2-EEB-28-C

Reviewer: Regina Hirsch 9/24/93

MRID No. 428373-02

JV-C-144S

DATA EVALUATION RECORD

1. **CHEMICAL:** Methoprene. Shaughnessey No. 105401.

2. **TEST MATERIAL:** (S)-Methoprene Technical; Lot No. 23698; CAS No. 65733-16-6; 91.829% active ingredient; a yellow-brown liquid.

3. **STUDY TYPE:** 72-4. Mysid Life-Cycle Toxicity Test. Species Tested: *Mysidopsis bahia*.

4. **CITATION:** Machado, M.W., W. Lima, J.M. Gibbons, S.P. Shepherd. 1992. (S)-Methoprene Technical - Chronic Toxicity to Mysid Shrimp (*Mysidopsis bahia*) Under Flow-Through Conditions. SLI Report No. 92-7-4328. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Zoecon Corporation, A Sandoz Company, Dallas, TX. EPA MRID No. 428373-02.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora

Date: 16 Sept 93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat

Date: 9/16/93

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

Signature:

Date:

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a mysid life-cycle toxicity test. The average number of young produced per female in the controls did not meet the minimum requirement. In addition, survival in the solvent control was 54%. Raw growth and reproduction data were not presented in the report. Based on the reviewer's analysis of survival data only, the MATC of (S)-Methoprene Technical to *Mysidopsis bahia* was >26 and <52 µg ai/l (geometric mean = 36.8 µg ai/l).

8. **RECOMMENDATIONS:** N/A.

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9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile *Mysidopsis bahia* (≤24 hours old) were obtained from laboratory cultures. The 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:** A constant-flow serial diluter delivered test solutions to glass test aquaria (39 x 20 x 25 cm). Each test vessel was equipped with a self-starting siphon which allowed the solution volume to fluctuate between 4 and 7 l. The flow rate provided 21 volume additions to each aquarium per day.

From days 0 to 12, two retention chambers (10-cm diameter glass petri dishes with 15-cm high nylon screen collars) were placed in each of two test vessels per treatment. From day 13 on, ten pairing chambers were placed in each replicate aquarium. The pairing chambers were cylindrical glass jars (5.1 cm diameter; 10 cm high) with two screen-covered holes.

The target test temperature was 25°C. A 16-hour light photoperiod (light intensity of 25-50 footcandles) was provided daily. 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 24-25 ppt and a pH of 7.8-8.0.

A stock solution (30 mg ai/ml) was prepared periodically by dissolving appropriate amounts of the test substance in acetone.

C. **Dosage:** Forty-nine-day flow-through test. Based on the results of an acute toxicity test, five nominal concentrations (9.4, 19, 38, 75, and 150 µg ai/l) were selected for this study. In addition, a dilution water control and a solvent control (5.0 µl acetone/l) were

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included. The acetone concentration in the solvent control was the same as that present in the highest test concentration.

D. **Design:** Thirty mysids were impartially selected and distributed to each of two replicate aquaria per treatment. Within each aquarium, the thirty mysids were evenly distributed into two retention chambers. The test aquaria were randomly arranged in a water bath. On day 13 of the exposure, the F_0 mysids were separated into pairs (1 male and 1 female). One pair was placed into each of ten chambers per treatment. Unpaired mysids were placed into an additional retention chamber in each replicate. The mysids were fed newly hatched *Artemia salina* nauplii three times daily. Starting on day 2 and every other day thereafter, the third feeding consisted of brine shrimp nauplii enriched with Selco (a complex fatty acid). The test system was cleaned daily.

Observations of mortality and sublethal responses were recorded every 24 hours. After pairing had begun, the offspring produced in each chamber were counted and removed daily. Dead adult females were removed when observed, while dead males were replaced. The total length (to the nearest 0.1 mm) and dry weight (to the nearest 0.01 mg) of individual adult mysids were determined at test termination.

Temperature, dissolved oxygen concentration (DO), salinity, and pH were measured daily in each aquarium. In addition, the temperature in one replicate of the dilution water control was recorded continuously during the study.

Samples were collected from each test vessel on days 0, 7, 16, 21, 28, 35, 42, and 49, and analyzed for (S)-Methoprene Technical using gas chromatography.

E. **Statistics:** Survival at 28 and 49 days, reproductive success, length, and dry weight data were statistically analyzed. Survival data were transformed (arcsine square-root percentage) prior to analysis. Bartlett's test was used to check assumptions of homogeneity of variance. William's test was used for all statistical analyses to determine treatment effects when compared to the pooled control data. Statistical conclusions were made at the 99% confidence level for Bartlett's test and at the 95% confidence level for William's test.

12. **REPORTED RESULTS:** No insoluble material was observed in any test vessel during the study. Mean measured concentrations were 6.4, 11, 26, 52, and 99 µg ai/l and averaged 66% of nominal concentrations (Table 2, attached).

Since reproduction of the control organisms did not meet the standard expectations, the study was extended from 28 days to 49 days in an effort to meet the established guideline criteria.

Survival in replicate A of the solvent control was significantly less than survival in the remaining control vessels. It was established that the low survival was due to "an unidentified condition (e.g., contamination) isolated to the individual exposure chamber." Therefore, the growth data from this replicate chamber were not included in statistical analyses. By test termination (day 49), mysid survival and reproductive success at 99 µg ai/l were significantly lower than the pooled control data (Table 3, attached). Since survival was significantly reduced in the highest test concentration, this concentration was not included in the statistical analysis of growth. Total length and dry weight in the remaining exposure solutions was not significantly lower than that of the pooled control (Tables 4 and 5, attached). After 49 days of exposure, the maximum acceptable toxicant concentration (MATC) was >52 and <99 µg ai/l. The geometric mean MATC was 72 µg ai/l.

During the study, the test solutions had a pH of 7.7-8.0, a temperature of 24-27°C, a salinity of 24-25 ppt, and a DO of 5.0-7.5 mg/l.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Springborn Laboratories, Inc. considers the calculations and estimation of toxicity, based on the data generated during the conducted exposures, to be accurate predictions of the response of *M. bahia* to chronic exposure to (S)-Methoprene Technical. However, due to the variation between the control organism performance (reproduction) as stated in the standard guidelines and the actual performance observed during this study, the exposure will be repeated to corroborate these reported results."

Good laboratory practice and quality assurance statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

A. **Test Procedure:** ASTM guidelines (1990) were used to evaluate this study. The test procedures generally followed the recommended protocols. The deviations are noted as follows:

Reproduction data were presented as the mean number of young per female reproductive day. Since no raw data were presented for this parameter, the reviewer estimated the mean number of young per female in the controls using the reported average reproduction days (i.e., 33 days). The value (approximately 1.9 young per female) obtained was less than that required by the guidelines (3 young per female).

According to the guidelines, a study is considered unacceptable if more than 30% of the first generation control mysids die between pairing and the end of the test. For this study, survival in both replicates of the solvent control was less than 70%. Raw survival data (i.e., daily records) were not presented in the report, therefore, the reviewer was unable to determine the percentage mortality that occurred between pairing and test termination.

The report states that individual length and dry weight measurements were recorded; however, only mean length and weight data were presented in the report. All raw data must be included in the report.

B. **Statistical Analysis:** Since raw growth and reproduction data were not presented in the report, statistical analysis for these parameters could not be verified. The reviewer used William's test (Toxstat 3.3) to compare 28- and 49-day survival of the dilution water control organisms with that of the treatment organisms. Due to the fact that survival in the solvent control was unacceptable and solvent was not present at the same concentration in all treatment levels, the treatment data were compared to those of the dilution water control. Survival at 52 and 99 μg ai/l was significantly reduced when compared to the dilution water control (printout, attached).

C. **Discussion/Results:** It is apparent that either the test organisms were not healthy or some unfavorable conditions existed during the test since both controls had low reproduction success and/or low survival rates. At the end of a typical exposure period (28 days) for a mysid life-cycle test, survival in one solvent replicate was only 58% and reproduction in all controls

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was reported as being unacceptable. The test was extended to 49 days and even then the number of young was still unacceptable.

This study is not scientifically sound and does not fulfill the guideline requirements for a mysid life-cycle toxicity test. The average number of young produced per female in the controls did not meet the minimum requirement. In addition, survival in the solvent control was 54%. Raw growth and reproduction data were not presented in the report. Based on the reviewer's analysis of survival data only, the MATC of (S)-Methoprene Technical to *Mysidopsis bahia* was >26 and <52 µg ai/l mean measured concentrations (geometric mean MATC = 36.8 µg ai/l).

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** Reproduction in both controls and survival in the solvent control did not meet the guideline requirements.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes; 31 August 1993.

REFERENCE:

ASTM. 1990. Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids. ASTM Designation: E 1191-90.

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Page ___ is not included in this copy.

Pages 7 through 10 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✔ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

(S)-Methoprene Technical: Survival of Exposed Mysids
File: 42837302.sur Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	0	6	0

Calculated Chi-Square goodness of fit test statistic = 12.7934
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

(S)-Methoprene Technical: Survival of Exposed Mysids
File: 42837302.sur Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 3.05
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

TITLE: (S)-Methoprene Technical: Survival of Exposed Mysids
FILE: 42837302.sur
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.9700	0.9700
1	Control	2	0.8000	0.8000
2	6.4 ug ai/l	1	0.7700	0.7700
2	6.4 ug ai/l	2	0.9000	0.9000
3	11 ug ai/l	1	0.8300	0.8300
3	11 ug ai/l	2	0.8700	0.8700
4	26 ug ai/l	1	0.7700	0.7700
4	26 ug ai/l	2	0.8300	0.8300
5	52 ug ai/l	1	0.6700	0.6700
5	52 ug ai/l	2	0.7300	0.7300
6	99 ug ai/l	1	0.5300	0.5300
6	99 ug ai/l	2	0.5000	0.5000

(S)-Methoprene Technical: Survival of Exposed Mysids
File: 42837302.sur Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	2	0.885	0.885	0.885
2	6.4 ug ai/l	2	0.835	0.835	0.843
3	11 ug ai/l	2	0.850	0.850	0.843
4	26 ug ai/l	2	0.800	0.800	0.800
5	52 ug ai/l	2	0.700	0.700	0.700
6	99 ug ai/l	2	0.515	0.515	0.515

(S)-Methoprene Technical: Survival of Exposed Mysids
File: 42837302.sur Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.885				
6.4 ug ai/l	0.843	0.625		1.94	k= 1, v= 6
11 ug ai/l	0.843	0.625		2.06	k= 2, v= 6
26 ug ai/l	0.800	1.249		2.10	k= 3, v= 6
52 ug ai/l	0.700	2.719	*	2.12	k= 4, v= 6
99 ug ai/l	0.515	5.438	*	2.13	k= 5, v= 6

s = 0.068
Note: df used for table values are approximate when v > 20.

(S)-Methoprene: Survival of Exposed Mysids at 49 days
File: 42837302.su Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	0	6	0

Calculated Chi-Square goodness of fit test statistic = 12.7934
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

(S)-Methoprene: Survival of Exposed Mysids at 49 days
File: 42837302.su Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 3.95
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

TITLE: (S)-Methoprene: Survival of Exposed Mysids at 49 days
FILE: 42837302.su
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.8000	0.8000
1	Control	2	0.7700	0.7700
2	6.4 ug ai/l	1	0.5000	0.5000
2	6.4 ug ai/l	2	0.6700	0.6700
3	11 ug ai/l	1	0.7700	0.7700
3	11 ug ai/l	2	0.8700	0.8700
4	26 ug ai/l	1	0.6700	0.6700
4	26 ug ai/l	2	0.7000	0.7000
5	52 ug ai/l	1	0.6300	0.6300
5	52 ug ai/l	2	0.5000	0.5000
6	99 ug ai/l	1	0.3300	0.3300
6	99 ug ai/l	2	0.3000	0.3000

(S)-Methoprene: Survival of Exposed Mysids at 49 days
File: 42837302.su Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	2	0.785	0.785	0.785
2	6.4 ug ai/l	2	0.585	0.585	0.703
3	11 ug ai/l	2	0.820	0.820	0.703
4	26 ug ai/l	2	0.685	0.685	0.685
5	52 ug ai/l	2	0.565	0.565	0.565
6	99 ug ai/l	2	0.315	0.315	0.315

(S)-Methoprene: Survival of Exposed Mysids at 49 days
File: 42837302.su Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.785				
6.4 ug ai/l	0.703	1.182		1.94	k= 1, v= 6
11 ug ai/l	0.703	1.182		2.06	k= 2, v= 6
26 ug ai/l	0.685	1.433		2.10	k= 3, v= 6
52 ug ai/l	0.565	3.153	*	2.12	k= 4, v= 6
99 ug ai/l	0.315	6.735	*	2.13	k= 5, v= 6

s = 0.070
Note: df used for table values are approximate when v > 20.

Ecological Effects Branch One-Liner 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.								
2.								
3.								
4.								
5.								
6.								
7.								

CHRONIC TOX.	% AI	MATC	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. *Mysidopsis bahia*	91.8	>26 <52 µg ai/l	49 d	survival	1992/1993	42837302 Invalid	SLI	RGM
2.								
3.								

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