

July 7/18/88

MRID 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 Fish, Flow-through
Species Tested: Rainbow Trout (Salmo gairdneri)
4. **CITATION:** Surprenant, D.C. 1986. Acute Toxicity of Ortho Fly Killer D to Rainbow Trout (Salmo gairdneri) Under Flow-Through Conditions. Bionomics Report #BW-85-12-1898. 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
KEN Engineering and
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Signature: *Isabel C. Johnson*

Date: *May 16, 1988*

for Henry T. Craven
Supervisor, EEB/HED
USEPA

Signature: *John Niles*

Date: *7/18/88*

7. **CONCLUSIONS:** This study is scientifically sound, but fails to fulfill the Guideline requirements for toxicity determination with a cold water fish species because it lacks a control for the inert carrier. The 96-hour LC50 value for Salmo gairdneri exposed for Ortho Fly Killer D under flow-through test conditions was 0.34 mg/L based on nominal concentrations of whole material. Ortho Fly Killer D is classified as highly toxic to rainbow trout.
8. **RECOMMENDATIONS:** N/A

9. BACKGROUND:10. DISCUSSION OF INDIVIDUAL TESTS: N/A11. MATERIALS AND METHODS:

- A. Test Animals: Rainbow trout (*Salmo gairdneri*) were obtained from a commercial supplier in Montana. The mean wet weight was 0.99 (0.45 - 1.46) g and a mean total length of 46 (39 - 52) millimeters. Fish were fed a dry commercial pelleted food, ad libitum, daily during holding.
- B. Test System: The test was conducted under flow-through conditions for 96 hours. Test aquaria were 39 X 20 X 25 cm with a 19-cm standpipe and providing for a test volume of 15 L. During the test, 0.5 L of test solution was delivered to each aquaria on an average of 178 times each 24-hour period; an average of 5.9 volume replacements per 24 hours. Dilution water used in the test had the following characteristics: total hardness of 28 - 29 mg/L as CaCO_3 ; alkalinity of 26 mg/L as CaCO_3 ; pH of 7.4 and specific conductance of 100 - 120 umhos/cm. The stock solution was prepared by adding 0.90 g of test material to 3 L of water for a 0.30 mg/ml stock concentration. Upon each cycle, 5.12 ml of the stock solution was introduced into the mixing chamber along with 2.65 L of dilution water. A portion of this 0.58 mg/L test solution was then diluted using a 65 percent dilution factor to prepare the lower test concentrations. The test temperature was maintained by a water bath at $12 \pm 1^\circ\text{C}$.
- C. Dosage: 96-hour acute flow-through test.
- D. Design: Ten rainbow trout were tested per test aquarium and all treatments were duplicated (20 per treatment level). A control and nominal Ortho Fly Killer D concentrations of (uncorrected for percent active ingredient) 0.10, 0.16, 0.24, 0.38, and 0.58 mg/L were maintained. The mean measured test concentrations, as Naled Technical, were 0.044, 0.069, 0.11, 0.15, and 0.27 mg/L. A sixth concentration was initiated at a nominal Ortho Fly Killer D concentration of 0.067 mg/L, but later discovered to actually be 0.17 mg/L; the mean measured concentration for this treatment was 0.074 mg/L.
- 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 0.34 (0.22 - 1.6) mg/L for rainbow trout (*Salmo gairdneri*) after 96 hours of exposure to Ortho Fly Killer D (36% as Naled Technical). The results of the test are reported in Table 2 (attached).
13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour LC50 for rainbow trout exposed to nominal Ortho Fly Killer D, as whole material, under flow-through test conditions was 0.34 mg/L with 95 percent confidence limits of 0.22 and 1.6 mg/L.

The data were audited by the 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 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 the Guidelines, except that test temperature was monitored daily, not every six hours as stated in the Guidelines for tests conducted in water baths. In addition, a control of inert carrier was not conducted concurrently as required.
- B. Statistical Analysis: The reviewer used the computer program developed by Stephan et al. to calculate the 96-hour LC50 value. These calculations are attached. The program does indicate that the selection of probit analyses, as done by the author, for reporting the 96-hour LC50 is not recommended. However, the use of the moving average method provides a similar LC50 and 95 percent confidence limits (0.38 mg/L with limits of 0.31 and 0.50 mg/L). The report did not specify the slope of the toxicity curve as required by the SEP, but the value calculated by Stephan's program was 3.00.
- C. Discussion/Results: The 96-hour LC50 value of 0.34 mg/L, as Ortho Fly Killer D uncorrected for active ingredient, for rainbow trout classify this substance as highly toxic. The toxicity test was conducted at a water hardness of 28 to 29 mg/L as CaCO₃ and a temperature of 12°C.
- 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

Table 2. Concentrations tested and corresponding mortalities of rainbow trout (*Salmo gairdneri*) exposed to Ortho Fly Killer D (36% Naled technical) during a 96-hour flow-through toxicity test. Mean measured concentrations are based on 0 and 96-hour analyses of the test solutions for Naled technical.

Nominal Concentrations as Ortho Fly Killer D (mg/L)	Nominal Concentrations as Naled technical (mg/L)	Mean Measured Concentrations as Naled technical (mg/L)	Cumulative Mortality (%)											
			24-hour			48-hour			72-hour			96-hour		
			A	B	\bar{x}	A	B	\bar{x}	A	B	\bar{x}	A	B	\bar{x}
0.58	0.21	0.27	0	0	0 ^{abcde}	70	70	70 ^{abc}	80	100	90 ^{abh}	80	100	90 ^{abh}
0.38	0.14	0.15	0	0	0 ^{ab}	20	10	15 ^{abfg}	30	40	35 ^{abf}	40	40	40 ^{ab}
0.24	0.086	0.11	0	0	0 ^b	0	0	0 ^{be}	10	0	5 ^{abf}	20	10	15 ^{bcl}
0.17 ^k	0.061 ^k	0.074	0	0	0	0	0	0	20	0	10	20	20	20
0.16	0.058	0.069	0	0	0	0	0	0	20	10	10 ^{efj}	40	20	30 ^e
0.10	0.036	0.044	0	0	0	0	0	0	0	0	0	10	0	5
control	—	—	0	0	0	0	0	0	0	0	0	0	0	10

^aAll fish were lethargic.
^bAll fish were respiring rapidly.
^cSeveral fish exhibited a partial loss of equilibrium.
^dSeveral fish exhibited a complete loss of equilibrium.
^eOne fish exhibited a darkened pigmentation.
^fOne fish exhibited a partial loss of equilibrium.
^gOne fish exhibited a complete loss of equilibrium.
^hAll fish exhibited a partial loss of equilibrium.
ⁱSeveral fish were lethargic.
^jSeveral fish were respiring rapidly.
^kNominal concentrations for this treatment level were corrected based on the results of the measured concentrations determined during this study.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(%)
.58	20	18	90	2.012253E-02
.38	20	8	40	25.17223
.24	20	3	15	.1288414
.17	20	4	20	.5908966
.16	20	6	30	5.765915
.1	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT .1 AND .58 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS 99.97788 PERCENT.
AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .4101885

>>>>>>>RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1735746	.3786737	.3122507	.5042656

>>>>>>>RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.6618753	2.464624	4.288018E-02

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

SLOPE = 3.00113
95 PERCENT CONFIDENCE LIMITS = .5595391 AND 5.442721

LC50 = .3426936
95 PERCENT CONFIDENCE LIMITS = .2157345 AND 1.638572
LC1 = 5.749119E-02
95 PERCENT CONFIDENCE LIMITS = 7.518325E-05 AND .1219983