July 7/18/88

MRID 263581

1. **CHEMICAL:** 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate; Naled Technical

2. **TEST MATERIAL:** Naled Technical; Lot No. SX-1554; 90% purity

3. **STUDY TYPE:** Acute Estuarine Fish, Flow-through
Species Tested: Cyprinodon variegatus

4. **CITATION:** Surprenant, D.C. 1986. Acute Toxicity of Naled Technical to Sheepshead Minnow (Cyprinodon variegatus) Under Flow-Through Conditions. Bionomics Report #BW-86-4-1971. Prepared by Springborn Bionomics, Inc., Wareham, Massachusetts. Submitted by Chevron Environmental Health Center, Inc. Richmond, California. MRID Number 263581.

5. **REVIEWED BY:**

Kimberly D. Rhodes
Aquatic Toxicologist
ESE

Signature: Kimberly D. Rhodes
Date: May 11, 1988

6. **APPROVED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
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Signature: Isabel C. Johnson
Date: May 12, 1988

for Henry T. Craven
Supervisor, EEB/HED
USEPA

Signature: John Noles
Date: 7/18/88

7. **CONCLUSIONS:** This study is scientifically sound. The 96-hour LC50 value for Cyprinodon variegatus exposed for 96 hours to Naled Technical under flow-through test conditions is 1.2 mg/L. Naled Technical is classified as moderately toxic to Cyprinodon variegatus.

8. **RECOMMENDATIONS:** N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Cyprinodon variegatus were obtained from a commercial fish supplier in Massachusetts and maintained for a minimum of 14 days. The fish ranged from 0.23 to 0.87 g wet weight with an average of 0.49 g wet weight. No mortality was observed during the 48-hour holding period immediately prior to testing. Adult [29 mm average total length] fish were selected for testing. Fish were fed a dry commercial pelleted food ad libitum, during holding.

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 flow rate provided 5.1 volume additions per day. The temperature was maintained by a water bath at 22 ± 1°C.

The dilution water was filtered natural seawater. The salinity of the seawater was 32 °/oo, the pH was 7.6-7.9 and the specific conductance was 30,000-35,000 umhos/cm.

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

D. Design: Ten sheepshead minnows were tested per test aquarium (20 per treatment level). A control, solvent control (acetone), and nominal Naled technical concentrations of (uncorrected for percent active ingredient) 0.54, 0.82, 1.3, 2.0, and 3.0 mg/L were maintained. The mean measured test concentrations were 0.30, 0.49, 0.70, 1.1, and 1.6 mg/L.

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

12. REPORTED RESULTS: "The test concentrations (nominal and mean measured), corresponding cumulative mortalities and observations made during the toxicity test are summarized in Table 2" (attached). "The 96-hour LC50 and corresponding 95% confidence interval based on mean measured concentrations of Naled technical were calculated by probit analysis to be 1.2 mg/L with a 95 percent confidence limits of 1.0 and 1.4 mg/L".

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 96-hour

LC50 for sheepshead minnow (Cyprinodon variegatus) exposed to mean measured concentrations of Naled Technical under flow-through test conditions was 1.2 mg/L with 95 percent confidence limits of 1.0 and 1.4 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 generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

Some of the test fish (0.23-0.87 g) were smaller than the recommended 0.5 to 5.0 g size range.

The SEP states that the salinity of the dilution water should be 10 to 17 °/oo for testing estuarine (euryhaline) fish species. The dilution water used during this study had a salinity of 32 °/oo.

The pH range reported for the holding seawater is not typical of the high salinity seawater and appears to be inaccurate, if not, it is very low. In any case this would not affect the test results.

B. Statistical Analysis: The reviewer used the computer program developed by Stephan et al. to calculate the LC50 values. 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 (1.15 mg/L with limits of 0.93 and 1.50 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 5.11.

C. Discussion/Results: The 96-hour LC50 value of 1.2 mg/L for Cyprinodon variegatus classifies Naled Technical as moderately toxic. The toxicity test was conducted at a salinity of 32 °/oo and a temperature of 22°C. These procedural modifications do not appear to have affected the validity of the test. Since the concentrations were measured and the LC50 values can be calculated from those measured concentrations, the results of the study are an accurate indication of the toxicity of the test material.

D. Adequacy of the Study:

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

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

Table 2. Concentrations tested and corresponding mortalities of sheepshead minnow (Cyprinodon variegatus) exposed to 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.

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	$\bar{x}$
3.0	1.6	40	70	55[abcd]	40	70	55[abd]	40	80	60[abcd]	50	90	70[abc]
2.0	1.1	10	20	15[abce]	10	30	20[abcd]	10	30	20[abd]	40	60	50[acgj]
1.3	0.70	0	0	0[abce]	10	0	5[abce]	10	0	5[abdh]	20	10	15[bdhj]
0.82	0.49	0	0	0[af]	0	0	0[abg]	0	0	0[abei]	0	0	0[abh]
0.54	0.30	0	0	0	0	0	0	0	0	0[ab]	0	0	0[ab]
solvent control	—	0	0	0	0	0	0	0	0	0	0	0	0
control	—	0	10	5	0	10	5	0	10	5	0	10	5

[a]Several fish were lethargic.
[b]Several fish exhibited anteriorly extended pectoral fins.
[c]Several fish exhibited partial loss of equilibrium.
[d]Several fish exhibited complete loss of equilibrium.
[e]One fish exhibited complete loss of equilibrium.
[f]One fish was dark.
[g]One fish exhibited partial loss of equilibrium.
[h]One fish was at the surface of the test solution.
[i]Several fish were at the surface of the test solution.
[j]One fish was lethargic.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(%)
1.6	20	14	70	5.765915
1.1	20	10	50	58.80986
.7	20	3	15	.1288414
.49	20	0	0	9.536743E-05
.3	20	0	0	9.536743E-05

THE BINOMIAL TEST SHOWS THAT .7 AND + INFINITY CAN BE USED AS STATISCALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 99.87116 PERCENT.
AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.1

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

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.2972176	1.14866	.9298539	1.496123

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

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1649676	.4208438	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 = 5.1071
95 PERCENT CONFIDENCE LIMITS = 3.032789 AND 7.18141

LC50 = 1.180365
95 PERCENT CONFIDENCE LIMITS = 1.006093 AND 1.458672
LC1 = .4134445
95 PERCENT CONFIDENCE LIMITS = .2135969 AND .5569134