CONDUCTING TOXICITY TESTS WITH FRESHWATER ORGANISMS EXPOSED TO DIELDRIN, FLUORANTHENE AND PHENANTHRENE

by

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For

U.S. EPA Contract No. 68-C1-0034
Work Assignment No. 5
$54,796

April, 1993

Several toxicity tests are needed to complete data sets for the numerical national water quality criteria for the protection of aquatic life. This report contains some of the needed tests which are: an acute renewal test with dieldrin exposing a cladoceran, *Daphnia magna*, acute and chronic flow-through tests with dieldrin exposing rainbow trout, *Oncorhynchus mykiss*, a chronic renewal test with fluoranthene (without additional UV light) exposing *D. magna* and acute flow-through and chronic renewal tests with phenanthrene exposing *D. magna*. All tests were conducted at the University of Wisconsin-Superior testing facilities according to the procedures recommended by the American Society for Testing and Materials (ASTM).

METHODS

Test Organisms

Daphnia magna originally obtained from the U.S. EPA Environmental Research Laboratory-Duluth, MN, (ERL-D) were cultured and reared at the UW-S laboratory. They were cultured in hard (160-180 mg/L as $CaCO_3$) reconstituted water (ASTM 1991a) which was renewed three times weekly (MWF). Cladocerans were fed green algae (*Selenastrum capricornutum*) and YTC (yeast-trout chow-cereal leaves) on a MWF regimen. Organisms from fourth broods were used to initiate the acute and chronic exposures to the test chemicals.

Certified disease free rainbow trout were acquired from two commercial suppliers. The chronic test was conducted with embryos supplied as ova and sperm from the Mount Lassen Trout Farms, Inc., Red Bluff, CA. Upon arrival at UW-S, 5,000 ova from three females and sperm from three males were combined in 1.0 L of water. Water hardening (imbibition of water into fertilized ova) was complete by two hours after fertilization. Embryos were immediately drawn into a glass tube, counted and placed in random order into incubation cups within the diluter. Fry for the acute test were acquired from Seven Pines Trout Farm, Lewis, WI., acclimated for forty seven days in laboratory water, and fed trout chow.

Exposure Conditions

The water supply for all acclimation and tests with rainbow trout was the municipal water supply for the City of Superior, WI. The city water was dechlorinated by passing through an activated charcoal column and by sodium sulfite addition. The water also passed through a cation exchange resin to remove heavy metals. This same water supply was used for the acute flow-through phenanthrene test with *D. magna*. The other *D. magna* tests were conducted in hard (160-180 mg/L as $CaCO_3$) reconstituted water (ASTM 1991a) which was renewed three times weekly (MWF).

Flow-through tests were conducted using proportional mini-diluter systems (Benoit, et al. 1982). All test systems delivered toxicant solutions at about 0.5 dilution factor. All surfaces exposed to test solutions were constructed of glass, plastic or stainless steel.

Cladocerans were acutely exposed to dieldrin in 100 mL plastic cups (Solo Cup Company; Chicago, IL) containing 80 mL of test solution. Four replicates were prepared for the control and each of the five toxicant concentrations before adding five organisms to each test chamber. The organisms were not fed during the exposure and were observed daily for mortalities. The test was conducted in a temperature and photoperiod (16L:8D) controlled chamber.

The rainbow trout acute exposure was conducted in a flow-through diluter with 2.4 L exposure chambers placed in a temperature controlled water bath. Four replicates of five exposure concentrations of dieldrin and control were used. Each replicate contained five fish. The photoperiod consisted of 16 h of light and 8 h of darkness. Fish were not fed during the 96-h exposure. Mortalities observed were recorded daily and dead organisms were removed from the test chamber.

The rainbow trout chronic test was conducted in a flow-through diluter with two-liter exposure chambers placed in a temperature controlled water bath. Four replicates of five exposure concentrations of dieldrin and control were used. Each contained 30 freshly fertilized embryos. The embryos were

placed in oscillating incubation chambers consisting of 100-mL beakers with the bottoms removed and replaced with nylon mesh (two per replicate with 15 embryos in each). The incubation chambers were slowly raised and lowered to facilitate movement of test solution around the embryos. The organisms were kept in the oscillating incubation chambers without direct lighting until swim-up of the fry occurred (approximately 45 days). Fifteen fry were randomly chosen from the two oscillating incubation chambers and released into each test chamber. At this time, photoperiod (16L:8D) was initiated and the fry were fed. Feeding consisted of a ration of trout chow three times a day. Test chambers were given equal amounts and the quantity was increased with time as the fry grew. Enough food was given to have excess on the bottom of the test chambers. The chambers were siphoned clean daily. Test duration was 90 days.

The cladoceran fluoranthene chronic test was conducted in 100 mL plastic cups (Solo Cup Co.; Chicago, IL) that were pre-conditioned with test solutions. The cups contained 80 mL of test solution which was renewed every MWF for 21 days. A single cladoceran (<24-h-old) was placed in each of ten replicate chambers for each exposure concentration and control. Five concentrations of fluoranthene and a control were tested. At renewal, the organisms were fed a mixture of YTC and green algae (ASTM 1991b), and the surviving adults were transferred to the fresh solution by pipet. If neonates were present, they were counted and discarded.

Cladocerans were acutely exposed to phenanthrene in a flow-through diluter with 2.0 L exposure chambers. The organisms were contained in 50 mL beakers with nylon mesh screens covering holes in the sides to allow exchange of solutions with the main chamber. Water exchange in the test chambers was enhanced by having the primary test chamber partially drain several times per hour using self-starting siphons. Two replicates of five phenanthrene concentrations and controls each contained two beakers with five organisms each. The photoperiod was 16L:8D. Organisms were not fed during the exposure and were observed daily for effects or mortalities.

Attempts were made to conduct a chronic toxicity test exposing phenanthrene with a cladoceran in a flow-through test system similar to that used for the acute test. Difficulty was encountered achieving uniform concentrations in the five replicate chambers containing the test organisms during the 21-d test period. Flow-through testing was abandoned when it was learned that phenanthrene solutions did not decline in static solutions to less than 50% of the original concentrations in the test chambers during a 72-h exposure period. Heating the exposure chambers to 200° C for 2 h before preconditioning the chambers with test solutions reduced the rate of phenanthrene concentration decline with time.

The cladoceran phenanthrene chronic test was conducted in 100 mL glass beakers that were pre-conditioned with test solutions. The beakers contained 80 mL of test solution which was renewed every MWF for 21 days. A single cladoceran (<24-h-old) was placed in each of ten replicate chambers for each concentration and control. Five concentrations of phenanthrene and a control were tested. At renewal, the organisms were fed a mixture of YTC and green algae (ASTM 1991b), and the surviving adults were transferred to the fresh solution by pipet. If neonates were present, they were counted and discarded.

Analytical Procedures

All stock solutions were prepared by dissolving the test compound in an appropriate reagent grade or better, water soluble organic solvent. A minimum of three working standards were prepared to cover the range of sample concentrations by dilution of the stock in deionized water (DIW) for the fluoranthene and phenanthrene chronic tests, or in hexane for the phenanthrene acute and dieldrin tests.

Dieldrin [CAS# 60-57-1] was obtained from Chem Service, Inc. West Chester, PA at 99.5% purity. Dieldrin was measured by gas chromatography (GC) with electron capture detection (ECD). The instrument used was a Hewlett Packard Model 5880 with a Model 7672A autosampler (Hewlett Packard, Avondale,

PA). The data was analyzed using a PE Nelson Turbochrom data system (Nelson Analytical, Inc., Cupertino, CA). A J & W DB-5 column (J & W Scientific, Folsom, CA) was used for the separation. The column was 0.32 mm I.D. with a 0.25 μm coating and was approximately 20 m in length. The injector temperature was 275 C and the detector was kept at 350 C. The column oven temperature program was 60 C for 1 min, 20 C/min to 225 C, holding for 1 min, then 30 C/min to 300 C, holding for 1 min. One microliter of sample or standard was injected.

Samples were collected in clean, solvent rinsed glass pipets from the center at mid-depth of the test chambers, and placed in clean, solvent rinsed glass bottles with PTFE lined screw caps. Dieldrin was extracted from 5 to 200 mL samples of test solution (depending on concentration) with 5 mL of hexane (B & J non-spectro grade; American Burdick & Jackson, Muskegon, MI) by vigorous stirring, with clean, solvent rinsed PTFE coated stir bars, for at least 30 min. Portions of the hexane layer were placed in clean, unused glass GC vials with PTFE lined crimp tops. Samples were generally analyzed immediately following extraction. A few samples were stored refrigerated due to instrument backlog, but were analyzed with standards stored with them, with no apparent degradation.

Fluoranthene [CAS# 206-44-0] was obtained from Aldrich Chemical Co. Milwaukee, WI at 98% purity. The compound was analyzed by high performance liquid chromatography (HPLC). The HPLC system consisted of two Waters M-45 pumps, a Waters automated gradient controller, a Waters M-490 variable wavelength UV detector (Waters Associates, Millford, MA), a Micromeritics Model 725 autoinjector (Micromeritics Instrument Corporation, Norcross, GA) with a 600 μL loop and a Spectra-Physics SP4270 integrator (Spectra-Physics, San Jose, CA). A Lichrocart 125-4 cartridge column with Lichrospher 100 RP-18 (5 μm) packing (EM Science, Cherry Hill, NJ) was used for the separation. The mobile phase was 85% acetonitrile (UV grade; American Burdick & Jackson, Muskegon, MI) and 15% DIW (Milli-Q; Millipore Corporation, Bedford, MA) and the flow rate was 1.5 mL/min. The wavelength used for detection was 236 nm.

Samples were taken with new glass pipets from the center of the test chamber, at mid-depth and placed in new clean, unused glass autoinjector vials. Samples were directly injected into the HPLC immediately after sampling.

Phenanthrene [CAS# 85-01-8] was obtained from Aldrich Chemical Company at 98+% purity. In the cladoceran acute test, the compound was analyzed by GC with flame ionization detection (FID). The instrument used was a Hewlett Packard Model 5794A with a Model 7671A autosampler. A J & W DB-1 30 m x 0.32 mm I.D. capillary column with a 1 μm coating was used. The injector was held at 250 C and the detector at 310 C. The column oven was 220 C isothermal. A Hewlett Packard Model 3390A integrator was used and the injection volume was about 2.8 μL.

Samples were taken with clean, solvent rinsed glass pipets from the center at mid-depth of the test chambers at mid-depth. The 50- or 100-mL samples were collected in clean, solvent rinsed glass bottles with PTFE lined screw caps. The phenanthrene was extracted into 5 mL of B & J non-spectro grade hexane by vigorous stirring for 30 min with PTFE coated stir bars. One milliliter of the hexane extract was transferred to new glass GC vials with PTFE lined crimp tops. Before analysis, 5 μL of hexadecane (5,200 mg/L) was injected into the vials as an internal standard. Samples were analyzed immediately after sampling and extraction.

The phenanthrene chronic test with the cladoceran was analyzed by HPLC. The instrument was the same as for fluoranthene cladoceran test but only one pump was used and the 85% acetonitrile (B & J UV grade) and 15% DIW mobile phase was pre-mixed. The flow rate was 1 mL/min and a 100 μL loop was used. The wavelength was 251 nm. Samples were collected by the same procedure used for the fluoranthene test.

Test Solution Sampling Schedule

For the cladoceran renewal tests (dieldrin acute and phenanthrene chronic), samples of the new (fresh) solutions were taken on the first day and

renewal days from the containers in which they were prepared, before dispensing into the individual test chambers. The old solutions were sampled from pooled replicates on renewal days and on the last day. For acute tests, new solutions were analyzed at 0 and 24 h and old solutions at 24 and 48 h. Chronic tests were sampled on MWF (renewal days) in old and new solutions. The phenanthrene acute, flow-through, cladoceran test was sampled in all chambers at 0, 24 and 48 h.

The rainbow trout acute flow-through test (dieldrin) was sampled as follows: one half of the test chambers (one set of replicates) each day, alternating the replicates at each sampling. This same pattern was followed for the chronic test but sampling was twice per week (generally on Tuesday and Thursday) with one set of replicates sampled on each day.

For all tests, at least 10% of the samples were run in duplicate and at least 10% were spiked to determine the percent recovery of the compound (Table 2).

Statistical Analysis of Data

LC50 and EC50 calculations with their 95% confidence limits were calculated by the trimmed Spearman Karber method (Hamilton, et al. 1977). Chronic exposure NOECs and LOECs were determined by a one-way analysis of variance (ANOVA) test. Concentrations causing significant differences from controls in growth or reproduction were identified by Dunnett's test (one-sided) at α=0.05 significance.

RESULTS

Acute Tests

Cladocerans (<24-h-old neonates) were exposed to five concentrations of dieldrin and a control (<0.7 μg/L), all in quadruplicate. Mean measured concentrations were 6.4 ± 1.4, 14.2 ± 5.3, 29.6 ± 9.7, 61.1 ± 18.7 and 111 ± 26.7 μg/L after 48 h and 7.2 ± 2.0, 15.9 ± 15.3, 33.5 ± 10.1, 66.1 ± 18.2 and

120 ± 35.4 µg/L after 72 h. Only 10% of the highest (111 µg/L) concentrations were affected at 24 h after initial exposure. After 48 h of exposure, the highest (111 µg/L) concentration had 95% affected, with very little movement. The test was renewed for another 24 h, at which time 90% mortality was observed in the highest (120 µg/L) concentration, 72 h after initial exposure. At this time, 15% mortality was observed in the second highest (66.1 µg/L) exposure. The three remaining exposures and controls showed no effects. The 48-h EC50 estimate and its 95% confidence limits for *Daphnia magna* are 79.5 (74.0-85.4) µg/L. The 72-h LC50 estimate and its 95% confidence limits are 81.4 (72.7-91.1) µg/L (Table 3).

Rainbow trout fingerlings (mean standard length, 49.0 ± 5.7 mm; mean weight, 1580 ± 591 mg) were exposed to five concentrations of dieldrin and a control (<1.0 µg/l), in quadruplicate, in flow-through exposures. Mean measured concentrations were 1.53 ± 0.19, 4.00 ± 0.40, 7.04 ± 0.94, 17.4 ± 4.11 and 31.0 ± 10.8 µg/L. Deaths began occurring within 24 h of initial exposure in the two highest (17.4 and 31.0 µg/L) concentrations with 10% and 70% mortality, respectively at that time. The highest (31.0 µg/L) concentration had 100% mortality by 48 h from initial exposure, and the second highest (17.4 µg/L) had 100% mortality at test termination. The third highest (7.04 µg/L) concentration had 30% mortality and the fourth highest (4.00 µg/L) had 10% mortality at test termination. There were no deaths or effects in the remaining exposure or control. The 96-h LC50 estimate and its 95% confidence limits for *Oncorhynchus mykiss* are 8.23 (6.86-9.87) µg/L (Table 3).

Cladocerans (<24-h-old neonates) were exposed to five mean measured concentrations (30 ± 0, 70 ± 10, 150 ± 20, 360 ± 20 and 650 ± 100 µg/L) of phenanthrene and a control (<10 µg/L), in duplicate in flow-through exposures. Affects were not observed until 48 h after initial exposure in the two highest (360 and 650 µg/L) concentrations, with the severity of effect increasing with concentration. There was no movement, just heartbeat in some organisms in the highest (650 µg/L) exposure. The third highest (150 µg/L) showed no effects,

and the two lowest exposures and control were not affected. The 48-h EC50 estimate for *Daphnia magna* is 230 µg/L (Table 3). The 95% confidence limits are not reliable because no intermediate exposure concentration had a partial effect.

Chronic Tests

Rainbow trout (freshly fertilized ova) were exposed to five nominal concentrations of dieldrin and a control (<0.02 µg/L), all in quadruplicate, in flow-through exposures. Mean measured concentrations were 0.04 ± 0.02, 0.15 ± 0.05, 0.30 ± 0.07, 0.55 ± 0.10 and 0.95 ± 0.14 µg/L. Fluoranthene exposures lasted for 90 days and began with ova and continued through the fry stage of development. Hatching began on day 30. Percent hatch was not affected by these concentrations. Deaths began occurring 28 days post-hatch with 20% mortality in the highest (0.95 µg/L) exposure at that time. There was 35% mortality in the highest (0.95 µg/L) and 2% mortality in the second and third highest (0.55 and 0.30 µg/L, respectively) exposures at test termination. There were no deaths in any of the remaining exposures or control. Mean standard length and wet weight are both significantly (α=0.05) different from controls at the highest (0.95 µg/L) concentration (Table 4). Scoliosis was observed in the highest (0.95 µg/L) exposure by day 56 and in the second and third highest (0.55 and 0.30 µg/L, respectively) exposures by day 87. The NOEC for *Oncorhynchus mykiss* exposed to dieldrin is 0.55 µg/L and the LOEC is 0.95 µg/L (Table 3). The chronic value (geometric mean of the NOEC and LOEC) for this test is 0.72 µg/L.

Cladocerans (<24-h-old neonates) were exposed to five concentrations of fluoranthene and a control (<3.8 µg/L) without additional UV light. Measured concentrations (mean of new solutions) were 6.9 ± 3.8, 17.0 ± 2.6, 35.3 ± 1.7, 73.2 ± 3.2 and 148 ± 7.0 µg/L. The decrease in fluoranthene concentrations averaged 74.3% for the five exposure concentrations (48 and 72 h between renewals). Ten replicates with one neonate per replicate were used for each exposure and control. Deaths began occurring within 72 h of initial exposure with 50% mortality in the highest (148 µg/L) and 10% mortality in the second

highest (73.2 μg/L) concentration and also in the control at that time. One organism was missing from a control chamber on day 7 and was presumed lost by adhering to the dissolved oxygen analyzer probe. The organisms remaining in the highest (148 μg/L) exposure continued to die until there was 100% mortality by test termination. The control had 20% mortality at the end of the exposure. Reproduction began by day 10 in all fluoranthene concentrations and control, except the highest (148 μg/L) exposure which had a few young by day 12. The mean length of surviving adults is significantly (α=0.05) different from controls at concentrations ≥35.3 μg/L (Table 5). The NOEC for *D. magna* exposed to fluoranthene without additional UV light is 17.0 μg/L and the LOEC based upon growth is 35.3 μg/L (Table 3). The chronic value (geometric mean of the NOEC and LOEC) for this test is 24.50 μg/L.

Cladocerans (<24-h-old neonates) were exposed to five measured concentrations of phenanthrene and a control (<4.7 μg/L). Measured concentrations (mean and new solutions) were 24 ± 7, 48 ± 4, 93 ± 7, 183 ± 11 and 345 ± 11 μg/L. During the 48 to 72 between test solution renewal, phenanthrene concentrations declined an average of 50.2 percent. Ten replicates with one neonate per replicate were used for each exposure and control. Deaths began occurring within 72 h of test initiation with 50% mortality in the highest (345 μg/L) concentration by day 5 and 90% mortality by test termination at the same concentration. The second highest (183 μg/L) concentration had 20% mortality at test termination with the deaths occurring during the last one-third of the test. The 48 and 93 μg/L exposures each had 10% mortality by test termination. There was 20% mortality in the control and no mortality in the lowest exposure (24 μg/L) at test termination. Reproduction began by day 9 in all concentrations and control, except in the highest (345 μg/L) exposure which had no young produced. The mean length of surviving adults is significantly (α=0.05) different from controls at the three highest (93, 183 and 345 μg/L) concentrations (Table 6). The NOEC for *D. magna* exposed to phenanthrene is 48 μg/L and the LOEC based upon growth is 93 μg/L (Table 3). The chronic value (geometric mean of NOEC and LOEC) for this test is 66.81 μg/L.

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TABLE 1. Water Characteristics of Dieldrin, Fluoranthene and Phenanthrene Exposures to the Cladoceran, *Daphnia magna*, and Rainbow Trout, *Oncorhynchus mykiss*. Mean ± Standard Deviation, Range (in Parentheses) and Number of Observations (n).

Compound	Organism & Test Type[1/]	Temperature (C)	Dissolved Oxygen (mg/L)	Total Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	pH	Specific Conductivity (µmhos/cm)
Dieldrin	Cladoceran R, Acute	19.9±0.4 (19.0-20.5) n=12	8.4±0.5 (7.7-8.8) n=12	170±2 (168-172) n=4	122±4 (120-128) n=4	8.46±0.06 (8.36-8.59) n=12	500±0 (500-500) n=4
	Rainbow trout FT, Acute	9.7±0.6 (8.3-10.9) n=120	8.9±0.8 (7.3-10.8) n=72	46.6±1.1 (44.6-48.0) n=8	51.9±1.3 (50.2-54.2) n=8	7.51±0.28 (7.11-8.19) n=71	90±2 (86-92) n=24
	Rainbow trout FT, Chronic	9.9±0.9 (7.4-14.7) n=2087	9.1±1.3 (5.2-11.5) n=537	50.4±4.9 (42.0-70.0) n=75	49.4±4.5 (36.0-64.0) n=75	7.54±0.22 (6.79±8.12) n=459	98±3 (85-109) n=445
Fluoranthene	Cladoceran R, Chronic	20.8±1.4 (18.8-24.5) n=104	9.0±0.5 (7.8-9.9) n=86	189±16 (164-226) n=22	115±12 (92-138) n=22	8.42±0.07 (8.22-8.58) n=34	694-54 (580-800) n=23
Phenanthrene	Cladoceran FT, Acute	23.3±0.7 (21.7-24.4) n=72	7.2±0.2 (7.0-7.5) n=12	51.3±1.3 (48.6-52.4) n=8	53.7±2.4 (51.0-58.0) n=8	7.80±0.11 (7.67-7.97) n=12	134±3 (131-138) n=8
	Cladoceran R, Chronic	21.2±0.9 (19.0-23.2) n=246	7.9±0.4 (7.2-9.1) n=240	162±10 (140-177) n=16	108±13 (84.0-120) n=16	8.42±0.14 (8.06-8.82) n=240	499±32 (300-600) n=240

[1] FT=Flow-through; R=Renewal.

TABLE 2. Percentage Agreement of Duplicates and Spike Recoveries, and Detection Limits for the Dieldrin, Fluoranthene and Phenanthrene Exposures of the Cladoceran, *Daphnia magna*, and Rainbow Trout, *Oncorhynchus mykiss*.

Compound	Organism	Test Type[1]	Mean ± Standard Deviation %Agreement	%Recovery	Detection Limit (μg/L)[2]
Dieldrin	Cladoceran	A/R	98.5±0.4	113.8±11.0	0.7
	Rainbow Trout	A/FT	97.2±3.1	102.6± 3.6	1.0
	Rainbow Trout	C/FT	92.5±9.1	106.0±20.4	0.018
Fluoranthene	Cladoceran	C/R	96.7±3.6	104.4±12.2	3.8
Phenanthrene	Cladoceran	A/FT	91.6±5.1	103.3± 7.7	10
	Cladoceran	C/R	98.6±1.8	95.6± 7.7	4.7

[1] A=Acute test; C=Chronic test; R=Renewal; FT=Flow-through.
[2] Calculated by the U.S. EPA recommended method published in *Federal Register*. Vol. 49, No. 209, October 26, 1984.

TABLE 3. Effect Concentrations (μg/L) of Acute and Chronic Exposures of Dieldrin, Fluoranthene and Phenanthrene to the Cladoceran, *Daphnia magna*, and Rainbow Trout, *Oncorhynchus mykiss*.

Compound	Organism	Test Type[1] and Duration	Effect	Concentration (95% Confidence Limits)
		ACUTE TESTS		
Dieldrin	Cladoceran	R (48 h)	EC50	79.5 (74.0-85.4)
		R (72 h)	LC50	81.4 (72.7-91.1)
	Rainbow trout	FT (96 h)	LC50	8.23 (6.86-9.87)
Phenanthrene	Cladoceran	FT (48 h)	EC50	230 [2]
		CHRONIC TESTS		
Dieldrin	Rainbow trout	FT (90 d)	NOEC	0.55
			LOEC[3]	0.95
Fluoranthene	Cladoceran	R (21 d)	NOEC	17.0
			LOEC[4]	35.3
Phenanthrene	Cladoceran	R (21 d)	NOEC	48
			LOEC[4]	93

[1] FT=Flow-through; R=Renewal; All tests with measured concentrations of toxicant.
[2] 95% Confidence limits are not reliable.
[3] Lowest Observed Effect Concentration based upon total length, wet weight and survival.
[4] Lowest Observed Effect Concentration based upon total length of surviving adults.

TABLE 4. Survival and Growth of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to Dieldrin for 90 Days Post-Fertilization.

	Mean Measured Dieldrin Concentration (μg/L)					
	<0.02	0.04	0.15	0.30	0.55	0.95
Mean percent survival at 55 days post-hatch	100	100	100	98.3	98.3	65.0*
Mean wet weight (g) at 55 days post-hatch	0.474	0.469	0.474	0.459	0.458	0.315*
Mean standard length (mm) at 55 days post-hatch	34.2	33.8	34.1	33.1	33.3	29.7*

*Significantly less than control at α=0.05.

TABLE 5. Survival, Reproduction and Growth of the Cladoceran, *Daphnia magna*, Exposed to Fluoranthene in a 21-Day Chronic Test.

	Fluoranthene Concentrations (μg/L)					
Nominal	0	10	20	40	80	160
Measured[1]	<3.8	6.9±3.8	17.0±2.6	35.3±1.7	73.2±3.2	148±7.0
Percent Adult Survival	80[2]	100	100	90	70	0
Mean Number of Young/Surviving Adult at 21 Days	53.4	60.1	53.7	55.8	33.7*	0*
Day of First Brood	10	10	10	10	10	12
Mean Total Length (mm) of Adults at 21 Days	4.8	4.5	4.5	4.0*	3.6*	---

[1] Mean of fresh test solutions and old solutions at renewal.
[2] One control organism disappeared from test chamber. Control survival may have been 90% otherwise.
* Significantly less than controls at α=0.05.
MATC=50.83 μg/L for reproduction and 24.50 μg/L for growth.

TABLE 6. Survival, Reproduction and Growth of the Cladoceran, *Daphnia magna*, Exposed to Phenanthrene in a 21-Day Chronic Test.

	Mean[1/] Measured Phenanthrene Concentration (µg/L)					
	<4.7	24±7	48±4	93±7	183±11	345±1
Percent Adults Survival	80	90	90	90	80	10
Mean Number of Young/ Surviving Adult at 21 Days	55.9	44.3	58.8	57.8	57.6	0*
Day of First Brood	9	9	8	9	9	[2/]
Mean Total Length (mm) of Adults at 21 Days	4.8	4.7	4.6	4.2*	3.9*	2.3*

[1/] Mean and standard deviation of fresh test solutions at renewal.
[2/] No young produced at this concentration.
* Significantly less than controls at α=0.05.
MATC=251.3 µg/L for reproduction and 66.81 µg/L for growth.