July 7/18/88

MRID No. 263580

DATA EVALUATION RECORD

1. **CHEMICAL:** Ortho Fly Killer D (36% Naled Technical)

2. **TEST MATERIAL:** Ortho Fly Killer D; Lot No. SX-1597; PN 3021-L; 36% as Naled Technical

3. **STUDY TYPE:** Acute Freshwater invertebrate, Flow-through Species Tested: Daphnia magna

4. **CITATION:** Surprenant, D.C. 1986. Acute Toxicity of Ortho Fly Killer D to Daphnia magna Under Flow-Through Conditions. Bionomics Report #BW-86-2-1938. Prepared by Springborn Bionomics, Inc., Wareham Massachusetts. Submitted by Chevron Environmental Health Center, Inc. Richmond, California. MRID Number 263580.

5. **REVIEWED BY:**

Brian A. Wade
Aquatic Toxicologist
ESE

Signature: Brian A. Wade
Date: 5/11/88

6. **APPROVED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
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Signature: Isabel C. Johnson
Date: May 16, 1988

Henry T. Craven
Supervisor, EEB/HED
USEPA

Signature: John Noles
Date: 7/19/88

7. **CONCLUSIONS:** This study is scientifically sound, but does not fulfill the Guideline requirements for toxicity determination with a freshwater invertebrate because an inert carrier control was not conducted. The 48-hour LC50 value for Daphnia magna exposed to Ortho Fly Killer D was 0.002 mg/L based on nominal concentrations. Ortho Fly Killer D is classified as very highly toxic to Daphnia magna.

8. **RECOMMENDATIONS:** N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Daphnia magna used in this test were obtained from laboratory cultures maintained at Springborn Bionomics in Wareham Massachusetts. Daphnia magna were less than twenty-four hours old at test initiation. Cultures were fed green algae (Ankistrodesmus sp. or Selenestrum sp.) and yeast suspension. Daphnia magna were not fed during exposure.

B. Test System: The test was conducted under flow-through conditions for 48-hours. Test vessels were glass jars with a volume capacity of 1.8 L. Test solutions were delivered at a rate of four volume replacements every 24 hours. Dilution water used in the test had the following characteristics: Total hardness of 160 mg/L as $CaCO_3$; Alkalinity of 120 mg/L as $CaCO_3$; pH of 8.0 and a specific conductance of 600 micromhos per 0.514 grams of test material to 100 mL. A secondary stock was prepared by dilution of 1.0 mL of the primary stock to 100 mL with distilled water. This 51.4 ug/mL test stock was delivered to the mixing chamber, and was diluted using a 50-percent dilution factor to provide the exposure concentrations.

The temperature was maintained at $20\pm^{o}C$ under fluorescent lighting on a 16-hour light and 8-hour dark photoperiod.

C. Dosage: 48-hour acute flow through test.

D. Design: Twenty $\leq$ 24-hour old Daphnia magna were tested in each vessel, and vessels were quadruplicated in each test concentration, (80 animals per treatment). A control and nominal Ortho Fly Killer D concentrations (uncorrected for percent active ingredient) of 0.62, 1.2, 2.5, 5.0 and 10 ug/L were maintained. Chemical analysis of the test substance could not be verified because concentrations were below the detectable limit for the method.

E. Statistics: The computer program developed by Stephan et al was used to calculate the LC50 values.

12. REPORTED RESULTS: The study reported an LC50 value of 2.0 (1.2-2.5) ug/L for Daphnia magna after 48-hours of exposure to Ortho Fly Killer D (36% as Naled Technical)

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 48-hour LC50 value for Daphnia magna exposed to nominal Ortho Fly Killer D, as whole material, under flow-through test conditions was 2.0 ug/L with 95 percent confidence limits of 1.2 and 2.5 ug/L.

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

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were in accordance with protocols recommended by Guidelines, except for the following:

Test temperature was recorded daily, not every six hours as required for tests utilizing water baths.

The dilution factor was 50 percent, not at least 60 percent as stated in the Guidelines.

Hard water was used in the test, although soft water is recommended.

B. Statistical Analysis: The computer program developed by Stephan et al was used to calculate the LC50 values. These calculations are attached. A LC50 value of 2.0 ug/L with confidence limits of 1.2-2.5 ug/L was calculatd using Non-linear interpolation. No slope is given.

C. Discussion/Results: The 48-hour LC50 value of 2.9 ug/L, as Ortho Fly Killer D, uncorrected for active ingredient, classify this substance as very highly toxic to Daphnia magna. The test was conducted at a water hardness of 160 mg/L as $CaCO_3$ and a temperature of 20°C. A concern does exist in this study because all test concentrations were below the limit of detection for the method and therefore, no test concentrations were verified.

D. Adequacy of the Study:

(1) Classification: Supplemental

(2) Rationale: N/A

(3) Repairability: Yes, provide inert carrier control data

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 5-11-88.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (%)
10	80	80	100	8.271806E-23
5	80	80	100	8.271806E-23
2.5	80	62	77.5	4.071334E-05
1.2	80	0	0	8.271806E-23
.62	80	0	0	8.271806E-23

THE BINOMIAL TEST SHOWS THAT 1.2 AND 2.5 CAN BE USED AS STATISCALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 99.99996 PERCENT.
AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2.032004

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.

Table 1. Concentrations tested, corresponding cumulative mortalities and observations made during the 48-hour flow-through exposure of Daphnia magna to Ortho Fly Killer D.

Nominal concentration (ug/L)	Percentage Mortality									
	24-hour					48-hour				
	A	B	C	D	$\bar{x}$	A	B	C	D	$\bar{x}$
10	30	40	25	30	31[a]	100	100	100	100	100
5.0	5	5	10	0	5[a]	100	100	100	100	100
2.5	0	0	0	0	0[b]	75	80	75	80	78[a]
1.2	0	0	0	0	0	0	0	0	0	0
0.62	0	0	0	0	0	0	0	0	0	0
control	0	0	0	0	0	0	0	0	0	0

[a]All surviving daphnids were lethargic.

[b]Several of the daphnids were lethargic.