

Reviewer: Regina Hirsch

9/13/93
MRID No. 428373-01
IV-a-MYS

DATA EVALUATION RECORD

1. **CHEMICAL:** Methoprene. Shaughnessey No. 105401.
2. **TEST MATERIAL:** (S)-Methoprene Technical; CAS No. 65733-16-6; Lot No. 23698; 91.829% active ingredient; a yellow brown liquid.
3. **STUDY TYPE:** 72-3. Estuarine Shrimp Acute Flow-Through Toxicity Test. Species Tested: Mysids (*Mysidopsis bahia*).
4. **CITATION:** Machado, M.W., W. Lima, J.M. Gibbons, S.P. Shepherd. 1992. (S)-Methoprene Technical - Acute Toxicity to Mysid Shrimp (*Mysidopsis bahia*) Under Flow-Through Conditions. SLI Report No. 92-3-4165. Performed by Springborn Laboratories, Inc., Wareham, MA. Submitted by Zoecon Corporation, A Sandoz Company, Dallas, TX. EPA MRID No. 428373-01.
5. **REVIEWED BY:**

Rosemary Graham Mora, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Rosemary Graham Mora</i> Date: 13 Sept 93
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6. **APPROVED BY:**

Pim Kosalwat, Ph.D. Senior Scientist KBN Engineering and Applied Sciences, Inc.	Signature: P. Kosalwat Date: 9/13/93
Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA	Signature: Date:
7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute flow-through toxicity study using estuarine shrimp. Based on mean measured concentrations, the 96-hour LC₅₀ was 106 µg ai/l, which classifies (S)-Methoprene Technical as highly toxic to mysids. The NOEC was 35 µg ai/l.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

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11. MATERIALS AND METHODS:

- A. Test Animals:** Mysids (*Mysidopsis bahia*) were obtained from laboratory cultures. The shrimp cultures were maintained in a 500-l tank under recirculating conditions with a photoperiod of 16 hours of light (intensity of 70-80 footcandles) and 8 hours of darkness. The culture water had a temperature of 25°C and a salinity of 25-26 parts per thousand (ppt). Mysids were fed live *Artemia salina* nauplii twice daily.
- B. Test System:** The test system consisted of a constant-flow serial diluter, a temperature-controlled water bath, and 19.5-l glass aquaria (39 X 20 X 25 cm) with self-starting siphons which maintained a solution volume of 7-11 l. Each replicate aquarium contained 2 retention chambers (10-cm Petri dishes with 15-cm high Nitex® screen collars) which housed five mysids each. The flow rate to each aquarium (100 ml/minute) provided 13 volume replacements every 24 hours.

The aquaria were impartially positioned in the water bath which was designed to maintain the test temperature at 25 ±1°C. The photoperiod during the test was the same as that used for culturing with a light intensity of 21-55 footcandles. Sudden transitions from light to dark and dark to light were avoided.

The dilution water was filtered (20 and 5 µm) natural seawater collected from the Cape Cod Canal, Bourne, MA. The seawater had a salinity of 25 ppt and a pH of 7.6-7.8. The salinity of the exposure solutions was maintained at 25 ±3 ppt by mixing natural seawater with soft laboratory well water.

A stock solution (2.7 mg ai/ml) was prepared by dissolving 1.448 g (1.3297 g as active ingredient) of test material with acetone to a final volume of 500 ml. The stock solution was delivered to a mixing chamber at a rate of 0.0376 ml/minute where it was diluted with seawater (0.40 l/minute) to provide the highest test concentration. Subsequent 50% dilutions provided the remaining 4 concentrations.

- C. Dosage:** Ninety-six-hour flow-through test. Based on the results of preliminary testing, five nominal concentrations (16, 31, 62, 120, and 250 µg ai/l) were selected for this study. In addition, a dilution water

control and a solvent control (94 μ l acetone/l) were included.

- D. **Design:** Ten mysids (≤ 24 hours old) were impartially loaded into each of two replicate aquaria (i.e., 20 mysids/treatment). During the test, the organism loading rate was 0.069 mg/l/day. Live brine shrimp nauplii were added twice daily during the study.

Biological observations and observations of physical characteristics of the test solutions were noted at test initiation and every 24 hours. Mortality was defined as lack of movement by exposed organisms. Dead mysids were removed at each observation interval.

The dissolved oxygen concentration (DO), pH, salinity, and temperature were measured daily in each replicate chamber. The temperature in one replicate of the dilution water control was monitored continuously using a Min/Max thermometer.

Chemical analysis of (S)-Methoprene Technical was performed using gas chromatography on each test solution collected on days 0 and 4 to verify the test concentrations.

- E. **Statistics:** The authors used a computer program by Stephan (1977, 1982) to calculate LC_{50} values. The probit analysis was used to calculate the 96-hour LC_{50} .

12. **REPORTED RESULTS:** Mean measured concentrations were 10, 17, 35, 84, and 150 μ g ai/l and averaged 61% of the nominal concentrations (Table 2, attached). The coefficients of variation averaged 6.3% for all mean measured concentrations.

Biological observations are presented in Table 3 (attached). The 96-hour LC_{50} for *Mysidopsis bahia* exposed to (S)-Methoprene Technical was 110 μ g ai/l (95% confidence interval of 88-120 μ g ai/l). The slope of the dose-response curve was 3.6. The NOEC was 35 μ g ai/l.

During the study, the test solutions had a temperature of 24-26°C, a pH of 7.5-7.8, a DO of 6.1-7.1 mg/l (85-99% of saturation), and a salinity of 25-26 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were presented by the authors.

A Good Laboratory Practice Compliance statement and a Quality Assurance statement were included in the report, indicating that the study was in accordance with GLP regulations (40 CFR, Part 160).

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

- A. Test Procedure: The test procedures were generally in accordance with the SEP, except for the following deviations:

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

During the study, the temperature of the test solution was 24-26°C. The SEP recommends a temperature at or around 22°C and that it should not deviate more than 1°C during the test.

- B. Statistical Analysis: The reviewer used EPA's Toxanal computer program to calculate the LC₅₀ value and obtained slightly more conservative results than the authors (printout, attached).
- C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for an acute flow-through toxicity study using estuarine shrimp. Based on mean measured concentrations, the 96-hour LC₅₀ was 106 µg ai/l, which classifies (S)-Methoprene Technical as highly toxic to *Mysidopsis bahia*. The NOEC was 35 µg ai/l.
- D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: N/A.
- (3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes; 30 August 1993.

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Pages 5 through 6 are not included in this copy.

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Rosemary Graham Mora (S)-Methoprene Mysidopsis bahia

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
150	20	17	85	.1288414
84	20	5	25	2.069473
35	20	0	0	9.536742E-05
17	20	0	0	9.536742E-05
10	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 84 AND 150 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 106.2087

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	8.643129E-02	103.4203	85.75252	131.5618

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
11	.2471967	1	.9996773

SLOPE = 6.852752
 95 PERCENT CONFIDENCE LIMITS = 3.44564 AND 10.25986

LC50 = 105.663
 95 PERCENT CONFIDENCE LIMITS = 88.31215 AND 124.8387

LC10 = 68.95978
 95 PERCENT CONFIDENCE LIMITS = 42.33978 AND 83.80238

Ecological Effects Branch One-Linear Data Entry Form

Chemical Methoprene Shaughnessy No. 105401 Pesticide Use

INVERTEBRATE ACUTE TOXICITY	% AI	EC ₅₀ (95%CL) SLOPE	HRS/TYPE	NOEC	STUDY/REVIEW DATES	MRID/CATEGORY	LAB	RC
1. Mysidopsis bahia	91.8	106 (88-125) µg ai/l 3.6	96 h flow thru	35 µg ai/l	1992/1993	42837301 Core	SLI	RGM
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	% AI	MATC LC ₅₀	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/CATEGORY	LAB	RC
1.								
2.								

COMMENTS: Based on mean measured concentrations. SLI=Springborn Laboratories, Inc.

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