

July 7/18/88

MRID 263578

**DATA EVALUATION RECORD**

1. **CHEMICAL:** Ortho Dibrom 8 Emulsive.
2. **TEST MATERIAL:** Formulation: Ortho Dibrom 8 Emulsive (SX 1598, PN 2738-M); 58% as Naled technical (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), 20% light aromatic petroleum distillate, 22% inert ingredients; a clear amber-colored liquid.
3. **STUDY TYPE:** Freshwater Fish 96-Hour Flow-Through Test.  
Species Tested: Lepomis macrochirus.
4. **CITATION:** Suprenant, D.C. 1986. Acute Toxicity of Ortho Dibrom 8 Emulsive to Bluegill (Lepomis macrochirus) Under Flow-Through Conditions. Prepared by Springborn Bionomics, Inc., Wareham, MA. Submitted by Chevron Environmental Health Center, Inc., Richmond, CA. Bionomics Report #BW-86-3-1958. MRID 263578.
5. **REVIEWED BY:**  
  
Prapimpan Kosalwat, Ph.D.  
Staff Toxicologist  
KEN Engineering and  
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Signature: Prapimpan Kosalwat  
Date: 5/13/88
6. **APPROVED BY:**  
  
Isabel C. Johnson, M.S.  
Principal Scientist  
KEN Engineering and  
Applied Sciences, Inc.  
  
Signature: Isabel C. Johnson  
Date: May 13, 1988  
  
for Henry T. Craven  
Supervisor, EEB/HED  
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Signature: John Niles  
Date: 7/18/88
7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for freshwater fish test. The 96-hour LC50 value of 0.24 mg a.i./L classifies Ortho Dibrom 8 Emulsive as highly toxic to Lepomis macrochirus. The NOEL was less than 0.16 mg a.i./L.
8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:
10. DISCUSSION OF INDIVIDUAL TESTS: N/A.
11. MATERIALS AND METHODS:

A. Test Animals: The bluegill sunfish, Lepomis macrochirus (Bionomics lot #85A30), were obtained from a commercial fish supplier in Nebraska and held in a 1700-L fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness. The well water which flowed into this tank was characterized as having a total hardness range of 30-32 mg/L as  $\text{CaCO}_3$ , an alkalinity range of 27-31 mg/L as  $\text{CaCO}_3$ , and a specific conductance range of 120-140 micromhos per centimeter (umhos/cm) (Weekly Gravity Feed Tank Water Quality Analysis Logbook). Other parameters monitored in the holding tank were a pH range of 6.5-6.9, a dissolved oxygen concentration range of 84-92% of saturation and a flow rate of 5-7 tank volume replacements/day (Weekly Record of Fish Holding Water Characteristics). Test fish were maintained under these conditions for a minimum of 14 days. The temperature range in the holding tank was 15-22°C during this 14-day period. All fish were fed a dry commercial pelleted food, ad libitum, daily. There was mortality of <0.10% of the test fish population during the 48 hours prior to testing (Daily Record of Fish Holding Conditions). The mean (range, n=30) wet weight and total length of the test fish population were 0.30 (0.19-0.43) grams and 28 (27-35) millimeters (Fish Weight and Lengths Log).

B. Test System: The exposure system used in this study was a modified, proportional diluter, similar to that described by Mount and Brungs (1967) with a 0.65 dilution factor. The dilution water was from the same source as the water which flowed into the fish holding tank and was characterized as having total hardness of 26-32 mg/L as  $\text{CaCO}_3$ , alkalinity of 22-28 mg/L as  $\text{CaCO}_3$ , pH of 6.9-7.5, and specific conductance of 110-120 umhos/cm during the study period.

The diluter delivered 6 nominal concentrations of test material and a dilution water control to duplicate test aquaria. Each glass test aquarium measured 39 x 20 x 25 cubic centimeters with a 19-cm-high standpipe which maintained a constant test water volume of 15 L. The diluter delivered 0.5 L of test water to each aquarium at an average rate of 164 times per day. This is equivalent to 5.5 aquarium volume replacements per 24-hour period. Illumination was provided by Cool White and Grow Lux fluorescent lights centrally located above the test aquaria. Sixteen hours of light at 3-10 hectolux at the water surface were provided each day. The aquaria rested in a water bath containing circulating water cooled by a refrigeration unit designed to maintain the test

water temperature at  $22 \pm 1^{\circ}\text{C}$ .

Stock solutions used during this study (1.0 mg/mL of Ortho Dibrom 8 Emulsive) were prepared by diluting the appropriate quantity of test material with distilled water (e.g., 4.0 grams of Ortho Dibrom 8 Emulsive/4000 mL of water). Every diluter cycle a calibrated Marriotte bottle and modified McAlister delivery system introduced 5.1 mL of the stock solution (1.0 mg/mL) to the diluter's mixing chamber containing 2.66 L of water. The resulting solution (1.9 mg/L) was subsequently diluted (65%) to provide the test concentration range (0.22 to 1.9 mg/L of Ortho Dibrom 8 Emulsive).

C. Dosage: 96-hour acute flow-through test.

D. Design: Procedures used in this acute toxicity test followed those described in the protocol entitled "Acute Toxicity of Ortho Dibrom 8 Emulsive to Rainbow Trout, Bluegill Sunfish and Daphnia magna in Flow-Through Test Systems," CEHC Protocol #S-2591; 4 February 1985. This protocol closely follows "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians (EPA, 1975).

The test was initiated when ten bluegill were impartially selected and distributed to each aquarium (20 per treatment level). The resulting test organism loading concentration did not exceed 0.27 g of biomass per liter of test solution at any time during the exposure period. The test concentrations were 0.22, 0.34, 0.53, 0.81, 1.2, and 1.9 mg/L as nominal concentrations of Ortho Dibrom 8 Emulsive.

Mortalities were recorded and removed from each aquarium every 24 hours during exposure. Biological observations of the fish and observations of the physical characteristics of the test solutions were also made at 0 hour and each subsequent 24-hour interval. The pH's, dissolved oxygen concentrations and temperatures were measured at 0, 24, 48, 72, and 96 hours in both replicates of the control and all test concentrations.

Prior to initiating the toxicity test, water samples (100 mL) were removed from each replicate aquarium of the control, low, middle and high treatment levels. These samples were extracted and analyzed for Naled technical concentration (active ingredient).

The concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals at each 24-hour interval of the exposure period. In addition, the no-discernible-effect concentration (NDEC), defined as the highest concentration tested at and below which there were no toxicant-

related mortalities or observed behavioral and physical abnormalities (e.g., loss of equilibrium, fish at surface, darkened pigmentation), was determined.

**E. Statistics:** A computer program by Stephan (1982) was used to calculate IC50 values. Three statistical methods, in the following order of preference, were available in the computer program: moving average angle analysis, probit analysis, and binomial probability.

12. **REPORTED RESULTS:** The diluter system which prepared and delivered the test solutions to the exposure aquaria functioned properly throughout the 96-hour study period. Daily observations of the test aquaria indicated that the test material was in solution at all treatment levels tested. The measured concentrations were consistent during the test period and an exposure concentration gradient ranging from 0.16 to 0.94 mg/L of Naled technical was maintained. Analyses of the QA samples resulted in recoveries of 92-115% of the nominal concentrations added. The water quality parameters measured remained within acceptable ranges (pH = 6.6-7.1, dissolved oxygen = 8.9-9.8 mg/L, and temperature = 22°C) for the survival of bluegill and were unaffected by the concentrations of Ortho Dibrom 8 Emulsive tested.

The following table summarizes the test concentrations (nominal and mean measured) with corresponding cumulative mortalities and observations made during the toxicity test.

Nominal Conc. as Ortho Dibrom 8 Emulsive (mg/L)	Mean Measured Conc. as Naled tech. (mg/L)	Cumulative Mortalities (%)			
		24-hour	48-hour	72-hour	96-hour
1.90	0.94	35 <sup>a,b,c</sup>	70 <sup>a,e</sup>	80 <sup>a,e</sup>	100
1.20	0.61	0 <sup>a,b,d</sup>	30 <sup>a,e</sup>	45 <sup>a,e</sup>	100
0.81	0.38	0 <sup>a,b,c</sup>	30 <sup>a,e</sup>	45 <sup>a,e</sup>	60 <sup>a,b,c,e</sup>
0.53	0.30	0 <sup>b</sup>	40 <sup>a,e</sup>	45 <sup>a,e</sup>	80 <sup>a,b,e</sup>
0.34	0.18	0 <sup>b</sup>	5	10 <sup>e</sup>	40 <sup>a,b,c,g</sup>
0.22	0.16	0 <sup>b</sup>	0	0	15 <sup>a,b,f,g</sup>
Control	—	0	0	0	0

a = Several fish had their pectoral fins anteriorly extended.

b = Several fish exhibited spasmodic behavior.

c = Several fish exhibited a complete loss of equilibrium.

d = Several fish were dark in coloration.

e = All fish were lethargic.

f = One fish exhibited a complete loss of equilibrium.

g = Several fish were lethargic.

The 24-, 48-, 72-, and 96-hour LC50 values with their 95% confidence interval and the no-discernible-effect concentrations were estimated as follows:

Based on	LC50 (mg/L)				No discern. effect conc. (mg/L)
	24-hour <sup>a</sup>	48-hour <sup>b</sup>	72-hour <sup>c</sup>	96-hour <sup>b</sup>	
Nominal conc. mg/L as Ortho Dibrom 8 Emulsive	>1.9	1.5 (>0.34)	0.89 (0.71-1.2)	0.41 (0.33-0.49)	<0.22 <sup>d</sup>
Mean measured conc. mg/L as Naled technical	>0.94	0.65 (0.51-0.98)	0.46 (0.36-0.62)	0.24 (0.20-0.27)	<0.16 <sup>d</sup>

a = LC50 value was empirically estimated as being greater than the highest concentration of Ortho Dibrom 8 Emulsive tested.

b = LC50 value was estimated by nonlinear interpolation; 95% confidence interval determined by binomial probability.

c = LC50 value was calculated by moving average angle analysis.

d = Lowest concentration tested.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour LC50 values and corresponding 95% confidence intervals, based on nominal and mean measured concentrations were 0.41 (0.33-0.49) mg/L of Ortho Dibrom 8 Emulsive and 0.24 (0.20-0.27) mg/L of Naled technical, respectively. The NDEC was determined to be 0.22 mg/L, the lowest concentration of Ortho Dibrom 8 Emulsive tested.

The data were audited by the Bionomics' Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and the pertinent EPA Good Laboratory Practice (GLP) Regulations. A GLP compliance statement was included and signed by the Quality Assurance Unit.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**  
 A. **Test Procedure:** The test procedures were in general accordance with the protocols recommended by the SEP's guidelines, except for the following deviations:

- o No solvent control was included in the test. When the

technical product is insoluble in water but the formulated product is soluble in water, the test design should include a control where organisms are exposed to just the inert ingredients and carriers. Naled technical is known to be insoluble in water, while Ortho Dibrom 8 Emulsive is soluble in water. Therefore, the test should include a solvent control.

o The hardness of test water was between 26 and 32 mg/L as  $\text{CaCO}_3$  which is slightly lower than the recommended water hardness of 40-48 mg/L as  $\text{CaCO}_3$ .

o Age of the test fish was not reported. It was not known if they were from the same year class.

o There was no 15- to 30-minute transition period between light and dark as recommended by the guidelines.

o The test temperature was measured every 24 hours, instead of every 6 hours as recommended by the guidelines for the test using a water bath to control the temperature.

B. Statistical Analysis: Statistical analysis used by the author is appropriate and verified by the reviewer.

C. Discussion/Results: The 96-hour  $\text{LC}_{50}$  calculated by the reviewer was 0.24 mg/L as mean measured concentration of Naled technical (95% confidence limits = 0.20-0.30 mg/L) which is similar to that calculated by the author. Ortho Dibrom 8 Emulsive is considered highly toxic to Lepomis macrochirus. The no-observed-effect level (NOEL) was <0.16 mg/L as Naled technical.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: No solvent control was included in the test.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes, May 12, 1988.

As Naled Technical  
measured conc.  
96-h.

KOSALWAT ORTHO DIBROM 8 EMULSIVE LEPOMIS MACROCHIRUS 5-6-88

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.94	20	20	100	9.536742E-05
.61	20	20	100	9.536742E-05
.38	20	12	60.00001	25.17223
.3	20	16	80	.5908966
.18	20	8	40	25.17223
.16	20	3	15	.1288414

THE BINOMIAL TEST SHOWS THAT .16 AND .61 CAN BE  
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT  
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL  
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .2034209

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	.23207	.2432137	.2008205 .2975437

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.5403801	2.413647	4.666579E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED  
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.261742  
95 PERCENT CONFIDENCE LIMITS = 1.128912 AND 7.394572

LC50 = .2388008  
95 PERCENT CONFIDENCE LIMITS = .1266939 AND .357094

LC10 = .1202363  
95 PERCENT CONFIDENCE LIMITS = 1.240984E-02 AND .1840972

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