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Effect of Temperature on the Chronic Toxicity of Hydrothol-191 to the Fathead Minnow (*Pimephales promelas*)

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Endothall herbicides (7-oxabicyclo [2,2,1] heptane-2,3-dicarboxylic acid) are used extensively in Florida to eliminate macrophyte infestations in canals and lakes. Inorganic salts of endothall, i.e. Aquathol and Aquathol-K, are both the most commonly used and least toxic formulations. However, Hydrothol-191, the alkylamine salt, is often preferred for algal and macrophyte control because it is effective at lower concentrations and for a longer time than Aquathol.

While the acute toxicity of Hydrothol to freshwater fish has been assessed (Keller et al. 1988; Mudge et al. 1986; Finlayson 1980; Johnson and Finley 1980), little information on its chronic toxicity is available (Keller et al. 1988). Several Florida state agencies predicted an increase in permit requests for Hydrothol use and, because of its potential impact on non-target organisms, were concerned by the lack of chronic toxicity data on this herbicide. The objectives of the current research were to: (1) provide such data using the Environmental Protection Agency fathead minnow subchronic toxicity test (Horning and Weber 1985) and (2) determine the effects of temperature on Hydrothol toxicity.

MATERIALS AND METHODS

The fathead minnow subchronic toxicity test is a 7-day, static renewal test that measures chronic toxicity based on survival and growth (weight increase) of fish larvae (Horning and Weber 1985). Fathead minnow (*Pimephales promelas*) embryos, obtained from EPA-Newtown, Ohio, for each test, hatched in transit or shortly after arriving in Gainesville and were used within 24 h. For each batch of larvae, a reference toxicant test was performed with $CdCl_2$ to verify nominal sensitivity (Horning and Weber 1985).

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Table 1. Dilution water quality parameters for fathead minnow larval growth and survival tests[a].

Temperature	pH	Cond. (uhmos/cm)	D.O. (mg/L)	Alk. (as mg/L $CaCO_3$)	Hard. (as mg/L $CaCO_3$)
15	7.85 (0.1)	240.2 (19.1)	9.13 (0.15)	57.73 (3.19)	80.03 (11.31)
25	7.80 (0)	289.3 (3.05)	7.10 (0.20)	62.93 (3.81)	78.43 (3.10

[a] Water quality parameters are reported as the mean (SD) of daily values taken over the six independent 7-d tests.

Liquid Hydrothol-191 (Pennwalt Corp., Philadelphia, Pennsylvania) was diluted with moderately hard reconstituted freshwater (Horning and Weber 1985). Hydrothol concentrations were calculated based on per cent endothall, the active ingredient. Twenty-four hour range finding tests performed at 15 and 25 C were used to determine concentrations appropriate for 7-d tests.

At the start of a test, 10 larvae were placed in duplicate 1-L chambers containing 500 mL of test solution at each concentration. Dissolved oxygen, temperature, conductivity, pH, alkalinity and hardness were measured at the beginning of each 24-h exposure for all test concentrations and in the control (Table 1). The fish were each fed 70-100 Artemia nauplii three times a day (Horning and Weber 1985). Test solutions were renewed daily.

Triplicate tests were conducted at 15 C and 25 C in a constant temperature room, with a photoperiod of 16 h light and 8 h of darkness. At the end of each test, surviving larvae from each chamber were removed and preserved in 4% formalin. At a later date, the preserved larvae were rinsed in distilled water and dried at 105 C for 2 hours. Dry weights of each test group of larvae were measured to the nearest 0.001 g.

Survival data were arcsine-transformed. The LC50 and 95% confidence intervals were calculated by moving average angle and the binomial method using EPA computer programs (Peltier and Weber 1985, Horning and Weber 1985). Significant differences in survival of test and control groups were determined using ANOVA and Dunnett's Procedure.

Sub-lethal effects on growth (dry weight) were analyzed by ANOVA and Dunnett's procedure. The NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration), and ChV (Chronic Value) were calculated based on the results. The ChV (Chronic Value), roughly equivalent to the maximum allowable toxicant concentration (MATC), is the geometric mean between the NOEC and LOEC (Horning and Weber 1985).

RESULTS AND DISCUSSION

Hydrothol toxicity to the fathead minnow was inversely related to temperature (Table 2). The 96-h LC50 increased from 393 ug/L at 15 C to 468 ug/L at 25 C. These values are similar to 4-day LC50s for bluegill (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*) which ranged from 490-940 ug/L Hydrothol (Johnson and Finley 1980). Although no assessment of toxicity relative to water hardness was included in the present study, its importance has been demonstrated in other studies and should be considered in subsequent investigations. For example, the 96-h LC50 for the

Table 2. Seven-day growth and survival of fathead minnow larvae in Hydrothol-191 at 15 and 25 C.

Hydrothol concentration (ug/L)	Mean weight[a] (± SD) (mg)	Mean percent survival
15 C		
Control	0.107 (± 0.024)	95
50	0.106 (± 0.023)	98
132	0.087 (± 0.012)	93
265	0.109 (± 0.038)	55[b]
530	-[c]	0[b]
1060	-[c]	0[b]
25 C		
Control	0.420 (± 0.074)	100
50	0.348 (± 0.049)	90
132	0.300[b] (± 0.016)	100
200	0.318[b] (± 0.059)	90
265	0.198[b] (± 0.084)	70[b]
530	0.255[b] (± 0.078)	5[b]
1060	-[c]	0[b]

[a] Mean values from three independent 7-d tests.
[b] Significantly different from control ($P \leq 0.05$).
[c] All died before 7 days, no weight determined.

golden shiner (*Notemigonus crysoleucas*) increased from 320 ug/L Hydrothol at 278 mg/L hardness to 1600 ug/L at 20 mg/L hardness (Finlayson 1980).

Test temperature had a noticeable effect on Hydrothol toxicity to the fathead minnow. The 7-d LC50 increased from 233 ug/L at 15 C to 304 ug/L at 25 C. These results suggest that the fish were stressed by the lower test temperature even though differences in per cent survival at the two temperatures were not statistically significant. The reverse effect of temperature has been demonstrated for Aquathol-K

Table 3. Chronic toxicity values (ug/L) determined for Hydrothol in the fathead minnow larval survival and growth test at 15 and 25 C.

Endpoint Designation	15 C			25 C		
	NOEC	LOEC	ChV	NOEC	LOEC	ChV
Survival	132	265	186	200	265	230
Growth	NA[a]	NA	NA	50	132	81

[a]Not available because there was no growth at 15 C.

toxicity to bluegills (Johnson and Finley 1980). The LC50 for bluegills decreased from 1740 mg/L to 343 mg/L at 7 and 22 C, respectively.

At 15 C, there was no statistically significant difference between growth (weight gain) of control and experimental fish (Table 2). Therefore, a ChV for growth at 15 C could not be calculated (Table 3). Further analysis of the results using test temperature (15 vs 25 C) and toxicant concentration in a 2-way ANOVA, confirmed that temperature rather than Hydrothol concentration was the significant factor controlling growth ($P \leq 0.05$).

Since fathead minnow larvae did not grow at 15 C, chronic toxicity could only be assessed on the basis of survival. At 15 C, the LOEC was 265 ug/L Hydrothol, while the NOEC was 132 ug/L (Table 3). Based on these data, the ChV for survival at 15 C was 186 ug/L.
At 25 C, the ChV for survival was 230 ug/L, calculated from an LOEC of 265 ug/L and an NOEC of 200 ug/L Hydrothol (Figure 1). Growth, inhibited by 132 ug/L

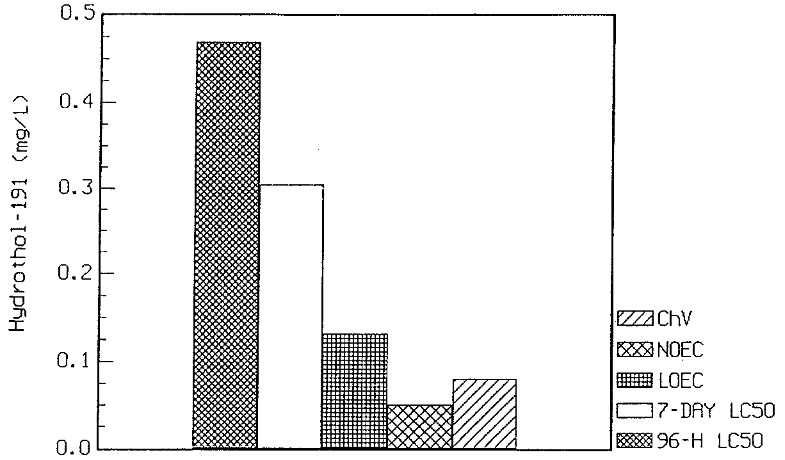

Figure 1. Toxicity of Hydrothol-191 to the fathead minnow at 25 C (ChV=Chronic Value, NOEC=No Observed Effect Concentration, LOEC=Lowest Observed Effect concentration).

Hydrothol, was twice as sensitive a measure of toxicity at 25 C as survival, the latter being significantly decreased at 265 ug/L. The ChV for growth at 25 C was 81 ug/L Hydrothol, three times more sensitive an endpoint than was survival at 25 C.

The finding that chronic toxicity values for survival and growth of fathead minnows were two (at 15 C) to six (at 25 C) times lower than those determined from acute tests (Figure 1) is significant. It underscores the need for more thorough evaluations of the longterm impact of xenobiotics in aquatic habitats than has been traditional. Use of subchronic toxicity tests in risk assessment programs provide simple, cost-effective means for determining species and ecosystem responses to chemical control of aquatic pests.

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REFERENCES

Finlayson, BJ (1980) Acute toxicities of the herbicides Komeen and Hydrothol-191 to golden shiner (Notemigonus crysoleucas). Bull Environ Contam Toxicol 25:676-681

Horning WB, Weber CI (1985) Short-term methods for estimating the chronic toxicity of effluents and

receiving waters to freshwater organisms. EPA-600/4-85-014, United States Environmental Protection Agency, Cincinnati, Ohio
Johnson WW, Finley MT (1980) Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. United States Department of Interior, Fish and Wildlife Service, Resource Publication 137, Washington, D.C.
Keller AE, Dutton RJ, Crisman TL, Bitton G (1988) Chronic toxicity of Hydrothol-191 to *Ceriodaphnia dubia* at 25 and 15 C. Bull Environ Contam Toxicol (In press)
Liguori VM, Zakour HR, Landolt ML, Felton SP (1983) Toxicity of endothall to juvenile chinook salmon (Oncorhynchus tshawytscha). In: Bishop WC, Cardwell RC, Heidolth BB, (eds) Aquatic toxicology and hazard assessment: Sixth symposium, American Society for Testing and Materials, STP 802, Philadelphia, Pennsylvania pp 530-544
Mudge JE, Northstrom TE, Stables TB (1986) Acute toxicity of Hydrothol-191 to phytoplankton and rainbow trout. Bull Environ Contam Toxicol 37:350-354
Norberg TJ, Mount DI (1985) A new fathead minnow (Pimephales promelas) subchronic toxicity test. Environ Toxicol Chem 4:711-718
Pennwalt Corp. (1980) Technical information manual: the uses and properties of endothall. AGCHEM Pennwalt, Philadelphia, Pennsylvania
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