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Chronic Toxicity of Hydrothol-191 to *Ceriodaphnia dubia* at 25 and 15°C

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Endothall (7-oxabicyclo [2,2,1] heptane-2,3-dicarboxylic acid) is one of the most commonly used aquatic herbicides in the State of Florida. It is used to control problem macrophytes, especially *Hydrilla verticillata*, and *Myriophyllum spicatum* (Dupes and Mahler 1982). While considerable data are available on the impact of the inorganic salts of this herbicide (e.g., Aquathol and Aquathol-K) on aquatic ecosystems (Johnson and Finley 1980; Holmberg and Lee 1976), less is known about the effects of the alkylamine salt of endothall, i.e., Hydrothol-191 (hereafter Hydrothol). The latter is more persistent in the environment and much more toxic to aquatic organisms than are inorganic forms (Blackburn et al. 1971; Pennwalt Corp. 1980; Sikka and Rice 1973).

Early toxicity tests which were short-term (48- to 96-h) assays, generally used death as the endpoint. Chronic life-cycle toxicity tests were designed to measure sublethal effects of exposure to toxicants e.g., changes in fecundity, growth, behavior or enzyme activity (Mayer et al. 1986). Depending on the organism used, a life-cycle test might require up to a year to complete. Shorter chronic toxicity tests became essential in the 1970's as interest in the impacts of xenobiotics on aquatic systems increased and the need arose for rapid test methods to verify compliance with National Pollutant Discharge Elimination System standards (Horning and Weber 1985; Birge and Black 1981).

The *Ceriodaphnia dubia* survival and reproduction test (Horning and Weber 1985) was developed as a substitute for the 3-4 week *Daphnia* chronic toxicity test. In the *Ceriodaphnia* test, toxicity is based on survival and reproduction over a 7-day period, with the data being comparable to the longer *Daphnia* chronic tests. *Ceriodaphnia dubia* reproduces faster, is ubiquitous, and is somewhat easier to culture in a laboratory (Horning and Weber 1985). Thus, the toxic effects of a

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substance may be more easily and rapidly determined using the 7-day test. Chronic toxicity is measured in two ways: a 7-day LC50 is calculated based on adult survival, and sublethal toxicity is measured as decreased fecundity.

The objectives of the present study were to: (1) determine the acute and chronic toxicity of Hydrothol-191 to *Ceriodaphnia dubia* using the new test and (2) measure the effect of temperature on the toxicity of this compound.

MATERIALS AND METHODS

Ceriodaphnia dubia, obtained from USEPA-Newtown, were cultured in the laboratory in 1-L beakers at 25 C under a 16-h light: 8-h dark lighting regime. Moderately hard reconstituted freshwater (Horning and Weber 1985) was used as the culture medium. Food consisted of a digested trout chow-Cerophyll-yeast (TCY) mixture (Horning and Weber 1985) provided at a rate of 3 mL/L of medium per day, supplemented by algae growing continuously in the beakers after innoculation with a *Euglena-Klebsomidium-Chlamydomonas* mixture. At least once monthly, a reference toxicant test using sodium dodecyl sulfate (SDS), was performed to verify that the in-house *Ceriodaphnia* culture was healthy and nominally sensitive.

Toxicity tests were performed at 15 and 25 C in a constant temperature room with a 16L:8D light regime. The liquid formulation of Hydrothol (Pennwalt Corp., Philadelphia, Pennsylvania) containing 53% endothall (active ingredient) was used. To begin each test, 5 toxicant solutions were prepared from a concentrated stock diluted with moderately hard reconstituted freshwater. A 48-h range-finding test was performed at each temperature using Hydrothol concentrations of 0.10-3.2 mg/L based on percent active ingredient.

In preparation for each chronic toxicity test, *C. dubia* neonates were isolated during a 4-8 hour period and held until the start of the test (< 24 h). Fifteen mL of toxicant or control water were put in each 30-mL test chamber, and the neonates (10 per test concentration) were randomly distributed among them, one to each chamber. Three replicate 7-day toxicity tests were then performed at each temperature. Hydrothol concentrations for chronic tests were 0.025-0.400 mg/L. Test solutions were prepared and renewed daily. The presence and number of young were recorded for each chamber prior to transferring the surviving adults to fresh test solutions.

Ceriodaphnia in the test vessels were fed 0.04 mL of TCY and 0.08 mL of a mixed algal culture (*Euglena, Klebsomidium* and *Chlamydomonas*) following transfer to clean vessels. Temperature, pH, alkalinity, hardness and conductivity of the dilution water were measured daily (Table 1). Each test was terminated after 7 days, and the mean production of young per adult was calculated for each treatment and the control.

Table 1. Dilution water quality parameters for *Ceriodaphnia dubia* survival and reproduction tests.

Parameter	Mean	S.D.
pH	6.84	0.08
Alkalinity (as mg/L $CaCO_3$)	53.21	1.55
Hardness (as mg/L $CaCO_3$)	86.83	1.56
Conductivity (umhos/cm)	349.3	3.4

LC50s were calculated by the moving-average angle and binomial methods (Horning and Weber 1985). Chronic sublethal toxicity was determined in 2 steps. First, Fisher's Exact Test was used to identify treatments in which adult survival was significantly different from that of the controls. Then, differences in reproductive rate were analyzed using ANOVA and Dunnett's procedure only for toxicant levels in which adult survival was *not* significantly different from the controls. From the results of these analyses, the No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC) and Chronic Value (ChV) were calculated. The ChV is the geometric mean of the NOEC and LOEC.

RESULTS AND DISCUSSION

The *Ceriodaphnia dubia* 48-h LC50 for Hydrothol was 0.49 mg/L at 25 C (Table 2). This agrees well with the published LC50 for *Daphnia sp.*--0.36 mg/L (Pennwalt Corp. 1980). At 15 C, the *C. dubia* 48-hour LC50 was 1.43 mg/L Hydrothol, comparable to the sensitivity of algae, rainbow trout (Mudge et al. 1986) and the golden shiner (Finlayson 1980) to Hydrothol at 17-21 C. Compared with other aquatic herbicides, Hydrothol is among the most toxic to daphnids (Table 3). For example, dichlobenil has a 48-h LC50 of 3.7-5.8 mg/L,

Table 2. Acute toxicity of Hydrothol to various aquatic organisms.

Organism	Temp. C	LC50 mg/L (95 % C.I.)	Test Time (h)
C. dubia	25	0.495[a] (0.363-0.7.65)	48
C. dubia	15	1.43[a] (1.09-2.00)	48
Daphnia sp.	25	0.360[b]	48
Algal mix	20.5	1.50[c]	120
Rainbow trout	20.5	1.70[c]	96
Golden shiner	17.5	3.20[d]	120

[a] Results of the current experiments.
[b] Pennwalt Corp. (1980). [c] Mudge et al. (1986).
[d] Finlayson (1980).

Table 3. Acute toxicities of several herbicides used to control submergent macrophytes in Florida lakes.

Herbicide	Organism	48-h LC50 mg/L	Temp C
Aquathol-K	*Daphnia magna*	316[a]	25
dichlobenil	*D. pulex*	3.7[b]	15
	Simocephalus	5.8[b]	15
2,4-D	*D. magna*	1.2[c,d]	21
diuron	*Simocephalus*	2.0[b]	15
	D. pulex	1.4[b]	15

[a] Pennwalt Corp. (1980). [b] Johnsqn and Finley (1980).
[c] Water hardness 272 ppm $CaCO_3$. [d] propylene glycol butyl ether ester of 2,4-D liquid.

while Aquathol-K is toxic only at much higher concentrations (48-h LC50 = 316 mg/L).

At 25 C, the 7-day LC50 for *C . dubia* was 0.18-0.19 mg/L Hydrothol. The 7-day LC50 was somewhat lower at 15 C (0.14-0.15 mg/L) than at 25 C, but the difference between the two ranges was not statistically significant ($p \leq 0.05$). This is approximately an order of magnitude lower than the suggested Hydrothol field application rate of 1-5 mg/L (Pennwalt Corp. 1980). Since its half-life is approximately 10 days (Blackburn et al. 1971; Reinert et al. 1985), the potential impact of Hydrothol on aquatic systems is obvious.

Seven-day reproduction data for *C. dubia* exposed to Hydrothol at 25 C indicated that even at concentrations as low as 0.025 mg/L (our lowest test concentration), Hydrothol significantly affected fecundity (Table 4). In order to determine the NOEC (No Observed Effect Concentration) used to calculate a chronic value (ChV), we ran 3 additional 7-day tests with controls and 0.010 mg/L Hydrothol test concentrations. Results of these tests (Figure 1) indicated that the LOEC was 0.025 mg/L, the NOEC was $\leq$ 0.010 mg/L and the chronic value (ChV) was $\leq$ 0.015 mg/L Hydrothol at 25 C.

There was no reproduction even after 7 days in the tests performed at 15 C. Therefore, no statistical analysis of sublethal effects was possible. The fact that no reproduction occurred at this low temperature is not surprising. McNaught and Mount (1985) found that the 7-day *C. dubia* reproduction test became a 28-day test at 18 C. Gophen (1976) measured a significant decrease in the metabolic rate of *C. reticulata* at 15 C versus 22 C. A lower metabolic rate can result in a lower reproductive rate.

The chronic toxicity of the aquatic herbicide Hydrothol-191 to the zooplankter *Ceriodaphnia dubia* was determined based on survival and reproduction. Our findings confirm previous conclusions that Hydrothol is considerably more toxic than some alternative compounds, e.g., Aquathol-K and diuron. *C. dubia* survival was significantly lower following a 7-day exposure to Hydrothol at concentrations as low as 0.20 mg/L. More importantly, reproduction was affected by Hydrothol concentrations as low as 0.01-0.03 mg/L. These results underscore the need for careful use of this herbicide in aquatic systems.

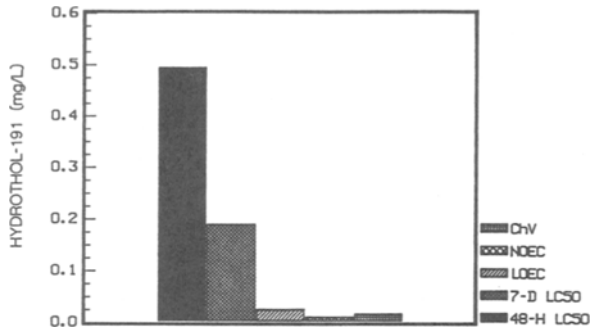

Figure 1. Chronic toxicity of Hydrothol-191 to *Ceriodaphnia dubia* at 25 C (ChV=Chronic Value, NOEC=No Observed Effect Concentration, LOEC=Lowest Observed Effect Concentration).

Table 4. Reproduction data for replicate tests of *Ceriodaphnia dubia* exposed to various concentrations of Hydrothol at 25 C for 7 days.

[Hydrothol] (mg/L)	Final Survival %	Mean (S.D.) young/female	Mean No. broods	
0	100	11.6(3.2)	2.50	*
.025	90	5.0(2.8)	1.33	*
.050	90	4.1(2.6)	0.80	*
.100	90	2.9(2.6)	0.80	
.200	70	0	0	
.400	0	0	0	
0	100	11.8(3.7)	2.50	*
.025	100	5.9(3.4)	1.10	*
.050	80	4.3(4.1)	0.90	*
.100	80	1.8(1.5)	0.70	
.200	80	0	0	
.400	0	0	0	
0	100	23.6(4.4)	2.9	*
.025	100	5.3(2.8)	1.1	*
.050	100	0.2	0.2	
.100	80	0	0	
.200	70	0	0	
.400	0	0	0	

* Indicates a significant difference from control at $p \leq 0.05$.

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