Acute and Chronic Toxicity of Several Pesticides to Five Species of Aquatic Organisms

by

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The Environmental Health Laboratory at the University of Wisconsin-Superior (UW-S) conducted tests to complete the data sets necessary to derive the national water quality criteria for seven pesticides. Acute tests with two plant species were also run.

Acute 96-h static tests with a green algae (*Selenastrum capricornutum*) and acute 96-h renewal tests with a duckweed (*Lemna minor*) were completed using carbaryl, dichlorvos and propoxur. The same tests were run with carbofuran, except that the duckweed test was static. An acute 96-h flow-through test with an amphipod (*Gammarus pseudolimnaeus*) was also completed using carbofuran. An acute 96-h renewal test and a chronic 7-day renewal test with p,p'-DDT were completed using a cladoceran (*Ceriodaphnia dubia*). Acute 96-h flow-through tests with the compounds dieldrin and endrin were completed using an annelid (*Lumbriculus variegatus*).

Methods

Test Organisms

Algae inoculum was obtained from Carolina Biological Supply, Inc. (Burlington, NC). The algae were cultured in nutrient media (ASTM 1991a) which was aseptically prepared. Cells were concentrated by centrifugation, resuspended in media solution and quantified with a hemocytometer for preparation of the desired cell density for test initiation.

Duckweed were from cultures maintained at the UW-S laboratory. They were cultured in a duckweed nutrient solution that was essentially the same as that recommended by ASTM (ASTM 1991b) under continuous light (100-300 ft candles). Plants at the two frond stage of development were used to initiate the tests.

Annelids were cultured and reared at the UW-S laboratory in flowing dechlorinated lab water. The stock culture originated from Eastman Kodak laboratories. The organisms were cultured on a substrate of shredded unbleached paper towels, which they used for cover and a food source. They were also fed adult brine shrimp (*Artemia salinus*) 3 times daily during

culturing.

Amphipods maintained at the UW-S laboratory were originally collected from feral populations in the Eau Claire River in Douglas County near Gordon, WI. They were fed YTC (yeast-trout chow-cereal leaves) and the water was renewed weekly.

Cladocerans were cultured and reared at the UW-S laboratory through many generations. They were cultured in hard (160-180 mg/L as $CaCO_3$) reconstituted water (ASTM 1991c) which was renewed three times weekly (MWF). Daphnids were fed YTC (yeast-trout chow-cereal leaves) and algae (*Selenastrum capricornutum*) on a MWF regime.

Exposure Conditions

All tests were performed at the UW-S campus laboratories. The water supply for the amphipod and annelid tests was the municipal water supply for the city of Superior, WI. The water was dechlorinated by charcoal filtration and subsequent sulfite addition. The water was then passed through a cation exchange column to remove heavy metals. Tests with algae, duckweed and cladocerans were carried out in deionized water with appropriate salts and nutrients added (ASTM 1991 a,b,c).

Flow-through tests were conducted in modified proportional mini-diluters (Benoit, et al. 1982). Primary exposure chambers were constructed of plate glass with dimensions 24 x 14 x 10 cm (L x W x H) which contained 2.1 or 2.4 L of water.

All tests consisted of five toxicant concentrations with a dilution factor of 0.5, plus a clean water control. Green algae tests consisted of three replicate chambers with 1 x 10^4 cells in each, except in the dichlorvos test which started with 2 x 10^5 cells in each. Duckweed tests consisted of four replicate chambers with ten 2-frond colonies in each. The gammarus and annelid tests consisted of two replicates with ten organisms in each. The cladoceran acute consisted of four replicate chambers with five organisms in each and the chronic consisted of ten replicates with one organism per

chamber.

Green algae were exposed in 300 mL Erlenmeyer flasks containing 100 mL of test solution. The flasks were loosely covered with aluminum foil and were continuously shaken on a shaker table in a temperature and light controlled chamber.

Duckweed were exposed in 100 mL beakers containing 40-100 mL of test solution containing the nutrients recommended by ASTM (ASTM 1991b). Solution was renewed daily in renewal tests.

Annelids were exposed in 250 mL glass beakers with 20 mm diameter holes in the sides covered with nylon mesh (30 μm openings). These were placed in the primary exposure chambers of the diluter to confine the organisms and permit easier observation of effects.

Cladoceran tests were conducted in 30 mL glass beakers containing 15 mL of solution which was renewed daily. Organisms were not fed during the 48-hour acute tests. Organisms in the chronic exposures were fed at each solution renewal with YTC and algae. Tests were conducted in a temperature and light regulated environmental chamber.

Amphipods were exposed in 30 mL beakers with the bottom removed and replaced with nylon mesh. The beakers were suspended by stainless steel wire in the primary exposure chamber. Each beaker was provided with an 11 mm leaf plug (*Betula* sp. and *Populus* sp.).

Organisms were not fed during the acute exposures and mortalities or effects observed were recorded daily.

Dissolved oxygen was measured in the control, low, middle and high exposures at least at the initiation of each test and every 48 h thereafter (Table 1), where possible. The control and high exposures were measured in the algae tests at test initiation.

Total hardness (EDTA), total alkalinity and pH were measured at least once during acute exposures and the seven day cladoceran chronic (Table 1). Temperatures were recorded daily (Table 1). Test organism biomass loading requirements stated by ASTM (1991a,b,c) were not exceeded. The initial cell

density (1 x 10^4 cells/mL) for the algae tests, except the dichlorvos test, was one half of the ASTM (1991a) recommended cell density.

The test chemical was added to the test water by either direct addition of a weighed quantity or by dissolution from a glass wool column coated with the chemical.

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 expected sample concentrations, by dilution of this stock in deionized water (DIW) for carbaryl, carbofuran and propoxur, in hexane for p,p'-DDT, endrin and dieldrin, and in toluene for dichlorvos.

Carbaryl [CAS #63-25-2] was obtained from Chem Service, Inc. (West Chester, PA) at 98.0% purity. It was analyzed by high pressure liquid chromatography (HPLC). The HPLC system consisted of two Waters M-45 pumps, a Waters automated gradient controller, a Waters M-490 UV detector (Waters Associates, Millford, MA), a Micromeritics Model 725 autoinjector (Micromeritics Instrument Corp., Norcross, GA), 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 consisted of 47 percent acetonitrile (UV grade, American Burdick & Jackson, Muskegan, MI) and 53% deionized water (Millipore Corp., Bedford, MA) at a flow rate of 1.5 mL/min. A five μL injection loop was used for the duckweed test and a ten μL loop was used for the algae test. The detector was set at 222 nm wavelength.

Carbofuran [CAS #1563-66-2] was obtained from Chem Service, Inc., at 98.5% purity. It was analyzed by HPLC on the above system. The mobile phase was as above for the duckweed test and 45:55 acetonitrile/DIW for the algae and amphipod tests. The flow rate was 1.5 mL/min for the duckweed and amphipod tests and 1.25 mL/min for the algae test. The loop sizes used were

100 μL for the algae test, 10 μL for the duckweed test and 10 mL for the amphipod test. The detector was set at 274 nm wavelength.

Propoxur [CAS #114-26-1] was obtained from Chem Service, Inc., at 99.0% purity. It was analyzed by HPLC on the above system. The mobile phase consisted of 60:40 acetonitrile/DIW at a flow rate of 1.0 mL/min. The loop was ten μL and the detector was set at 220 nm wavelength.

Samples for the HPLC tests were taken with glass pipets from the center of the test chamber at mid depth. They were placed in new glass autoinjector vials or, for the carbofuran amphipod test, in clean, solvent-rinsed glass culture tubes with teflon-lined screw caps. Samples were directly injected into the HPLC immediately after sampling.

The p,p'-DDT [CAS #50-29-3] was obtained from Chem Service, Inc. at 98.5% purity. It was analyzed by gas chromatography (GC) with electron capture detection (ECD). A Hewlett Packard Model 5880A GC with an HP 7672A autosampler (Hewlett Packard, Avondale, PA) was used. The column was a J & W 60 m x 0.32 mm capillary column with a 0.25 μm DB-5 coating (J & W Scientific, Folsom, CA). The injector temperature was 250°C and the detector was 350°C. The column oven temperature program was 60°C for 1 min, 20°C/min to 260°C, holding for 6 minutes. A one μL injection was used.

Dichlorvos [CAS #62-73-7] was obtained from Chem Service, Inc., at 99% purity. It was analyzed by GC using an HP 5794A with an HP 7671A autosampler with flame ionization detection (FID). The column was a J & W 30 m x 0.32 mm capillary with a 1 μm DB-1 coating. The injector temperature was 250°C and the detector was 310°C. The column oven was held at 145°C. The split ratio was 18:1. A one μL injection was used.

Dieldrin [CAS #60-57-1] was obtained from Chem Service, Inc. at 99.5% purity. It was analyzed by GC using the HP 5880A with the HP 7672A autosampler and ECD detector. The column was a J & W clipped to 20 m x 0.32 mm with a 0.25 μm DB-5 coating. The injector temperature was 275°C and the detector was 350°C. The column oven temperature program was 60° C for one minute, 20°C/min to 225°C, holding for one minute, then 30°C/min to 300°C,

holding for one minute. A one μL injection was used.

Endrin [CAS #72-20-8] was obtained from Chem Service, Inc., at 99.0% purity. It was analyzed by GC using the system employed for dieldrin. The column was a J & W 60 m x 0.32 mm capillary with a 0.25 μm DB-5 coating. The injector temperature was 200°C and the detector was 350°C. The column oven temperature program was 60°C for one minute, 20°C/min to 260°C, holding for 6 min. A one μL injection was used.

Samples for GC analysis were taken with clean glass pipets from the center of the exposure chamber at mid-depth and placed in clean glass extraction bottles with teflon-lined caps. The compound was extracted into either hexane or toluene by vigorous stirring for at least 30 min. The extract was then placed in a GC vial for analysis.

Test Solution Sampling Schedule

The algae and duckweed static tests were sampled on the first, middle and last days of the test (MWF), except the propoxur algae test which was sampled daily. Algae tests were sampled from surrogate flasks which did not contain algae. The duckweed renewal with carbaryl was sampled as follows: new solutions on day of preparation and one-half of the old solutions alternating odds and evens, except the last day when all the old solutions were sampled. The duckweed renewal with propoxur was sampled the same except that all the old solutions were sampled every day. The flow-through tests with amphipods and annelids were sampled as follows: all of the replicates on MWF and one-half of the replicates T and the other half TH. The cladoceran renewal tests were sampled as follows: all new solutions on the first day and renewal days from the containers in which they were prepared before dispensing into the test chambers; the old solutions were sampled from pooled replicates on renewal days and on the last day.

In 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, IC50 and EC50 calculations with their 95% confidence limits were calculated by the trimmed Spearman-Karber method (Hamilton, et al. 1977) (Table 3). For the plant tests and the cladoceran chronic exposure, the highest observed no-effect concentration (NOECs) and the lowest observed effect concentration (LOECs) were determined by a one-way analysis of variance (ANOVA) test. The tests were analyzed for equality of variances by Bartlett's test before the 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 (Steele and Torrie, 1980) (Table 3). The chronic value for the 7-day cladoceran test was calculated as the geometric mean of the LOEC and the NOEC.

Results

Carbaryl

Green algae were exposed to five measured concentrations (50 ± 60, 90 ± 100, 170 ± 170, 330 ± 350 and 720 ± 790 µg/L) of carbaryl, plus controls (<21 µg/L), in triplicate static exposures. The mean values are approximately 53% of the initial concentrations, and the lowest two exposures were below the detection limit (<21 µg/L) by 96 h. The final cell count was significantly (α=0.05) different from control in the four highest exposures (90, 170, 330 and 720 µg/L) at 96 h (Table 4). The NOEC was 50 µg/L and the LOEC was 90 µg/L (Table 3). The 96-h IC50 estimate and its 95% confidence limits for *Selenastrum capricornutum* exposed to carbaryl are 490 (210-1,120) µg/L (Table 3).

Duckweed (2-frond stage) were exposed to five measured concentrations (3,300 ± 1,400, 5,800 ± 500, 12,000 ± 900, 23,200 ± 1,000 and 45,200 ± 4,000 µg/L) of carbaryl, plus controls (<290 µg/L), in quadruplicate renewal exposures. The final frond count was significantly (α = 0.05) different from control in all exposures (3,300, 5,800, 12,000, 23,200 and 45,200 µg/L) at 96 h (Table 5). The NOEC was <3,300 µg/L and the LOEC was 3,300 µg/L

(Table 3). The 96-h IC50 estimate and its 95% confidence limits for *Lemna minor* exposed to carbaryl are 23,900 (16,500-34,600) µg/L (Table 3).

Carbofuran

Green algae were exposed to five measured concentrations (400 ± 100, 1,000 ± 200, 2,200 ± 200, 4,100 ± 400 and 9,000 ± 700 µg/L) of carbofuran, plus controls (<60 µg/L), in triplicate static exposures. The mean values are approximately 92% of the initial concentrations. The final cell count was significantly (α=0.05) different from control in the four highest exposures (1,000, 2,200, 4,100, and 9,000 µg/L) at 96 h (Table 6). The NOEC was 400 µg/L and the LOEC was 1,000 µg/L (Table 3). The 96-h IC50 estimate and its 95% confidence limits for *Selenastrum capricornutum* exposed to carbofuran are 1,980 (1,260-3,110) µg/L (Table 3).

Duckweed (2-frond stage) were exposed to five measured concentrations (17,100 ± 1,800, 36,600 ± 700, 70,500 ± 8,900, 149,000 ± 14,200 and 292,000 ± 17,200 µg/L) of carbofuran, plus controls (<430 µg/L), in quadruplicate static exposures. The mean values are approximately 98% of the initial concentrations. The final frond count was significantly (α=0.05) different from control in the four highest exposures (36,600, 70,500, 149,000 and 292,000 µg/L) at 96 h (Table 7). The NOEC was 17,100 µg/L and the LOEC was 36,600 µg/L (Table 3). The 96-h IC50 estimate and its 95% confidence limits for *Lemna minor* exposed to carbofuran are 236,000 (170,000-326,000) µg/L (Table 3).

Amphipods (mean weight 47 ± 11 mg) were exposed to five measured concentrations (0.12 ± 0.11, 0.61 ± 0.34, 2.8 ± 1.0, 5.8 ± 0.84 and 10.4 ± 0.91 µg/L) of carbofuran, plus controls (<0.08 µg/L), in duplicate flow-through exposures. Deaths began occurring in the highest exposure (10.4 µg/L) and third highest (2.8 µg/L) by 24 h after initial exposure (Table 8). There was 100% mortality in the highest exposure (10.4 µg/L) by 48 h after test initiation. At test termination, the second highest exposure (5.8 µg/L) had 85% mortality and the third highest (2.8 µg/L) had 15% mortality. No deaths

occurred in any of the remaining exposures or control. The 96-h LC50 estimate and its 95% confidence limits for *Gammarus pseudolimnaeus* exposed to carbofuran are 3.76 (3.06-4.64) µg/L (Table 3).

p,p'-DDT

Cladocerans (<24-h-old) were exposed to five measured concentrations (means of old and new solutions, 0.49 ± 0.09, 0.89 ± 0.10, 1.18 ± 0.38, 2.37 ± 1.02 and 5.31 ± 3.65 µg/L of p,p'-DDT, plus controls (<0.16 µg/L), in quadruplicate renewal exposures. Effects (lying on the bottom, barely moving) or mortalities began occurring by 24 h after test initiation in the three highest exposures (1.18, 2.37 and 5.31 µg/L) (Table 9). By 48 h, the percent dead or affected was 100% in the three highest exposures (1.18, 2.37 and 5.31 µg/L), 45% in the fourth highest (0.89 µg/L), 15% in the lowest (0.49 µg/L) and 5% in the control. The 48-h EC50 estimate and its 95% confidence limits for *Ceriodaphnia dubia* exposed to p,p'-DDT were 0.83 (0.71-0.97) µg/L (Table 3).

Cladocerans (<24-h-old neonates) were exposed for seven days to five measured concentrations (means of old and new solutions, <0.53, 0.62 ± 0.34, 0.99 ± 0.54, 1.74 ± 0.82 and 3.57 ± 2.06 µg/L of p,p'-DDT, plus controls (<0.53 µg/L). Ten replicates with one neonate per replicate were used for each exposure and control. Deaths began occurring within 48-h of initial exposure with 100% mortality in the highest (3.57 µg/L) exposure at 96 h (Table 10). At test termination, the percent mortality was 40% in the second highest (1.74 µg/L) exposure, 20% in the third highest (0.99 µg/L), 10% in the fourth highest (0.62 µg/L) and also in the control. No deaths occurred in the lowest (<0.53 µg/L) exposure. In the controls, 60% of the adults had three broods with an average of 17.3 young per female. Reproduction was not significantly (α=0.05) different from control in any exposure where adults survived. Although the reproduction in the second highest (1.74 µg/L) exposure is much lower than in the control, the high variability (reflected in the standard deviations) makes the difference insignificant at α=0.05. The

percent survival in the highest (3.57 µg/L) exposure was significantly (α=0.05) different from control at test termination. The NOEC for *Ceriodaphnia dubia* exposed to p,p'-DDT was 1.74 µg/L and the LOEC based upon survival was 3.57 µg/L (Table 3). The chronic value for this test is 2.49 µg/L.

Dichlorvos

Green algae were exposed to five measured concentrations (13,800 ± 6,400, 29,000 ± 7,800, 56,800 ± 8,300, 119,000 ± 11,000 and 239,000 ± 14,000 µg/L) of dichlorvos, plus controls (<1,200 µg/L), in triplicate static exposures. The mean values are approximately 84% of the initial concentrations. The dry weight of the standing crop was not significantly (α=0.05) different from control in any of the exposures at 96 h (Table 11). The 96-h IC50 estimate and its 95% confidence limits for *Selenastrum capricornutum* exposed to dichlorvos are 110,000 (93,700-130,000) µg/L (Table 3).

Duckweed (2-frond stage) were exposed to five measured concentrations (24,200 ± 5,500, 52,800 ± 12,000, 105,000 ± 13,900, 228,000 ± 19,500 and 471,000 ± 21,300 µg/L) of dichlorvos, plus controls (<1,200 µg/L), in quadruplicate static exposures. The mean values are approximately 90% of the initial concentration. The final frond count was significantly (α=0.05) different from control in all exposures at 96 h (Table 12). The NOEC was <24,200 µg/L and the LOEC was 24,200 µg/L (Table 3). The 96-h IC50 estimate and its 95% confidence limits for *Lemna minor* exposed to dichlorvos are 398,000 (293,000-541,000) µg/L (Table 3).

Dieldrin

Annelids (adult, mean weight 3.4 ± 0.4 mg) were exposed to five measured concentrations (15.9 ± 3.3, 29.8 ± 3.8, 46.8 ± 2.4, 88.1 ± 7.7 and 159 ± 15.5 µg/L) of dieldrin, plus controls (<1.0 µg/L), in duplicate flow-through exposures. No deaths occurred in any of the exposures or controls (Table 13).

Effects (coiled and inactive organisms) were observed in the highest (159 µg/L) exposure by 24 h after test initiation. At 72 h the three highest (46.8, 88.1 and 159 µg/L) exposures were affected and by 96 h the four highest (29.8, 46.8, 88.1 and 159 µg/L) concentrations were affected. The 96-h EC50 estimate for *Lumbriculus variegatus* exposed to dieldrin was 21.8 µg/L (Table 3). The 95% confidence limits could not be determined because no partial effects were observed in any exposure concentration.

Endrin

Annelids (adult, mean weight 1.7 mg) were exposed to five measured concentrations (17.6 ± 1.7, 34.9 ± 3.1, 52.1 ± 3.2, 88.5 ± 3.4 and 165 ± 7.3 µg/L) of endrin, plus controls (<4.2 µg/L), in duplicate flow-through exposures. No deaths occurred in any exposure or control (Table 14). Effects were observed (coiled tightly together and inactive) in the four highest (34.9, 52.1, 88.5 and 165 µg/L) exposures by 24 h after test initiation. By 96 h all exposures showed effects, but the three highest (52.1, 88.5 and 165 µg/L) were much more affected. When the organisms were prodded to uncoil, no movement was observed in these except under magnification, whereas in the two lowest (17.6 and 34.9 µg/L) concentrations the organisms reacted to gentle prodding. The 96-h EC50 estimate for *Lumbriculus variegatus* exposed to endrin was 42.6 µg/L (Table 3). The 95% confidence limits could not be determined because no partial effects were observed in any exposure concentration.

Propoxur

Green algae were exposed to five measured concentrations (1,620 ± 370, 3,350 ± 820, 7,430 ± 1,540, 14,400 ± 2,620 and 27,500 ± 5,330 µg/L) of propoxur, plus controls (<150 µg/L), in triplicate static exposures. The mean values are approximately 83% of the initial concentrations. The final cell count was significantly (α=0.05) different from control in the three highest (7,430, 14,400 and 27,500 µg/L) exposures at 96 h (Table 15). The NOEC was 3,350 µg/L and the LOEC was 7,430 µg/L (Table 3). The 96-h IC50 estimate and

its 95% confidence limits for *Selenastrum capricornutum* exposed to propoxur were 6,320 (5,420-7,360) µg/L (Table 3).

Duckweed (2-frond stage) were exposed to five measured concentrations (means of old and new solutions, 11,700 ± 500, 24,600 ± 400, 47,600 ± 3,500, 98,500 ± 5,400 and 198,000 ± 6,100 µg/L) of propoxur, plus controls (<1,710 µg/L), in quadruplicate renewal exposures. The final frond count was significantly (α=0.05) different from control in the three highest (47,600, 98,500 and 198,000 µg/L) exposures at 96 h (Table 16). The NOEC was 24,600 µg/L and the LOEC was 47,600 µg/L (Table 3). The 96-h IC50 estimate for *Lemna minor* exposed to propoxur was greater than 198,000 µg/L (Table 3). The 95% confidence limits could not be determined because the % inhibition was not greater than 50% in any exposure concentration.

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Table 1. Water Characteristics of Pesticide Exposures of Various Freshwater Organisms. Mean ± Standard Deviation, Range in Parentheses and Number of Observations (n).

Chemical Compound	Organism and 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)
Carbaryl	Green Algae S, Acute	25.0±0.4 (24.4-25.6) n=30	8.5±0.1 (8.4-8.6) n=2	81.9±5.5 (78.0-85.8) n=2	36.0±5.6 (32.0-40.0) n=2	7.14±0.02 (7.12-7.15) n=2	246±6 (242-250) n=2
	Duckweed R, Acute	24.5±1.9 (19.6-26.2) n=28	8.1±0.4 (7.4-8.9) n=16	171±6 (156-176) n=8	90.5±5.2 (84.0-100) n=8	7.56±0.28 (6.60-7.81) n=16	782±5 (780-790) n=4
Carbofuran	Green Algae S, Acute	24.8±0.3 (24.3-25.3) n=30	8.4±0.1 (8.4-8.5) n=2	91.5±30.4 (70.0-113) n=2	49.0±29.7 (28.0-70.0) n=2	7.48±0.14 (7.38-7.58) n=2	327±153 (219-435) n=2
	Duckweed S, Acute	23.9±0.2 (23.6-24.2) n=30	8.1±0.3 (7.8-8.4) n=12	175±3 (172-179) n=4	91.0±3.8 (88.0-96.0) n=4	7.81±0.60 (7.71-7.94) n=12	755±26 (720-780) n=4
	Amphipod FT, Acute	17.6±0.9 (15.8-19.2) n=60	9.9±0.3 (9.2-10.3) n=11	50.7±3.2 (46.8-54.6) n=4	40.0±0 (40.-40.0) n=4	6.70±0.23 (6.45-7.04) n=11	114±2 (112-115) n=4
p,p'-DDT	Cladoceran R, Acute	25.3±0.1 (25.1-25.4) n=10	8.1±0.3 (7.6-8.6) n=10	182±6 (178-186) n=2	113±1 (112-114) n=2	8.19±0.06 (8.15-8.23) n=2	ND[2/]
	Cladoceran R, Chronic	24.8±0.3 (24.0-26.0) n=77	7.9±0.2 (7.6-8.2) n=48	194±29 (160-218) n=4	104±20 (80.0-120) n=4	7.90±0.16 (7.65-8.12) n=32	520±25 (495-550) n=29
Dichlorvos	Green algae S, Acute	25.5±0.3 (24.7-26.1) n=40	ND	54[3/]	16[3/]	7.0±0.8 (4.3-7.7) n=30	ND

Table 1. (continued)

	Duckweed R, Acute	22.3±0.7 (21.5-24.0) n=52	8.4±0.6 (7.0-10.2) n=39	187±8 (170-200) n=12	96.5±18.2 (60.0-126) n=12	8.47±0.22 (8.00-8.80) n=39	ND
Dieldrin	Annelid FT, Acute	21.0±0.7 (19.9-22.1) n=72	8.3±0.5 (7.9-9.1) n=12	61.2±2.0 (58.3-62.2) n=4	49.0±2.0 (48.0-52.0) n=4	7.20±0.07 (7.13-7.30) n=12	123±1 (122-124) n=8
Endrin	Annelid FT, Acute	21.0±1.0 (19.1-22.6) n=60	9.1±0.3 (8.6-9.5) n=12	62.2±4.5 (58.3-66.1) n=4	48.0±0.0 (48.0-48.0) n=4	7.23±0.22 (6.87-7.44) n=12	112±2 (110=115) n=4
Propoxur	Green Algae S, Acute	24.6±0.2 (24.3-24.9) n=30	8.3±0.1 (8.2-8.4) n=2	68.2±13.8 (58.5-78.0) n=2	40.0±0 (40.0-40.0) n=2	7.76±0.14 (7.66-7.86) n=2	238±4 (235-240) n=2
	Duckweed R, Acute	24.8±1.0 (23.3-26.5) n=30	7.9±0.2 (7.5-8.3) n=39	208±19 (187-230) n=8	106±12 (88-120) n=8	8.02±0.13 (7.84-8.23) n=15	852±45 (800-920) n=7

[1/] FT = Flow-through, R = Renewal, S = Static.
[2/] Not Determined.
[3/] Measured in dilution water.

Table 2. Percentage Agreement of Duplicates, Spike Recoveries, and Detection Limits for the Exposures of Various Aquatic Organisms to Several Pesticides.

Compound	Organism	Test Type[a]	Mean ± Standard Deviation		Detection Limit (µg/L)
			% Agreement	% Recovery	
Carbaryl	Green Algae	A/S	95.5±4.8	85.8±12.2	21
	Duckweed	A/R	99.2±0.4	90.0±10.3	290
Carbofuran	Green Algae	A/S	99.6±0.7	96.4±3.7	60
	Duckweed	A/S	96.5±4.9	96.9±7.4	430
	Amphipod	A/FT	92.4±9.5	101.0±12.9	0.08
p,p'-DDT	Cladoceran	A/R	94.9±2.5	95.7±10.7	0.16
	Cladoceran	C/R	93.2±4.2	86.5±14.2	0.53
Dichlorvos	Green algae	A/S	98.4±1.5	92.4±16.0	1,200
	Duckweed	A/R	98.5±0.6	99.1±1.2	1,200
Dieldrin	Annelid	A/FT	98.5±1.0	101.6±4.9	1.0
Endrin	Annelid	A/FT	94.1±6.3	102.3±6.4	4.2
Propoxur	Green Algae	A/S	97.2±3.2	95.3±8.8	150
	Duckweed	A/R	97.2±3.2	95.3±8.8	1,710

[a] A = Acute Test, C = Chronic Test, FT = Flow Through, R = Renewal, S = Static.

Table 3. Effect Concentrations of Acute and Chronic Exposures of Various Aquatic Organisms to Several Pesticides.

Compound	Organism	Test Type[a] & Duration	Concentration (μg/L)			
			Effect	(95% Confidence Limits)	NOEC	LOEC
Carbaryl	Green Algae	S (96 h)	IC50	490 (210-1,120)	50	90
	Duckweed	R (96 h)	IC50	23,900 (16,500-34,600)	<3,300	3,300
Carbofuran	Green algae	S (96 h)	IC50	1,980 (1,260-3,110)	400	1,000
	Duckweed	S (96 h)	IC50	236,000 (170,000-326,000)	17,100	36,600
	Amphipod	FT (96 h)	LC50	3.76 (3.06-4.64)	-	-
p,p'-DDT	Cladoceran	R (48 h)	EC50	0.83 (0.71-0.97)	-	
	Cladoceran	R (7 d)	Chronic Value	2.49	1.74	3.57
Dichlorvos	Green Algae	S (96 h)	IC50	110,000 (93,700-130,000)	-	-
	Duckweed	R (96 h)	IC50	398,000 (293,000-541,000)	<24,200	24,200
Dieldrin	Annelid	FT (96 h)	EC50	21.8 -	-	-
Endrin	Annelid	FT (96 h)	EC50	42.6 -	-	-
Propoxur	Green Algae	S (96 h)	IC50	6,320 (5,420-7,360)	3,350	7,430
	Duckweed	R (96 h)	IC50	198,000 -	24,600	47,600

[a] FT = Flow-Through, R = Renewal, S = Static.

Table 4. Effect of Carbaryl on Green algae (*Selenastrum capricornutum*) Cell Production in a 96-Hr Static Test.

	Mean Carbaryl Concentration ± s.d. (µg/L)					
	<21	50±60	90±100	170±170	330±350	720±790
Initial cell count	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$
Final mean cell count	$2.1x10^5$	$2.1x10^5$	$1.8x10^{5a}$	$1.6x10^{5a}$	$1.2x10^{5a}$	$0.9x10^{5a}$
Net difference	$2x10^5$	$2x10^5$	$1.7x10^{5a}$	$1.5x10^{5a}$	$1.1x10^{5a}$	$0.8x10^{5a}$
% Inhibition[b]	-	0	14	24	43	57

[a] Significantly different from control at $\alpha = 0.05$.
[b] % Inhibition (%I) - [(C-X)/C] x 100 where: C = Final mean cell count in the control.
X = Final mean cell count in a treatment.

Table 5. Effect of Carbaryl on Duckweed (*Lemna minor*) Frond Production in a 96-Hr Renewal Test.

	Mean Carbaryl Concentration ± s.d. (µg/L)					
	<290	3,300±1,400	5,800±500	12,000±900	23,200±1,000	45,200±4,000
Initial frond count	20	20	20	20	20	20
Final mean frond count	57.8	47.8[a]	43.8[a]	35.5[a]	31.0[a]	20.5[a]
Net difference	37.8	27.8[a]	23.8[a]	15.5[a]	11.0[a]	0.5[a]
% Inhibition[b]	-	17.3	24.2	38.6	46.4	64.5

[a] Significantly different from control at $\alpha = 0.05$.
[b] % Inhibition (%I) = [(C-X)/C] x 100 where: C = Final mean frond count in controls
X = Final mean frond count in treatments.

Table 6. Effect of Carbofuran on Green Algae (*Selenastrum capricornutum*) Cell Production in a 96-Hr Static Test.

	Mean Carbofuran Concentration ± s.d. (µg/L)					
	<60	400±100	1,000±200	2,200±200	4,100±400	9,000±700
Initial cell count	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$
Final mean cell count	$2.6x10^5$	$2.6x10^5$	$1.4x10^{5a}$	$1.5x10^{5a}$	$0.8x10^{5a}$	$0.6x10^{5a}$
Net difference	$2.5x10^5$	$2.5x10^5$	$1.3x10^{5a}$	$1.4x10^{5a}$	$0.7x10^{5a}$	$0.5x10^{5a}$
% Inhibition[b]	-	0	46	42	69	77

[a] Significantly different from control at α=0.05.
[b] % Inhibition (%I) = [(C-X)/C] x 100 where: C = Final mean cell count in controls
X = Final mean cell count in treatments.

Table 7. Effect of Carbofuran on Duckweed (*Lemna minor*) Frond Production in a 96-Hr Static Test.

	Mean Carbofuran Concentration ± s.d. (μg/L)					
	<430	17,100±1,800	36,600±700	70,500±8,900	149,000±14,200	292,000±17,200
Initial frond count	20	20	20	20	20	20
Final mean frond count	51.2	49.8	47.2[a]	42.8[a]	32.8[a]	22.2[a]
Net difference	31.2	29.8	27.2[a]	22.8[a]	12.8[a]	2.2[a]
% Inhibition[b]	-	2.7	7.8	16.4	35.9	56.6

[a] Significantly different from control at α=0.05.
[b] % Inhibition (%I) = [(C-X)/C] x 100 where: C = Final mean frond count in controls.
X = Final mean frond count in treatments.

Table 8. Concentrations of Carbofuran in the Amphipod (*Gammarus pseudolimnaeus*) 96-Hr Flow-Through Test with Percent Mortality in Parentheses.

Exposure Time (Hr)	Measured Concentrations (µg/L)					
0	<0.8/[a] <0.08	<0.08/ <0.08	<0.08/ <0.08	4.4/ 4.6	7.3/ 6.9	11.0/ 11.3
24	<0.08/ <0.08 (0)[b]	0.34/ 0.22 (0)	0.84/ 0.90 (0)	2.1/ 2.0 (5)	5.2/ 5.1 (0)	9.1/ 9.6 (30)
48	<0.08/ <0.08 (0)	0.09/ <0.08 (0)	0.81/ 0.72 (0)	2.2/ 2.2 (10)	5.9/ 5.6 (30)	11.1/ 10.9 (100)
72	<0.08 (0)	<0.08 (0)	0.50 (0)	1.9 (10)	4.7 (60)	-
96	<0.08/ <0.08 (0)	<0.08/ 0.20 (0)	084/ 0.86 (0)	3.0/ 3.1 (15)	6.2/ 6.1 (85)	-
% Spike Recovery 101.0±12.9						
Corrected[c] Concentration Mean ± (s.d.)	<0.08	0.12 (0.11)	0.61 (0.34)	2.8 (1.0)	5.8 (0.84)	10.4 (0.91)
Nominal	0	0.88	1.75	3.5	7	14

[a] Detection limit.
[b] Percent mortality of twenty organisms per treatment.
[c] Means and standard deviations corrected for percent recovery of spiked samples.

Table 9. Concentrations of p,p'-DDT in the Cladoceran (*Ceriodaphnia dubia*) 48-Hr Renewal Test with Percent Mortality in Parentheses.

Exposure Time (Hr)	Measured Concentrations (µg/L)					
0	<0.16[a]	0.55	1.00	1.40	3.28	7.55
24	<0.16[b]/ <0.16(0)[c]	0.37[b]/ 0.43 (0)	0.62[b]/ 0.78 (0)	0.84[b]/ 1.50 (0)	1.43[b]/ 2.94 (45)	2.61[b] (100)
48	<0.16[b](5)	0.52[b](15)	0.54[b](45)	0.80[b](100)	1.43[b](100)	-
% Spike Recovery 95.7±10.7						
Corrected[d] Concentration Mean ± (s.d.)	<0.16	0.49 (0.09)	0.89 (0.10)	1.18 (0.38)	2.37 (1.02)	5.31 (3.65)
Nominal	-	0.5	1.0	2.0	4.0	8.0

[a] Detection limit.
[b] Old solutions at renewal.
[c] Percent mortality of twenty organisms per treatment.
[d] Means and standard deviations corrected for percent recovery of spiked samples.

Table 10. Survival and Reproduction in the Cladoceran (*Ceriodaphnia dubia*), p,p'-DDT 7-Day Chronic Exposures.

	Mean[a] p,p'-DDT Concentrations ± s.d. (µg/L)					
	<0.53[b]	<0.53	0.62±0.34	0.99±0.54	1.74±0.82	3.57±2.06
Percent Survival of Adults at 7 Days	90	100	90	80	60	0[c]
Day of First Brood	4	4	4	4	5	–[d]
Number of Young Produced	156	187	102	151	63	0
Mean Number of Young/Surviving Adult at 7 Days	17.3	18.7	11.3	18.9	10.5	0[c]

[a] Mean of old and new solutions at renewal.
[b] Detection limit.
[c] Significantly different from control at α=0.05.
[d] No young produced.

Table 11. Effect of Dichlorvos on Green Algae (*Selenastrum capricornutum*) Biomass in a 96-Hr Static Test.

	Mean Dichlorvos Concentration ± s.d. (µg/L)					
	<1,200[a]	13,800±6,400	29,000±7,800	56,800±8,300	119,000±11,000	239,000±14,000
Mean Standing Crop Dry Weight (mg)	6.1±0.8	8.2±5.9	6.9±4.7	6.8±3.9	2.3±0.3	1.2±1.0
% Inhibition[b]	-	-34	-13	-11	62	80

[a] Detection limit.
[b] % Inhibition (%I) = [(C-X)/C] x 100 where: C = dry weight of cells in control.
X = Dry weight of cells in a treatment.

Table 12. Effect of Dichlorvos on Duckweed (*Lemna minor*) Frond Production in a 96-Hr Static Test.

	Mean Dichlorvos Concentration ± s.d. (μg/L)					
	<1,200[a]	24,200±5,500	52,800±12,000	105,000±13,900	228,000±19,500	471,000±21,300
Initial Frond Count	20	20	20	20	20	20
Final Mean Frond Count	47.8	41.0[b]	40.2[b]	39.0[b]	34.2[b]	21.5[b]
Net Difference	27.8	21.0[b]	20.2[b]	19.0[b]	14.2[b]	1.5[b]
% Inhibition[c]	-	14.2	15.9	18.4	28.4	55.0

[a] Detection limit.
[b] Significantly different from control at α=0.05.
[c] % Inhibition (%I) = [(C-X)/C] x 100 where: C = final mean frond count in control.
X = final mean frond count in a treatment.

Table 13. Concentrations of Dieldrin in the Annelid (*Lumbriculus variegatus*) 96-Hr Flow-Through Test with Percent Mortality in Parentheses.

Exposure Time (Hr)	Measured Concentrations (µg/L)					
0	<0.1[a]/ <1.0	18.2/ 20.2	32.3/ 35.3	44.1/ 45.2	82.7/ 83.0	156/ 165
24	<1.0 (0)[b]	21.7 (0)	35.3 (0)	47.7 (0)	80.7 (0)	135 (0)[c]
48	<1.0/ <1.0 (0)	13.1/ 13.7 (0)	29.4/ 31.1 (0)	48.2/ 49.3 (0)	88.4/ 90.2 (0)	160/ 182 (0)[c]
72	<1.0 (0)	14.1 (0)	26.7 (0)	51.9 (0)[c]	93.3 (0)[c]	181 (0)[c]
96	<1.0/ <1.0 (0)	13.7/ 14.6 (0)	25.3/ 27.2 (0)[c]	47.5/ 46.7 (0)[c]	93.0/ 105 (0)[c]	149/ 160 (0)[c]
% Spike Recovery	101.6±4.9					
Corrected[d] Concentration Mean ± (s.d.)	<1.0	15.9 (3.3)	29.8 (3.8)	46.8 (2.4)	88.1 (7.7)	159 (15.5)

[a] Detection limit.
[b] Percent mortality of twenty organisms per treatment.
[c] Affected organisms-coiled and inactive.
[d] Means and standard deviations corrected for percent recovery of spiked samples.

Table 14. Concentrations of Endrin in the Annelid (*Lumbriculus variegatus*) 96-Hr Flow-Through Test with Percent Mortality in Parentheses.

Exposure Time (Hr)	Measured Concentrations (µg/L)					
0	<4.2[a]/ <4.2	15.3/ 17.3	35.1/ 38.5	54.1/ 56.1	90.9/ 94.3	178/ 182
24	<4.2 (0)[b]	16.7 (0)	41.2 (0)[c]	48.0 (0)[c]	89.4 (0)[c]	159 (0)[c]
48	<4.2/ <4.2 (0)	17.4/ 18.8 (0)	30.9/ 33.8 (0)[c]	50.0/ 52.6 (0)[c]	87.1/ 89.9 (0)[c]	168/ 168 (0)[c]
72	<4.2 (0)	18.2 (0)[c]	33.3 (0)[c]	56.9 (0)[c]	85.4 (0)[c]	161 (0)[c]
96	<4.2/ <4.2 (0)	20.5/ 20.0 (0)[c]	36.1/ 36.6 (0)[c]	56.9/ 51.7 (0)[d]	91.0/ 96.0 (0)[d]	167/ 169 (0)[d]
% Spike Recovery	102.3±6.4					
Corrected[e] Concentration Mean ± (s.d.)	<4.2	17.6 (1.7)	34.9 (3.1)	52.1 (3.2)	88.5 (3.4)	165 (7.3)

[a] Detection limit.
[b] Percent mortality of twenty organisms per treatment.
[c] Affected organisms - coiled and less active.
[d] Affected organisms - tightly coiled and inactive - do not respond to gentle prodding.
[e] Means and standard deviations corrected for percent recovery of spiked samples.

Table 15. Effect of Propoxur on Green Algae (*Selenastrum capricornutum*) Cell Production in a 96-Hr Static Test.

	Mean Propoxur Concentration ± s.d. (µg/L)					
	<150[a]	1,620±370	3,350±820	7,430±1,540	14,400±2,620	27,500±5,330
Initial Cell Count	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$
Final Mean Cell Count	$2.8x10^5$	$2.8x10^5$	$2.8x10^5$	$0.8x10^{5b}$	$0.2x10^{5b}$	$0.1x10^{5b}$
Net Difference	$2.7x10^5$	$2.7x10^5$	$2.7x10^5$	$0.7x10^{5b}$	$0.1x10^{5b}$	0[b]
% Inhibition[c]	-	0	0	71	93	96

[a] Detection limit.
[b] Significantly different from control at α=0.05.
[c] % Inhibition (%I) = [(C-X)/C] x 100 where: C = final mean cell count in control.
X = final mean cell count in a treatment.

Table 16. Effect of Propoxur on Duckweed (*Lemna minor*) Frond Production in a 96-Hr Renewal Test.

	Mean[a] Propoxur Concentration ± s.d. (μg/L)					
	<1,710[b]	11,700±500	24,600±400	47,600±3,500	98,500±5,400	198,000±6,100
Initial Frond Count	20	20	20	20	20	20
Final Mean Frond Count	62.0	65.0	53.8	47.2[c]	44.0[c]	40.5[c]
Net Difference	42.0	45.0	33.8	27.2[c]	24.0[c]	20.5[c]
% Inhibition[c]	-	-4.8	13.2	23.9	29.0	34.7

[a] Mean of old and new solutions at renewal.
[b] Detection limit.
[c] Significantly different from control at α=0.05.