July 7/18/88

MRID 263579

DATA EVALUATION RECORD

1. **CHEMICAL:** Ortho Dibrom LVC 10.

2. **TEST MATERIAL:** Formulation: Ortho Dibrom LVC 10 (SX 1599, PN 5196); 15% as Naled technical (1,2-dibromo-2.2-dichloroethyl dimethyl phosphate); a clear amber-colored liquid.

3. **STUDY TYPE:** Freshwater Fish 96-Hour Flow-Through Test. Species Tested: Salmo gairdneri.

4. **CITATION:** Suprenant, D.C. 1986. Acute Toxicity of Ortho Dibrom LVC 10 to Rainbow Trout (Salmo gairdneri) Under Flow-Through Conditions. Prepared by Springborn Bionomics, Inc., Wareham, MA. Submitted by Chevron Environmental Health Center, Inc., Richmond, CA. Bionomics Report #BW-86-4-1972. MRID 263579.

5. **REVIEWED BY:**

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

6. **APPROVED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
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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 and meets the guideline requirements for freshwater fish test. The 96-hour LC50 value of 0.21 mg a.i. (Naled technical) per liter classifies Ortho Dibrom LVC 10 as highly toxic to Salmo gairdneri. The NOEL was less than 0.076 mg a.i./L.

8. **RECOMMENDATIONS:** N/A.

9. [illegible]

10. DISCUSSION OF [illegible] TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: The rainbow trout, Salmo gairdneri (Bionomics lot #85A34), were obtained from a commercial fish supplier in Montana and held in a 500-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 29-33 mg/L as $CaCO_3$, an alkalinity range of 25-26 mg/L as $CaCO_3$, and a specific conductance range of 110-120 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 7.0-7.3, a dissolved oxygen concentration range of 79-90% of saturation and a flow rate of 19-21 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 11-13°C during this 14-day period. All fish were fed a dry commercial pelleted food, ad libitum, daily. There was no mortality in 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 (0.08-0.51) grams and 32 (24-39) 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 35-36 mg/L as $CaCO_3$, alkalinity of 25-26 mg/L as $CaCO_3$, pH of 7.5-7.6, and specific conductance of 120-170 umhos/cm during the study period.

The diluter delivered 5 nominal concentrations of test material, a solvent control, 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 163 times per day. This is equivalent to 5.4 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 12 ±1°C.

Stock solutions used during this study (73 mg/mL of Ortho Dibrom LVC 10) were prepared by diluting the appropriate quantity of test material with acetone (e.g., 3.65 grams of Ortho Dibrom LVC 10 per 50 mL of acetone). In every diluter cycle, a calibrated Marriotte bottle and modified McAlister delivery system introduced 0.0348 mL of the stock solution (73 mg/mL) to the diluter's mixing chamber containing 2.52 L of water. The resulting solution (1.0 mg/L) was subsequently diluted (65%) to provide the test concentration range (0.18 to 1.0 mg/L of Ortho Dibrom LVC 10). In addition to the dilution water control, a solvent control containing the greatest amount of acetone present in any treatment level (14 uL/L) was also maintained during this study.

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 LVC 10 to Rainbow Trout, Bluegill Sunfish and Daphnia magna in Flow-Through Test Systems," CEHC Protocol #S-2593; 1 February 1985. This protocol closely follows "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians (EPA, 1975).

The test was initiated when ten rainbow trout were impartially selected and distributed to each aquarium (20 per treatment level). The resulting test organism loading concentration did not exceed 0.20 g of biomass per liter of test solution at any time during the exposure period. The test concentrations were 0.18, 0.27, 0.42, 0.65, and 1.0 mg/L as nominal concentrations of Ortho Dibrom LVC 10.

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 controls and all test concentrations.

Water samples (100 mL) were removed prior to test initiation from both replicate aquaria of the solvent control and the low, middle, and high treatment levels. Analyses of these pretest samples were used to determine if sufficient concentrations of the test material were being delivered and maintained in the exposure aquaria to initiate the 96-hour test. Samples were removed from one replicate aquarium of each treatment level and the controls at test initiation and termination. All 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, 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 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 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 mean measured concentrations during the test period ranged from 0.033 to 0.23 mg/L of Naled technical. Analyses of the QA samples during pretest and at 96 hours resulted in recoveries of 108 and 94% of the nominal concentrations added. The water quality parameters measured remained within acceptable ranges (pH = 7.0-7.6, dissolved oxygen = 9.5-10.8 mg/L, and temperature = 12-13°C) for the survival of rainbow trout and were unaffected by the concentrations of Ortho Dibrom LVC 10 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 LVC 10 (mg/L)	Mean Measured Conc. as Naled tech. (mg/L)	Cumulative Mortalities (%)			
		24-hour	48-hour	72-hour	96-hour
1.00	0.23	0[a]	0[abcd]	45[abcdefg]	65[abcdg]
0.65	0.13	0	0[ae]	0	0[cdg]
0.42	0.076	0	0	0	0
0.27	0.068	0	0	0	0
0.18	0.033	0	0	0	0
Solvent Control	---	0	0	0	0
Control	---	0	0	0	0

a = Several fish were dark.
b = Several fish exhibited complete loss of equilibrium.
c = Several fish were at the surface of the test solution.
d = Several fish were lethargic.
e = Several fish exhibited partial loss of equilibrium.
f = Several fish exhibited anteriorly extended pectoral fins.
g = Several fish were respiring rapidly.

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[a]	48-hour[a]	72-hour[a]	96-hour[b]	
Nominal conc. mg/L as Ortho Dibrom LVC 10	>1.0	>1.0	>1.0	0.90 (0.8-1.1)	0.42
Mean measured conc. mg/L as Naled technical	>0.23	>0.23	>0.23	0.20 (0.17-0.25)	0.076

a = LC50 value was empirically estimated as being greater than the highest concentration of Ortho Dibrom LVC 10 tested.
b = LC50 values and 95% confidence interval were calculated by probit analysis.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour LC50, based on nominal concentrations of Ortho Dibrom LVC 10 was calculated by probit analysis to be 0.90 mg/L with a 95% confidence interval between 0.8 and 1.1 mg/L. Calculations based on mean measured concentrations of Naled technical resulted in a 96-hour LC50 (95% confidence interval) of 0.20 (0.17-0.25) mg/L.

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 The hardness of test water was between 35 and 36 mg/L as $CaCO_3$ which is slightly lower than the recommended water hardness of 40-

48 mg/L as $CaCO_3$.

o Age of the test fish were 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: The author used probit analysis to determine the 96-hour LC50 values. When there were less than two concentrations at which the percent dead was between 0 and 100, the probit method would not give any statistically sound results. The approximate 96-hour LC50 determined by binomial test was 0.21 mg/L as Naled Technical.

C. Discussion/Results: The 96-hour LC50 calculated by binomial test was 0.21 mg/L as mean measured concentration of Naled technical. Ortho Dibrom LVC 10 is considered highly toxic to Salmo gairdneri. The no-observed-effect level (NOEL) was 0.076 mg/L as Naled technical.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

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

Measured Active ingredient

KOSALWAT ORTHO DIBROM LVC 10 SALMO GAIRDNERI 5-6-88

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.23	20	13	65	13.1588
.13	20	0	0	9.536742E-05
.076	20	0	0	9.536742E-05
6.800001E-02		20	0	0
9.536742E-05				
.033	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY 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 .2079309

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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