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 colorless liquid.

3. STUDY TYPE: Daphnia magna 48-Hour Flow-Through Test.
Species Tested: Daphnia magna.

4. CITATION: Suprenant, D.C. 1986. Acute Toxicity of Ortho Dibrom 8 Emulsive to Daphnia magna Under Flow-Through Conditions. Prepared by Springborn Bionomics, Inc., Wareham, MA. Submitted by Chevron Environmental Health Center, Inc., Richmond, CA. Bionomics Report #BW-86-3-1965. MRID 263578.

5. REVIEWED BY:

Prapimpan Kosalwat, Ph.D.
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Signature: Prapimpan Kosalwat
Date: 5/13/88

6. APPROVED BY:

Isabel C. Johnson, M.S.
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Signature: Isabel C. Johnson
Date: May 13, 1988

for Henry T. Craven
Supervisor, EEB/HED
USEPA

Signature: John Noles
Date: 7/18/88

7. CONCLUSIONS: This study is scientifically sound but does not meet the guideline requirements for freshwater invertebrate test. The 48-hour LC50 value of 1.5 ug/L nominal concentration classifies Ortho Dibrom 8 Emulsive as very highly toxic to Daphnia magna. The NOEL was 0.62 ug/L nominal concentration of Ortho Dibrom 8 Emulsive.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: The daphnids used in this toxicity test were obtained from laboratory cultures maintained at Springborn Bionomics, Inc., Wareham, MA. The culture water was prepared by fortifying well water based on the formula for hard water (EPA, 1975) and filtering it through a carbon filter and an Amberlite XAD-7 resin column to remove any potential organic contaminants. This water had total hardness and alkalinity ranges as $CaCO_3$ of 160-180 mg/L and 110-130 mg/L, respectively. Other parameters monitored were a pH range of 7.9-8.3, a temperature of 20 $\pm 1^{o}C$, a dissolved oxygen concentration of greater than 60% of saturation and a specific conductance range of 400-600 micromhos per centimeter (umhos/cm).

The daphnid culture area received a regulated photoperiod of 16 hours of light and 8 hours darkness. Light at an intensity of 5-10 hectolux at the culture solution surfaces was provided by a combination of Sylvania Growlux and Cool White fluorescent bulbs. Daphnids were fed a solution of green algae (Ankistrodesmus sp. or Selenastrum sp.) and yeast suspension once daily. The ambient air temperature in the culture area was controlled in order to maintain the culture solution temperatures at 20 $\pm 1^{o}C$.

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 0.50 dilution factor. The dilution water was from the same source as the water used in the culture vessels and was characterized as having total hardness of 160-170 mg/L as $CaCO_3$, alkalinity of 120 mg/L as $CaCO_3$, pH of 7.9-8.0, and a specific conductance of 600 umhos/cm during the study period.

The diluter delivered 5 nominal concentrations of Ortho Dibrom 8 Emulsive and a dilution water control to the test aquaria. Test vessels were glass battery jars having a volume capacity of 1.8 liters. Test solutions drained from aquaria through a 3 x 8 cm notch cut on the upper edge of the jars which maintained a solution depth of 15 cm. Drains were covered with a Nitex 40-mesh screen to prevent loss of the daphnids. Test solutions were delivered to the aquaria at a rate of 5 aquarium volumes per 24 hours. The ambient air temperature in the laboratory was controlled in order to maintain test solution temperatures at 20 $\pm 1^{o}C$. Test solutions were not aerated. The test area was illuminated with Cool White and Grow Lux fluorescent lights at an intensity of 3-6 hectolux during a photoperiod of 16 hours light

2

and 8 hours darkness.

A primary stock solution of 2570 ug/milliliter (mL) was prepared by diluting the 0.257 g of Ortho Dibrom 8 Emulsive with distilled water to a volume of 100 mL. A secondary stock solution of 25.7 ug/mL was formulated by diluting 1.0 mL of the primary stock to 100 mL with distilled water. A 50-mL gas-tight syringe with a stainless steel needle was activated during each diluter cycle by a mechanical injector to deliver 0.0759 mL of the 25.7 ug/mL Ortho Dibrom 8 Emulsive stock solution into the chemical mixing chamber of the diluter. The solution in the mixing chamber served as the highest treatment level which was subsequently diluted (0.5 dilution factor) to provide the exposure concentration range.

C. Dosage: 48-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-2592; 4 February 1985. This protocol closely follows "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians (EPA, 1975).

Eighty daphnids, ≤24 hours old, were impartially distributed to each concentration (20 daphnids per replicate) at the initiation of the study. The test concentrations were 0.31, 0.62, 1.2, 2.5, and 5.0 ug/L as nominal concentrations of Ortho Dibrom 8 Emulsive. All treatments and the control were conducted in quadruplicate. The test daphnids were not fed. Biological observations and observations of the physical characteristics of each replicate test solution were also made and recorded at 0, 24, and 48 hours of exposure. The pH, dissolved oxygen concentration and temperature were measured at 0, 24, and 48 hours in one replicate vessel of all treatment levels and the control.

Prior to initiating the definitive test, water samples (500 mL) were removed from each replicate solution of the control, low, middle and high treatment levels on the two days prior to test initiation. These pretest samples were analyzed for Naled technical (active ingredient) to confirm that the proper concentration of test material was being delivered and maintained in the exposure aquaria. During the definitive test composite water samples (total volume of 500 mL) were removed from two replicates of each treatment level and the control at 0, 24, and 48 hours. Alternate replicate solutions were sampled at each interval to provide a minimum of one analysis of each replicate.

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., lethargy, flared carapace), was determined.

E. Statistics: A computer program by Stephan (1982) was used to calculate LC50 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 method validation-recovery study conducted at Springborn Bionomics, Inc. for Naled technical in freshwater demonstrated that the minimum concentration of Naled technical which could accurately be measured in the exposure solutions was approximately 10 ug/L. During the definitive test exposing D. magna, the nominal concentration range was 0.31 to 5.0 ug/L of Ortho Dibrom 8 Emulsive (0.18 to 2.9 ug/L as Naled technical). Since the concentration of Naled technical in all exposure solutions was considerably lower than the established minimum detection limit, the amount of test material could not be analytically verified.

The diluter system which prepared and delivered the test solutions to the exposure aquaria functioned properly throughout the 48-hour study period. Daily observations of the test aquaria indicated that the test material was in solution at all treatment levels tested. The water quality parameters (pH = 7.8=-8.0, dissolved oxygen = 88-93% of saturation, and temperature = 20-21^{o}C) 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. of Ortho Dibrom 8 Emulsive (ug/L)	Cumulative Mortality (%)	
	24-hour	48-hour
5.0	39[a]	99[c]
2.5	30[b]	100
1.2	0[b]	16[b]
0.62	0	0
0.31	0	0
Control	0	0

a = All of the surviving daphnids were lethargic.
b = Several daphnids were lethargic.
c = One daphnid was lethargic.

The 24- and 48-hour LC50 values with their 95% confidence intervals estimated were >5.0 and 1.5 (1.4-1.6) ug/L, respectively. The no-discernible-effect concentration was determined to be 0.62 ug/L of Ortho Dibrom 8 Emulsive.

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 or inert ingredients.

o The hardness of test water was between 160 and 170 mg/L as $CaCO_3$ which is much higher than the recommended water hardness of 40-48 mg/L as $CaCO_3$.

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 measuring continuously (hourly) as recommended by the guidelines.

o Each treatment concentration was only 50% of the next highest concentration. The SEP's guidelines recommend each designated treatment group to be exposed to a concentration of toxicant that is at least 60% of the next highest concentration.

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

C. Discussion/Results: The 48-hour LC50 calculated by the reviewer was 1.5 ug/L as nominal concentration of Ortho Dibrom 8 Emulsive (95% confidence limits = 1.4-1.7 ug/L) which is similar to that calculated by the author. Ortho Dibrom 8 Emulsive is considered very highly toxic to Daphnia magna. The no-observed-effect level (NOEL) was 0.62 ug/L as nominal concentration of Ortho Dibrom 8 Emulsive. The test concentrations could not be verified with the analytical method used.

D. Adequacy of the Study:
(1) Classification: Supplemental.

(2) Rationale: The hardness of the test water was much higher than the recommended hardness in the guidelines and no solvent control was included in the test. The test concentrations could not be verified with the method used.

(3) Repairability: No.

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

KOSALWAT ORTHO DIBROM 8 EMULSIVE DAPHNIA MAGNA 5-6-88

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
5	80	79	98.75	0
2.5	80	80	100	0
1.2	80	13	16.25	0
.62	80	0	0	0
.31	80	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.532661

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.251917E-02	1.512162	1.363478	1.68574

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	23.83586	198.7777	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 8.033027

95 PERCENT CONFIDENCE LIMITS =-31.1858 AND 47.25186

LC50 = 1.545601

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 1.07399

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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