2-EEB-25-E

Reviewer: Regina Hirsch 9/24/93

MRID No. 428373-03

DATA EVALUATION RECORD IV-e-MyS

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

2. **TEST MATERIAL:** (S)-Methoprene Technical; Lot No. 23698; CAS No. 65733-16-6; 91.829% active ingredient (ai) for initial test, 91.39% ai for final test; 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. 1993. (S)-Methoprene Technical - Chronic Toxicity to Mysid Shrimp (*Mysidopsis bahia*) Under Flow-Through Conditions. SLI Report No. 92-11-4518. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Zoecon Corporation, A Sandoz Company, Dallas, TX. EPA MRID No. 428373-03.

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:** These studies are not scientifically sound and do not meet the guideline requirements for a mysid life-cycle toxicity test. Survival in both controls of the initial test and in the solvent control of the final test was less than 70%. In addition, the average number of young produced per female in the controls of the final test was less than required. Raw growth and reproduction data were not presented in the report. Based on the authors' results, the MATC of (S)-Methoprene Technical to *Mysidopsis bahia* was >48 and <97 µg ai/l (geometric mean = 68 µg ai/l).

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

23 L 9 1

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 two 500-l tanks 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 24-26°C and a salinity of 23-32 parts per thousand (ppt). Mysids were fed live *Artemia salina* nauplii at least twice daily. One of the two daily feedings was enriched with Selco every other day.

B. **Test System:** Two definitive tests were performed during the course of this study. These tests are henceforth referred to as the initial and final tests.

In each test, 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.

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. When the mysids began to reach sexual maturity, 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 ±2°C. A 16-hour light photoperiod (light intensity of 24-60 footcandles for the initial test and 20-50 footcandles for the final test) 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 for the initial test had a salinity of 24-26 ppt and a pH of 7.7-7.9. For the final test, the seawater had a salinity of 24-25 ppt and a pH of 7.9-8.2.

2

A stock solution (10 mg ai/ml for the initial test and 30 mg ai/ml for the final test) was prepared periodically by dissolving appropriate amounts of the test substance in acetone.

C. **Dosage:** Twenty-eight-day and 34-day flow-through tests. Based on the results of an acute toxicity test, five nominal concentrations (3.1, 6.2, 12, 25, and 50 µg ai/l) were selected for the initial test. Based on the results of the same acute toxicity test and the results of the initial test, five nominal concentrations (9.4, 19, 38, 75, and 150 µg ai/l) were selected for the final test. In addition, a dilution water control and a solvent control (5.0 µl acetone/l) were included in each test. 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 to two retention chambers. The test aquaria were randomly arranged in a water bath. When the mysids began to reach sexual maturity, they were separated into pairs (1 male and 1 female). One pair was placed into each of ten pairing chambers per treatment. Unpaired mysids were placed into an additional retention chamber in each replicate. The mysids were fed brine shrimp nauplii. One of the daily feedings (every other day during the final test) was enriched with Selco (a complex mixture of fatty acids). 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 F_0 mysids were removed when observed, while dead adult males were replaced. The total length (to the nearest 0.1 mm) of individual adult mysids was determined at the end of the initial test. At the end of the final test, total length (to the nearest 0.1 mm) and dry weight (to the nearest 0.01 mg) of individual adult mysids were determined.

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, 8, 14, 21, and 28 for the initial test and on days 0, 7, 14, 21, and 28. The samples were analyzed for (S)-Methoprene Technical using gas chromatography.

E. **Statistics:** Survival (at 28 for the initial test and at 28 and 34 days for the final test), reproductive success, length, and dry weight data were statistically analyzed. Survival data were transformed (arcsine square-root percentage) prior to analysis. Student's t-test was used to compare the dilution water control to the solvent control. If no significant difference was demonstrated, then the data were pooled for comparison to the treatments; otherwise, the treatment data were compared to the solvent control. 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:**

Initial Test

No insoluble material was observed in any test vessel during the study. Mean measured concentrations were 2.2, 2.9, 5.4, 12, and 24 µg ai/l and averaged 52% of nominal concentrations (Table 2, attached).

Mean survival in the dilution water control and solvent control was 67 and 52%, respectively (Table 3, attached). The mean number of young per female per reproductive day was 0.36 and 0.14 for the control and the solvent control, respectively (Table 3, attached). Survival, reproduction and growth (i.e., length) were not significantly reduced in any test concentration when compared to the pooled control data (Table 4, attached). "Since the initial test failed to satisfy all of the acceptance criteria, the study was repeated, but at a higher concentration range in an attempt to define a Lowest-Observed-Effect Concentration (LOEC)."

During this test, the test solutions had a pH of 7.6-8.0, a temperature of 23-26°C, a salinity of 24-26 ppt, and a DO of 5.3-7.3 mg/l.

Final Test

No insoluble material was observed in any test vessel during the study. Mean measured concentrations were 5, 11, 24, 48,

and 97 µg ai/l and averaged 61% of nominal concentrations (Table 6, attached).

Since reproduction of the control organisms did not meet the standard expectations, the study was extended to 34 days in an effort to meet the established guideline criteria.

Mean survival in the dilution water control and solvent control was 84 and 72% on day 28, and 80 and 55% on day 34, respectively (Table 7, attached). The number of young per female per reproductive day in the control and solvent control was 0.0 and 0.04 by day 28, and 0.0 and 0.03 by day 34, respectively (Table 7, attached). Survival on day 28 and 34 was significantly affected at 97 µg ai/l. Growth (i.e., length and weight) at 97 µg ai/l was not statistically analyzed, since survival at this concentration was significantly affected. Growth was not significantly affected at the remaining test concentrations (Tables 8 and 9, attached). Reproduction during the extended test (i.e., 34 day duration) did not meet standard expectations, therefore, the data were not analyzed.

During this test, the test solutions had a pH of 7.9-8.2, a temperature of 23-27°C, a salinity of 24-25 ppt, and a DO of 5.7-7.5 mg/l.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
"The results of the two life-cycle exposures were used to determine the chronic effect of (S)-Methoprene Technical on the survival, growth and reproduction of mysid shrimp. The reduction in survival at the 97 µg ai/l treatment level during the final test was the only chronic effect of (S)-Methoprene Technical observed in either test...Because reproduction during the final test did not meet standard expectations, this study did not provide data sufficient to evaluate the effect of Methoprene on mysid reproduction."

"As a conservative estimate, therefore, the No-Observed - Effect Concentration (NOEC) for (S)-Methoprene Technical and *Mysidopsis bahia* was 24 µg ai/l. In the absence of reproduction data at concentrations above the NOEC, the Lowest-Observed-Effect Concentration (LOEC) cannot be determined; therefore, a conservative estimate of the Maximum Acceptable Toxic Concentration (MATC) would be 24 µg ai/l."

"..the MATC was estimated to be 24 µg ai/l which is equivalent to the highest exposure level tested which did not adversely affect organism performance in a study in which reproduction data for controls were acceptable (which

5

was not the case in the final study). If it were assumed that exposure to 48 µg ai/l Methoprene did not adversely affect the reproductive performance of mysid shrimp, the MATC established for the chronic study would be >48 µg ai/l and <97 µg ai/l (Geometric Mean MATC = 68 µg ai/l)."

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:

Raw growth and reproduction data were not presented in the report.

In the both tests, 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., 14 days for the initial test and 16 days for the final test). The value obtained for reproduction in the solvent control in the initial test and in both controls for the final test was less than that required by the guidelines (3 young per female).

By termination of the initial test, survival in both replicates of the solvent control (63 and 40%) and one replicate of the dilution water control (57%) was less than 70%. In the final test, survival in one replicate of the solvent control at day 28 (67%) and both replicates in the solvent control at test termination (67 and 43%) was less than 70%. According to the guidelines, a study is unacceptable if more than 30% of the first generation control mysids die between pairing and the end of the test.

Contamination was observed in both controls of the initial test and in the dilution water control of the final test. However, it could have been background noise since the detected amounts were close to detection limits.

6

B. **Statistical Analysis:** Since raw growth and reproduction data were not presented in the report, statistical analysis could not be verified. Due to the fact that solvent was not present at the same concentration in all test levels, the treatment data were compared to those of the dilution water control. The reviewer compared survival of the dilution water control organisms with that of the treatment organisms using William's test and Kruskal-Wallis test (Toxstat 3.3) for the initial and final tests, respectively. Survival in both tests was not significantly reduced when compared to the dilution water control (printouts, attached). The authors' results for survival were more conservative than the reviewer's.

C. **Discussion/Results:** It is apparent that either the test organisms were not healthy or some unfavorable conditions existed during the test since both tests had low control survival and low reproductive success rates.

These studies are not scientifically sound and do not meet the guideline requirements for a mysid life-cycle toxicity test. Survival in both controls of the initial test and in the solvent control of the final test was less than 70%. In addition, the average number of young produced per female in the controls of the final test was less than required. Raw growth and reproduction data were not presented in the report. Based on the authors' results, the MATC of (S)-Methoprene Technical to *Mysidopsis bahia* was >48 and <97 µg ai/l (geometric mean = 68 µg ai/l).

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** These test did not meet the guideline requirements for survival or reproduction.

(3) **Repairability:** No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes; 3 September 1993.

REFERENCE:

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

Page ___ is not included in this copy.

Pages 8 through 14 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: Survival of Exposed Mysids - Initial
File: 42837303.int 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 - Initial
File: 42837303.int Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 2.23
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 - Initial
FILE: 42837303.int
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.5700	0.5700
1	Control	2	0.7700	0.7700
2	2.2 ug ai/l	1	0.7700	0.7700
2	2.2 ug ai/l	2	0.6300	0.6300
3	2.9 ug ai/l	1	0.8000	0.8000
3	2.9 ug ai/l	2	0.7000	0.7000
4	5.4 ug ai/l	1	0.7700	0.7700
4	5.4 ug ai/l	2	0.6000	0.6000
5	12 ug ai/l	1	0.8300	0.8300
5	12 ug ai/l	2	0.7300	0.7300
6	24 ug ai/l	1	0.4000	0.4000
6	24 ug ai/l	2	0.8000	0.8000

(S)-Methoprene: Survival of Exposed Mysids - Initial
File: 42837303.int Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	2	0.670	0.670	0.717
2	2.2 ug ai/l	2	0.700	0.700	0.717
3	2.9 ug ai/l	2	0.750	0.750	0.717
4	5.4 ug ai/l	2	0.685	0.685	0.717
5	12 ug ai/l	2	0.780	0.780	0.717
6	24 ug ai/l	2	0.600	0.600	0.600

(S)-Methoprene: Survival of Exposed Mysids - Initial
File: 42837303.int 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	0.717				
2.2 ug ai/l	0.717	0.314		1.94	k= 1, v= 6
2.9 ug ai/l	0.717	0.314		2.06	k= 2, v= 6
5.4 ug ai/l	0.717	0.314		2.10	k= 3, v= 6
12 ug ai/l	0.717	0.314		2.12	k= 4, v= 6
24 ug ai/l	0.600	0.468		2.13	k= 5, v= 6

s = 0.150
Note: df used for table values are approximate when v > 20.

(S)-Methoprene: Survival of Exposed Mysids - Final
File: 42837303.fin Transform: ARC SINE(SQUARE ROOT(Y))

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	5	2	5	0

Calculated Chi-Square goodness of fit test statistic = 6.0902
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

(S)-Methoprene: Survival of Exposed Mysids - Final
File: 42837303.fin Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

TITLE: (S)-Methoprene: Survival of Exposed Mysids - Final
FILE: 42837303.fin
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.7300	1.0244
1	Control	2	0.8700	1.2019
2	5.0 ug ai/l	1	0.8300	1.1458
2	5.0 ug ai/l	2	0.6300	0.9169
3	11 ug ai/l	1	0.8000	1.1071
3	11 ug ai/l	2	0.6700	0.9589
4	24 ug ai/l	1	0.8000	1.1071
4	24 ug ai/l	2	0.8000	1.1071
5	48 ug ai/l	1	0.7700	1.0706
5	48 ug ai/l	2	0.7300	1.0244
6	97 ug ai/l	1	0.4000	0.6847
6	97 ug ai/l	2	0.4300	0.7152

(S)-Methoprene: Survival of Exposed Mysids - Final
File: 42837303.fin Transform: ARC SINE(SQUARE ROOT(Y))

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	1.113	0.800	17.500
2	5.0 ug ai/l	1.031	0.730	14.000
3	11 ug ai/l	1.033	0.735	13.000
4	24 ug ai/l	1.107	0.800	18.000
5	48 ug ai/l	1.048	0.750	12.500
6	97 ug ai/l	0.700	0.415	3.000

Calculated H Value = 5.735 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

(S)-Methoprene: Survival of Exposed Mysids - Final
File: 42837303.fin Transform: ARC SINE(SQUARE ROOT(Y))

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 6	2	3	5	4	1
6	97 ug ai/l	0.700	0.415	\					
2	5.0 ug ai/l	1.031	0.730	.	\				
3	11 ug ai/l	1.033	0.735	.	.	\			
5	48 ug ai/l	1.048	0.750	.	.	.	\		
4	24 ug ai/l	1.107	0.800	.	.	.	.	\	
1	Control	1.113	0.800	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.574

78

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 LC_{50}**	**DAYS**	**AFFECTED PARA.**	**STUDY/REVIEW DATES**	**MRID/ CATEGORY**	**LAB**	**RC**
1. *Mysidopsis bahia*	91.39	>48 <97 µg ai/l	34	survival	1993/1993	42837303 Invalid	SLI	RGM
2.								
3.								

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

GL