Bull. Environ. Contam. Toxicol. (1994) 52:298–304

Environmental Contamination and Toxicology

Lethal and Sub-lethal Toxicity of Lindane to *Pimephales promelas* and *Ceriodaphnia dubia*

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Received: 2 February 1993/Accepted: 7 July 1993

The insecticide lindane, the gamma isomer of hexachlorocyclohexane, is known to have toxic effects on aquatic organisms at very low concentrations. The survival of *Daphnia sp.* continuously exposed for three generations to 19 µg lindane/L was significantly reduced in the 3rd generation, indicating a generation to generation cumulative effect (Macek et al. 1976). Populations of rotifers, primarily *Polyarthra sp.* exposed for 6 d to 20 µg/L were reduced by 75% (Lay et al. 1987). The midge *Chironomus tentans* exhibits larval mortality, severe developmental retardation and reduction in the number of emerging adults when exposed to 7.3 µg/L of lindane for two generations. At 5.0 µg/L lindane still caused a significant delay in emergence of adults. The fresh water scud, *Gammarus fasciatus*, does not successfully reproduce when exposed to lindane concentrations of 2.6 µg/L (Macek et al. 1976) and have a 96-hr LC_{50} of 10 µg/L (Mayer and Ellersieck 1986). Exposure of the fish *Anabas testudineus* to 75 µg/L for 1 hr caused irreversible damage to gills and intestinal tissues (Bakthavathsalam et al. 1987). The fish, *Barbus stigma*, exhibits high sensitivity to lindane with a 96-hr LC_{50} of 1.5 µg/L (Khillare and Waghs 1988).

Lindane is frequently detected at levels of 0.001 - 0.02 µg/L in western Canadian rivers (Environment Canada 1980, 1982, 1984, 1985, 1986 - 1989). This low, but continuous level of contamination could pose a sub-lethal toxic threat to freshwater life. This potential toxic threat needed to be assessed with more sensitive, up-to-date toxicity tests than have been applied in the past. The 7-day lethal and sub-lethal tests with *P. promelas* and *C. dubia* are a rapid means of estimating the effects of low concentrations of chemicals on aquatic animals (Norberg and Mount 1985).

MATERIALS AND METHODS

The Fathead Minnow Survival and Growth Test was conducted following the procedure of Norberg and Mount (1985). Test concentrations (control water with acetone (1 mL acetone/L water), control water with acetone plus 2.7, 6.6, 11, 21, 45, 72, or 173 µg lindane/L) were selected for testing based on preliminary test results. Each concentration was tested in duplicate. Eggs were cultivated from in-house rearing stock obtained from a certified fish hatchery. The larvae were removed from egg incubation tanks and put into rearing tanks as they hatched. The tests were initiated by placing ten, newly hatched (< 1 d old) fathead minnow larvae into each test vessel. Acclimation was minimal due to the requirements of the test for very young larvae. Test vessels were 1 L, glass

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vessels containing 500 mL of laboratory dilution water and were capped to prevent volatilization of lindane. All test containers were held under a photoperiod of 16 hr of light and 8 hr of dark. The larvae were fed 0.1 mL of a concentrated suspension of newly hatched brine shrimp (*Artemia salina*) three times a day. The test solutions were renewed daily with freshly prepared concentrations. The larval survival and the physical and chemical parameters of the larval survival exposure water (dissolved oxygen, temperature, conductivity, hardness and alkalinity) were monitored daily. After 7 d of exposure, the tests were terminated, the larvae from each test concentration were killed by removal from water, rinsed in distilled water, placed on an aluminum foil dish and dried at 105 °C in a Fisher Isotemp oven for 24 hr. The dishes and dried fish were cooled in a desiccator prior to weighing to determine dry weight.

The *Ceriodaphnia dubia* 7-day Survival and Reproduction Test was conducted as outlined by the U.S. EPA (1985). The test organisms were obtained from the U.S. EPA Environmental Research Laboratory in Duluth, Minnisota. Ten *C. dubia* were exposed to each test concentration (control water, control water with acetone (1 mL acetone/L water), and control water with acetone plus 6.6, 11, 21, 45, 72, 173, 353, 743, or 1570 µg lindane/L) by putting one neonate (< 12 hr old) each into a 20 mL scintillation vial with 15 mL of test solution. Each toxicity test was accompanied by controls containing dilution water only and dilution water plus acetone equivalent to the volume of acetone (1 mL/L) used in each exposure solution. All tests were conducted at 25 °C under a photoperiod of 16 hr light and 8 hr of dark. All organisms in the test vessels were fed daily at a rate of 0.1 mL of food suspension (fermented trout chow, bakers yeast, and Cerophyl^{R})/15 mL of test solution. Each surviving test organism was transferred daily into a new test vessel. Mature *C. dubia* produced young on about the 3rd d and the adult was again transferred to fresh test solution and fed. The remaining young were killed with 2 drops of HCl and counted. The number of surviving adults, the number of young produced, the physical and chemical parameters of the exposure water (dissolved oxygen, temperature, conductivity, hardness and alkalinity) were monitored daily.

Survival of *C. dubia* females was analyzed using Fisher's Exact Test (to determine significant differences between responses at different concentrations) and the moving average method to determine the concentration that results in a 50% kill of exposed organisms (Bennett 1952). The number of neonates produced per female at each concentration were statistically compared by analysis of variance (ANOVA), followed by Dunnett's procedure (Dunnett 1955). Comparisons were based on the performance of organisms in solvent control (lab water plus acetone) after it had been determined that reproduction by *C. dubia* in blank and solvent control solutions were statistically similar (t-test, p=0.05). Survival and growth of *P. promelas* was analyzed in a similar fashion except that Dunnett's test was performed on transformed (arc sine) survival data. The geometric mean of the highest No-Observed-Effect-Concentration (NOEC) and the Lowest-Observed-Effect-Concentration (LOEC) was calculated to give the Maximum-Acceptable-Toxicant-Concentration (MATC). Data from both sub-lethal tests were also analyzed by a monotonic smoothing method to determine an interpolated point estimate called the Inhibition Concentation Percentage (ICp) (Norberg-King, 1988). By this method an inhibition of 50% in growth or reproduction is reported as the IC_{50} and is analagous to the MATC.

The technical formulation of lindane (97% gamma isomer of hexachlorocyclo-hexane) was obtained from May and Baker Inc., Burlington, Ontario. Analytical grade (99.7%) acetone was used as a carrier (solvent).

Laboratory dilution water characteristics ranged as follows: pH 7.3 - 8.8; dissolved oxygen 8.2 - 9.8 mg/L; conductivity 304 - 329 µmhos/cm; alkalinity 90 - 100 mg/L as $CaCO_3$; and hardness 150 - 160 mg/L as $CaCO_3$. This water was used for both tests.

Test samples (500 - 800 mL) were stored at 4 °C in opaque glass containers until analysis. The samples were carried through an in-bottle extraction with dichloromethane (3 X 50 mL) using a magnetic stirrer. Sample bottles were rinsed three times with dichloromethane and the rinses were added to the extracts. The combined extracts were concentrated by evaporation to a volume of approximately 25 mL. Extracts were then dried by passing them through Na_2SO_4. The extracts were further concentrated to a volume of 2 mL utilizing a Kuderna-Danish apparatus, transferred to culture tubes and made up to 10 mL using *iso*-octane.

Lindane analyses were performed on a Hewlett-Packard 5890 gas chromatograph with dual electron capture detectors. The dual columns were a J & W 30-meter DB-1 and a 30-meter DB-1701, both with 0.25 mm id and 0.25 µm bonded phase on fused silica. The conditions of the analysis were as follows: oven temperature was held at 60 °C for one minute; temperature was increased to 150 °C at 20 °C per minute then to 300 °C at 10 °C per minute; injector temperature was 220 °C; and detector temperature was 300 °C.

Stock solutions of lindane were made up in acetone and stored in amber bottles at 4 °C for the test duration. Each day, 2 mL of lindane stock solution was pipetted into a glass flask and diluted to 2 L. The 2 L exposure concentrations prepared for that day were analyzed for gamma-HCH concentration and represented the daily replacement concentration used for both *P. promelas* and *C. dubia* test solutions. Exposure concentrations for a complete dilution series were analyzed on the first day (t=0) and the sixth day of the test (t=6). The first day's exposure solution was recovered from the respective exposure vessels and analyzed to determine pesticide losses after 8 and 24 hr. The 8 hr samples were collected from duplicate test vessels to ensure sufficient sample volume. There was no evidence of pesticide loss from test vessels after 24 hr of static exposure nor from stock solutions stored over one week. The data were, therefore, combined to derive the actual exposure concentrations in calculating effect concentrations for each organism. The data for each concentration showed from 4.4 - 13% coefficient of variation and close agreement with nominal levels.

Blanks of distilled water and spiked samples were prepared for each batch of samples processed. Recoveries were in excess of 95%.

RESULTS AND DISCUSSION

The fathead minnow survival data was suspect for calculating an MATC as the reduction in survival was significant at 45 and 173 µg/L, but not at 72 µg/L. This irregularity made the survival data less reliable than the growth data for calculating an MATC for fathead minnows and thus only the growth data was used for this purpose.

The 7-day LC_{50} for fathead minnows was 112 µg/L (Table 1 and Figure 1) (Bennett 1952). Growth of fathead minnow larvae was reduced by 29% at a lindane concentration of 45 µg/L (the LOEC), no significant effects were observed at 21 µg/L (the NOEC) (Dunnett's procedure, $p<0.05$). The minimum significant difference in reduction of minnow dry weight was 24.5%. The MATC

was calculated to be 31 µg/L (see Table 1 and Figure 2). The 7-day IC_{50} was determined to be 58.5 µg/L (95% Confidence Interval = 45 - 63.5 µg/L).

The 7-day LC_{50} for *C. dubia* was 45.5 µg/L (25 - 76 µg/L). The survival NOEC was determined to be 10.5 µg/L, the LOEC was 21 µg/L and the MATC was estimated to be 15 µg/L (see Table 1 and Figure 3). At the LOEC survival was reduced by 50%, a 30% reduction in survival was not significant at the NOEC (Fisher's Exact Test, p=0.05).

The 7-day IC_{50} for *C. dubia* was 13 µg/L (9.5 - 16 µg/L). The rate of reproduction was significantly reduced (45 %, t-test, p=0.05) at a concentration of 10.5 µg/L (the LOEC), but not at 6.6 µg/L (the NOEC). The resulting estimated MATC for *C. dubia* reproduction was 8.3 µg/L (see Table 1 and Figure 4).

By comparing the reproduction and survival data it appears that the reproduction of *C. dubia* is more sensitive to the toxic effects of lindane than is survival. The IC_{50} was significantly lower at 13 µg/L than the LC_{50} at 45.5 µg/L. Between species, *C. dubia* has been shown to be more sensitive than *P. promelas* in 7-day static renewal tests for a variety of chemicals (Stewart et al. 1990). This trend is continued as the sub-lethal MATC for *C. dubia* (8.3 µg/L) is significantly (t-test, p=0.05) lower than the sub-lethal MATC for *P. promelas* (31 µg/L).

Table 1. Summary of toxicity results

Test	7-day LC_{50} or IC_{50}* (µg/L)	Lindane Concentration (µg/L)		
		NOEC	LOEC	MATC
P. promelas survival (LC_{50})	112 (97 - 128)	na	na	na
P. promelas growth (IC_{50})	58.5 (45 - 63.5)	21	45	31
C. dubia survival (LC_{50})	45.5 (25 - 76)	10.5	21	15
C. dubia reproduction (IC_{50})	13 (9.5 - 16)	6.6	10.5	8.3

* - figures in brackets are 95 % confidence intervals n/a - not available

Lindane has been shown to be sub-lethally toxic to *P. promelas* and brook trout (*Salvelinus fontinalis*) at levels below 50 µg/L (Macek et al 1976). *S. fontinalis* exhibited significant reductions in weight gain and growth during 261 d of continuous exposure to 16.6 µg/L of lindane. They also exhibited unusual behaviour that inhibited fertilization, leading to total reproductive failure. In this study the growth rate of larval *P. promelas* was significantly reduced (29 %) at 45 µg/L.

The conclusion that aquatic arthropods are more sensitive to lindane than fish is supported by this work. *Daphnia sp.* are affected at 19 µg/l; *Polyarthra sp.* at 20 µg/L; *C. tentans* at 5 µg/L; and *G. fasciatus* at 2.6 µg/L. This study determined that the Maximum Acceptable Toxicant Concentration of lindane for *C. dubia* reproduction is in the same range at 8.3 µg/L.

The Canadian guideline for protection of aquatic wildlife from

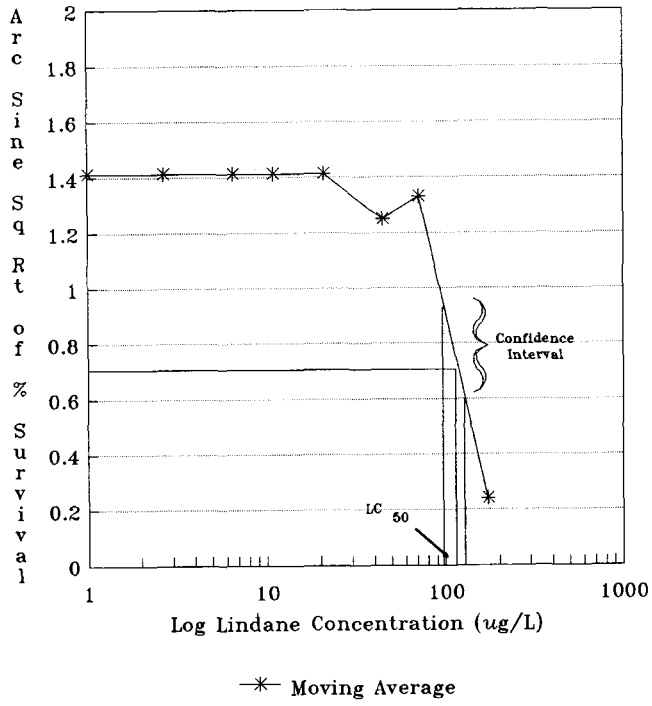

Figure 1. Lethal Toxicity of Lindane to *Pimephales promelas*

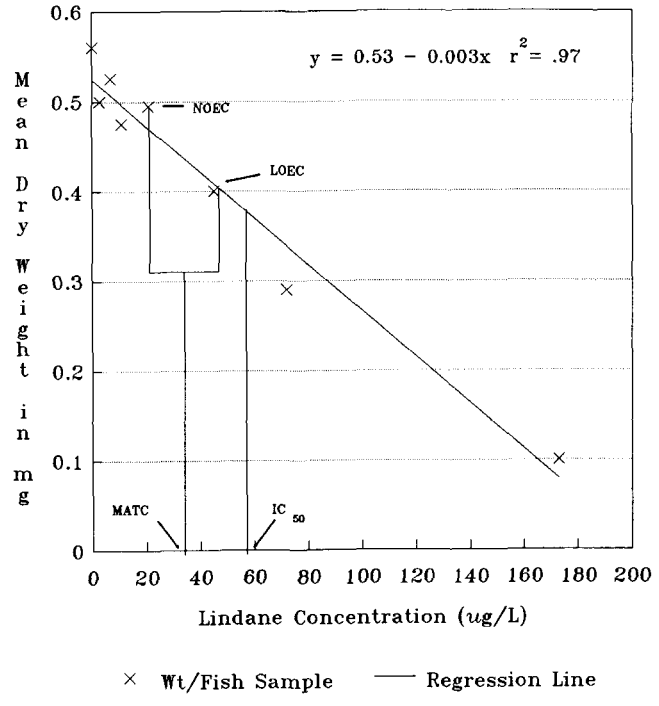

Figure 2. Sub-Lethal Toxicity of Lindane to *Pimephales promelas*

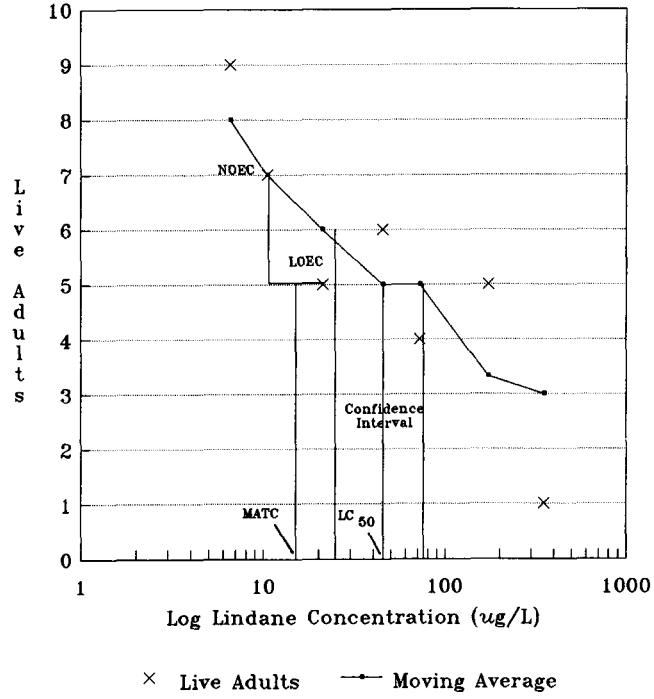

Figure 3. Lethal Toxicity of Lindane to *Ceriodaphnia dubia*

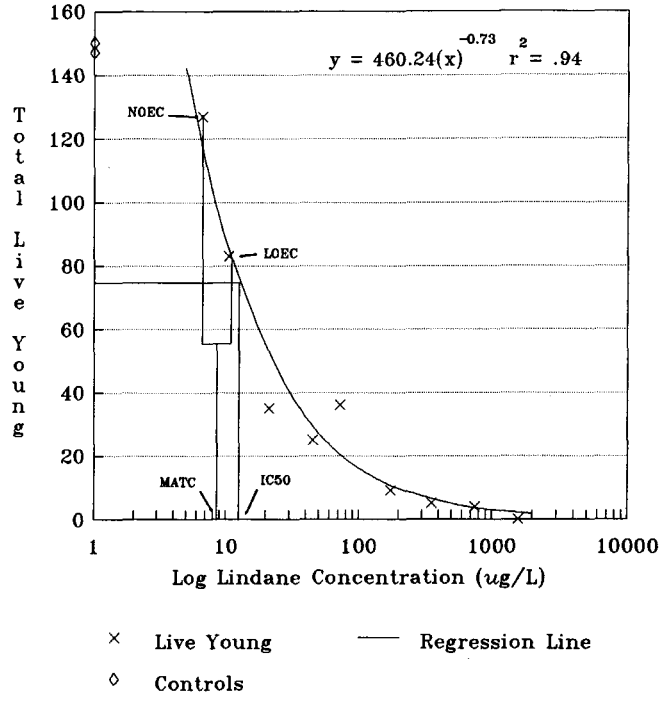

Figure 4. Sub-Lethal Toxicity of Lindane to *Ceriodaphnia dubia*

hexachlorocyclohexane (HCH) isomers is 0.01 µg/L (C.C.M.E. 1985). The combined levels of alpha- and gamma-HCH isomers found in Canadian prairie rivers occasionally exceed the guideline concentration. This is due primarily to the higher levels of alpha-HCH in western Canadian rivers than gamma-HCH (Environment Canada 1980, 1982, 1984, 1985, 1986 - 1989). Alpha-HCH has little insecticidal activity and is not a toxic threat to aquatic life at the levels detected in western Canada (Canton et al. 1975). The maximum detected ambient levels of HCH isomers was 0.039 µg/L, of which 0.009 µg/L was lindane, the gamma isomer (Environment Canada 1980, 1982, 1984, 1985, 1986 - 1989). This lindane concentration is 922 times lower than the MATC for *C. dubia* reproduction, and 3444 times lower than the MATC for *P. promelas* growth. Based on this evidence, aquatic resources in western Canadian rivers are likely not at risk from lindane.

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