.

Rainbow Trout 90-Day Early Life-Stage Test

Rainbow trout embryos were exposed to five concentrations of phenanthrene (0.005 ± 0.003, 0.008 ± 0.006, 0.014 ± 0.010, 0.032 ± 0.023, and 0.066 ± 0.052 $mg.L^{-1}$) plus controls, all in duplicate.

Trout embryos started to hatch on day 28 post-fertilization and hatching continued to day 35. No difference from the controls was observed in either length of incubation or hatching success (Table 4) at any phenanthrene exposure level. The mean percent of abnormal and dead fry at hatch was significantly increased ($p \leq 0.01$) at the highest exposure (0.066 $mg.L^{-1}$). Sac fry were underdeveloped in the three highest exposures (≥ 0.014 $mg.L^{-1}$), and continued to remain underdeveloped throughout the test. Swim-up was also delayed at these three highest exposures.

Upon termination of the test, survival was significantly affected ($p \leq 0.05$) at concentrations ≥ 0.008 $mg.L^{-1}$ (Table 4). Mean wet weights and standard lengths at the termination of the test were significantly reduced ($p \leq 0.01$) from the control

Table 4. Hatchability, Abnormal Development, Survival and Growth of Rainbow Trout (Salmo gairdneri) Exposed to Phenanthrene for 90 Days Post-Fertilization

	Phenanthrene Concentration (mean + s.d. $mg \cdot L^{-1}$)					
	Control <0.001	0.005± 0.003	0.008± 0.006	0.014± 0.010	0.032± 0.023	0.066± 0.052
Mean percent hatch[1/]	86.0	82.0	84.0	83.0	76.0	92.0
Mean percent abnormal and dead fry at hatch	2.2	1.2	9.6	10.8	12.6	52.2**
Mean percent survival through 90 days[2/]	96.6	80.0	56.7*	50.0**	46.7**	0**
Mean wet weight (g) at 90 days post-fertilization	0.626	0.676	0.644	0.610	0.294**	-
Mean standard length (mm) at 90 days post-fertilization	37.2	37.3	37.1	36.1	28.9**	-
Mean wet weight (g)/chamber at 90 days post-fertilization	8.137	7.434	5.476*	4.576**	2.056**	-

1/ Live fry/total eggs.

2/ Percent survival of 30 individuals transferred from egg cups to exposure chambers.

* $p \geq 95\%$.

** $p \geq 99\%$.

fish at phenanthrene concentrations $\geq$0.032 mg.L^{-1}. The mean wet weight of fish per chamber was also significantly reduced ($p \leq 0.05$) from that of the control fish weight at concentrations $\geq$0.008 mg.L^{-1}. This is a reflection of reduced survival, as the weights of individual fish were not affected. The NOEC for phenanthrene with rainbow trout was 0.005 mg.L^{-1} and the LOEC was 0.008 mg.L^{-1}. The geometric mean of these values was 0.006 mg.L^{-1} and is the MATC.

An acute-chronic toxicity ratio was derived for rainbow trout exposed to phenanthrene. The 96-h LC50 was 0.375 mg.L^{-1} and the MATC was 0.006 mg.L^{-1}. The resultant ratio was 62.50.

Daphnia magna 21-Day Flow-Through Chronic Test

Cladoceran neonates $\leq$24-h old were exposed to four concentrations of phenanthrene (0.046 $\pm$ 0.016, 0.057 $\pm$ 0.035, 0.163 $\pm$ 0.118 and 0.378 $\pm$ 0.088 mg.L^{-1}), plus a control, in eight replicates. Treatment deaths were evident up to day 21 except for the highest exposure (0.378 mg.L^{-1}) where all mortality occurred within 8 d. All eight cladocerans died at the highest exposure, and seven of the eight organisms died at 0.163 mg.L^{-1}.

Significant reductions ($p \leq 0.01$) in reproduction when compared to the control occurred at concentrations $\geq$0.163 mg.L^{-1} (Table 5). Reproduction was measured by the mean number of young produced throughout the test and per reproductive day. The geometric mean of the NOEC (0.057 mg.L^{-1}) and the LOEC (0.163 mg.L^{-1}) was ~~0.090~~ 0.096 mg.L^{-1}. This is considered the MATC.

An acute-chronic toxicity ratio was calculable from these tests. The 48-h EC50 was 0.117 mg.L^{-1} and the MATC was ~~0.090~~ 0.096 mg.L^{-1}. The resulting ratio was ~~1.30~~ 1.219.

Table 5. Chronic Toxicity of Phenanthrene to Daphnia magna in a 21-Day Flow-Through Exposure in UW-Superior Laboratory Water

	Phenanthrene Concentration (Mean ± s.d. $mg \cdot L^{-1}$)				
	Control <0.008	0.046± 0.016	0.057± 0.035	0.163± 0.118	0.378± 0.088
Mean percent survival	75	62.5	75	12.5	0
Mean ± s.d. number of young per starting female	57.5±12.8	31.1±21.4	31.6±29.2	0±1.0**	0±0**
Mean ± s.d. number of young per female per reproductive day	5.0±1.0	4.0±3.0	4.0±3.0	0±1.0**	0±0**

** Significantly different from the controls at 99% confidence level.

MATC = ~~0.090~~ 0.096 $mg \cdot L^{-1}$.

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