6-4-91

MRID No. 417769-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Propanil-3,4-Dichloropropionilide. Shaughnessey No. 028201.

2. **TEST MATERIAL:** Propanil; Batch No. 01; 98% active ingredient; a blue-gray crystal.

3. **STUDY TYPE:** Marine Shrimp Acute Flow-Through Toxicity Test. Species Tested: Mysid shrimp (Mysidopsis bahia).

4. **CITATION:** Sousa, J.V. 1990. (Propanil) - Acute Toxicity to Mysid Shrimp (Mysidopsis bahia) Under Flow-Through Conditions. SLI Report No. 90-4-3275. Performed by Springborn Laboratories, Inc., Wareham, Massachusetts. Submitted by The Propanil Task Force, c/o John M. Wise, Liberty, Missouri. EPA MRID No. 417769-01.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 6/4/91

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 6/4/91

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature:
Date:

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute flow-through toxicity study. The 96-hour LC_{50} of Propanil for Mysidopsis bahia was 0.40 mg a.i./L, based on mean measured concentrations. Therefore, Propanil is classified as highly toxic to mysid shrimp. The NOEC could not be determined.

→ See page 3 & 18

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The test organisms (*Mysidopsis bahia*) were obtained from cultures maintained at Springborn Laboratories, Inc. The culture water was from the same source as that used in the test. The temperature of the cultures was maintained at 25°±1°C. A 16-hour light/8-hour dark photoperiod was maintained. The shrimp were fed twice daily with brine shrimp nauplii.

B. **Test System:** The test system consisted of an intermittent-flow proportional diluter and 14 glass aquaria (30 X 15 X 19 cm) fitted with self-starting siphons. The system flow rate was adequate to fully exchange the solution in each tank 10 times/day. The total volume in the tanks fluctuated between 3.6 and 5.4 L. Each aquarium contained two mysid retention chambers which were constructed of Nitex screen and glass petri dishes. The aquaria were impartially positioned in a circulating water bath designed to maintain the test temperature at 25°±1°C.

C. **Dosage:** Ninety-six-hour flow-through test. Based on the results of a preliminary test, five nominal concentrations were used (0.12, 0.18, 0.27, 0.42, and 0.65 mg a.i./L). In addition, a dilution water control and a solvent control were used.

A stock solution was prepared using 2.392 g of Propanil mixed to a total volume of 50 mL with acetone. The test substance stock solution was mechanically injected into the mixing chamber of the diluter. A concentration equal to the highest test concentration was produced in the mixing chamber and was subsequently diluted to produce the remaining test concentrations. A similar system was used to distribute solution to the solvent control. Each solvent replicate received the greatest amount of solvent (14 µL acetone/L) introduced into any test solution.

D. **Design:** Five mysids were loaded into each of two retention chambers in each replicate aquarium. Each concentration level consisted of two replicate aquaria, resulting in 20 mysids per test level. At any level, the organism loading concentration was less than 3 mg/L. Live brine shrimp nauplii were added twice daily to each retention chamber containing live shrimp.

Mortality, biological observations, and observations of physical characteristics were noted every 24 hours. Dead mysids were removed at each observation interval.

The dissolved oxygen, pH, salinity, and temperature were measured daily in each replicate of the test levels and controls. The temperature was also monitored continuously using a minimum/maximum thermometer. Chemical analyses of the solutions from Day 0 and 4 of the test were performed to verify the test concentrations.

E. **Statistics:** The author used the computer program by Stephan (1977, 1980) to calculate the LC_{50} (EC_{50}). For the results of this study the moving average angle method was used.

12. **REPORTED RESULTS:** Records of test parameters indicate that acceptable test conditions were maintained throughout the study (Table 1, attached).

Based on the mean measured concentrations, the dilution series was as follows: 0.12, 0.19, 0.29, 0.42, 0.63 mg a.i./L (Table 2, attached). The measured concentrations were fairly consistent between sampling days.

The mortality and observation data, based on mean measured concentrations, demonstrated mortality among mysids exposed to 0.12 and 0.19 mg a.i./L was 5-10%. However, since 5% mortality was also observed in the solvent control solution, mortality at the two levels was considered incidental and unrelated to treatment (Table 3, attached).

The 96-hour LC_{50} (EC_{50}) for mysid shrimp exposed to Propanil was 0.40 mg a.i./L (95% confidence interval of 0.35-0.48 mg a.i./L) (Table 4, attached). The slope was 1.2. The NOEC was 0.18 mg a.i./L, "the lowest measured concentration tested."

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based on EPA (1985) criteria, Propanil would be classified as highly toxic to Mysidopsis bahia."

A Good Laboratory Practice Compliance Statement was included in the report, indicating that the study was in accordance with GLP regulations with the following exceptions: "stability, characterization and verification of the test substance identity and maintenance of record on the test substance is the responsibility of the test Sponsor." This

statement was signed by representatives of the performing laboratory and the sponsor. In addition, a Quality Assurance Statement was presented in the report and signed by a quality assurance representative of the performing laboratory.

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, except for the following deviations:

The test organisms were "impartially selected" for loading into the test chambers. The SEP recommends random assignment of test organisms to test chambers.

The report does not indicate the age of the test organisms.

The recommended photoperiod for a shrimp acute toxicity study is 16-hour light/8-hour dark with a 15- to 30-minute transition period. No transition period was mentioned in the report.

The continuous temperature measurement indicated that the temperature was 25°±2°C. The SEP recommends a temperature which is at or around 22°C and should not deviate more than 1°C or so during the test.

Overall health of the test organisms was not reported during acclimation.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the EC_{50} value and obtained the same results as the author (printout, attached). The reviewer concludes that the NOEC could not be determined since mortality was demonstrated in all concentrations tested.

C. **Discussion/Results:** Deviations listed in Section 14A probably did not affect the results of the study.

The conclusions as stated by the author indicate that the NOEC was 0.18 mg a.i./L, "the lowest measured concentration tested." This is an error since the lowest mean concentration tested was 0.12 the lowest measure concentration tested.

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The author concluded that the mortality demonstrated in the two lowest concentrations levels was considered incidental and unrelated to the test substance. The reviewer does not support the author's assumption, since it can not be proven that the mortality was not toxicant related. Therefore, it should be considered that the NOEC could not be determined. However, this does not effect the validity of the test.

This study is scientifically sound and meets the guideline requirements for a acute flow-through toxicity study. The 96-hour LC_{50} of 0.40 mg a.i./L, based on mean measured concentrations, indicates that Propanil is highly toxic to mysid shrimp. The NOEC could not be determined. or (<.12) lowest dose ?

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A

(3) Repairability: N/A

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, April 19, 1991.

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RIN 1876-95

PROPANIL EEB REVIEW

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Pages 6 through 9 are not included.

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Louis M. Rifici Propanil-4 Mysidopsis bahia 5-30-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.65	20	18	90	2.012253E-02
.42	20	9	45	41.19014
.27	20	5	25	2.069473
.18	20	1	5	2.002716E-03
.12	20	2	10	2.012253E-02

THE BINOMIAL TEST SHOWS THAT .27 AND .65 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 .4385492

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1031078	.3940176	.3378962	.4757851

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1282568	1	.1002291

SLOPE = 3.68492
95 PERCENT CONFIDENCE LIMITS = 2.365241 AND 5.004599

LC50 = .3859346
95 PERCENT CONFIDENCE LIMITS = .3210047 AND .4865967

LC10 = .1745283
95 PERCENT CONFIDENCE LIMITS = .1147938 AND .2203193

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