10-3-96

DATA EVALUATION REPORT

(S)-METHOPRENE TECHNICAL

STUDY TYPE: LIFE-CYCLE - MYSID SHRIMP (72-4)

Prepared for

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464

(S)-METHOPRENE TECHNICAL

Mysid Life-Cycle Study (72-4)

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DATA EVALUATION REPORT

MRID# & TITLE OF STUDY: MRID 44022101, (S)-Methoprene Technical - Chronic Toxicity to Mysids (*Mysidopsis bahia*) Under Flow-Through Conditions

DP BARCODE: D226999 **CASE**: 003099
REG./FILE#: 002724-00375 **CHEMICAL/BIOL#**: 105401 Methoprene

COMPANY/SPONSOR: Sandoz Agro, Inc., 1300 E. Touhy Avenue, Des Plaines, Illinois 60018

TEST MATERIAL: (S)-Methoprene Technical

REVIEW CONCLUSION: This study was conducted according to acceptable procedures and determined the following values for (S)-methoprene technical to mysid shrimp: LOEC of 25 μg a.i./L, NOEC of 14 μg a.i./L, and MATC >14 and <25 μg a.i./L (geometric mean MATC = 19 μg a.i./L). This study was adequately conducted and provided useful data.

RECOMMENDATIONS: None

ADEQUACY OF STUDY: Core

MATERIALS & METHODS: The study procedures followed those of the Springborn Laboratories, Inc. (Wareham, MA) protocol entitled "(S)-Methoprene - Life-Cycle Toxicity Test with Mysids (*Mysidopsis bahia*), Following FIFRA Guideline 72-4" (Springborn Laboratories Protocol #:081295/FIFRA/530/s-methoprene [1995] and Protocol Amendment #1 [1995]). The study was conducted in accordance with GLP 40 CFR 160 with the exception of routine water screening and food analyses for pesticides, PCB's, and toxic metals. The water screening and food analyses were conducted using standard U.S. EPA procedures by Lancaster Laboratories (Lancaster, PA). No protocol deviations were noted and the study was acceptably conducted. The test material, (S)-Methoprene technical (Lot No. 5S1008, CAS# 40596-69-8), was received from Sandoz Agro, Inc. (Dallas, TX) and was stored frozen. The test material was an amber liquid with a purity of 95.311%, molecular weight of 310.5 g/mol, water solubility of 0.52 ppm, and vapor pressure of <1 mm Hg. An analytical standard of (S)-Methoprene (Lot No. 95-24) was received from the same source and was an amber liquid with a purity of 95.21 $\pm$ 0.01%. The analytical standard was also stored frozen.

The mysids ($\leq$24 hours old) used in these tests were obtained from laboratory cultures maintained at Springborn Laboratories (SLI Lot #95A107) and were kept in recirculated, filtered artificial seawater for 14 days prior to the test. Juvenile mysids ($\leq$24 hours old) were collected and fed brine shrimp (*Artemia salina*) nauplii, *ad libitum*, twice daily, with one feeding supplemented with Selco®, a liquid food supplement. Food sources were analyzed routinely and found to be acceptably free of pesticides, PCB's, and metals considered toxic to mysids.

Artificial seawater used as dilution water during these tests was prepared by the addition of a commercially prepared salt formula (hw-MARINEMIX®) to filtered soft freshwater having a hardness of 20 to 40 mg/L as $CaCO_3$, with a final salinity of 25 ± 3‰. The prepared dilution was aerated vigorously for approximately 24 hours, then allowed to aerate for an additional 24 hours prior to use. Routine analyses found no toxic concentrations of pesticides, PCBs, or toxic metals in the dilution water source. Mysids maintained in artificial seawater prepared from the same source as the artificial seawater used in this study have successfully survived and reproduced over several generations.

Nominal concentrations selected for the test material were 9.4, 19, 37, 75, and 150 µg a.i./L. A 30 mg a.i./mL stock solution was prepared by dissolving 1.584 g of test material with acetone to volume in a 50 mL volumetric flask. Additionally, a 0.50 mL/mL solvent stock solution was prepared by diluting 50 mL of acetone with distilled water to volume in a 100 mL volumetric flask.

The life-cycle test was conducted using an exposure system consisting of a constant-flow serial diluter, a temperature-controlled water bath, and a set of 14 exposure aquaria (two per test concentration level). Each aquarium contained two mysid retention chambers made of glass Petri dishes covered with screen which were used to maintain non-paired mysids during the study. Pairing chambers, used to house sexually mature male and female organisms, were cylindrical glass jars having two screen-covered holes. The aquaria systems allowed for adequate solution exchange via siphon drains. The 150 µg a.i./L nominal treatment was attained by delivering 0.0015 mL/min of the test material stock solution to a mixing chamber which also received 0.302 L/min of dilution water. The stock solution was proportionally diluted (50% dilution factor) to provide the remaining nominal test concentrations. A similar system was used to deliver the acetone stock solution to the diluter system of the solvent test chambers, providing an acetone concentration equivalent to the acetone concentration in the highest test solution. The solution exchange system operated at a rate of approximately 15 aquarium volume additions per day to provide a 90% test solution replacement rate of approximately 3.5 hours. The entire operating system was illuminated with fluorescent lighting for 16 hours daily followed by 8 hours darkness.

"Mysids, ≤24 hours old, were collected form the Springborn culture unit and divided among 28 beakers. The beakers contained culture water and were held in a waterbath maintained at 25 ± 2°C. The organisms were impartially selected and distributed to the beakers by adding five organisms at a time to each beaker until each beaker contained 15 mysids. Each group of 15 mysids was then transferred to one of the 28 labeled retention chambers (two per aquarium). The test was initiated when the retention chambers were placed in their respective test aquaria. Each test aquarium contained two retention chambers, yielding 30 mysids per replicate vessel and 60 organisms for each treatment level and control."

Upon reaching sexual maturity (Day 15), mature male/female pairs within each exposure aquarium were transferred from the retention chambers to the 10 glass pairing jars (one pair per jar). The remaining mysids were all placed in one of the initial retention chambers within each aquarium and maintained for the duration of the chronic test. Male mysids from this pool were used to replace dead males removed from the paired groups. Females that died in pairing jars were not replaced. If development of brood pouches, distinguishing females from males, was delayed due to toxicant exposure, all test organisms were maintained in the retention chambers until maturity was observed or until test termination. Mysids were fed live brine shrimp (*Artemia salina*) nauplii twice daily. Before pairing, at least one of the daily feedings was enriched with Selco®. After pairing, the mysids were fed Selco®-enriched brine shrimp nauplii once every other day.

During the first 14 days, observations were made for mortality and any abnormal appearance or behavior. After pairing (Day 15), mortality of the paired mysids, the number of offspring produced by each female, and any abnormal appearance or behavior was recorded. Observations were made daily throughout the study. Dead mysids were removed and discarded.

At test termination, all mysids were sacrificed and measured for individual body length (nearest 0.1 mm) and total dry body weight (nearest 0.01 mg). Reproductive success was calculated for each replicate aquarium as the ratio of the total number of offspring produced to the total number of females contained within each chamber per reproductive day. The number of female reproductive days was determined as the number of days that an individual was alive, counting the day that offspring were first observed in any control (i.e., Day 18 represents reproductive day 1).

Daily measurements were made for water temperature, dissolved oxygen concentration, pH, and salinity in each replicate of each treatment. Samples were removed from each replicate test solution and control on days 0, 7, 14, 21, and 28 and analyzed for test material concentration.

Data from the paired and unpaired mysids were statistically analyzed for treatment effects. Endpoints analyzed for first generation (F_0) mysids included survival, growth (i.e., body weights and lengths), and reproduction. Reproductive success was determined only for the paired organisms. Bartlett's Test was used to test for homogeneity of variance (99% certainty level). Student's t-test was conducted for each endpoint to compare solvent and negative controls, resulting in no significant difference. Therefore, solvent and negative control endpoints were pooled for the remaining comparisons between controls and treatments. The Williams' Test was used to determine treatment level effects (95% certainty level). The Maximum-Acceptable-Toxicant-Concentration (MATC), or the theoretical threshold concentration of the test material expected to produce no deleterious effects to mysids, was estimated at the 95% certainty level. Also determined were the Lowest-Observed-Effect Concentration (LOEC) and the No-Observed-Effect Concentration (NOEC).

REPORTED RESULTS: Water quality parameters measured during the 28-day exposure remained within acceptable limits. Analyses of test material concentrations in the aquaria exhibited consistency between replicates and sampling intervals and the expected concentration gradient across treatment levels was maintained throughout the 28-day test. However, mean measured concentrations ranged from 66 to 77% of the nominal concentrations and defined the concentrations tested as 7.2, 14, 25, 50, and 98 μg a.i./L. Coefficients of variation averaged 15% for all mean measured concentrations.

Survivals of the F_0 mysids were 90 and 92% for the control and solvent control, respectively, with no statistical difference between the two (pooled control survival = 91%). Survivals of 78, 78, 82, 83, and 57% were observed for mysids exposed to mean measured test material levels of 7.2, 14, 25, 50, and 98 μg a.i./L, respectively. Only the 98 μg a.i./L concentration was determined to be statistically different from the pooled control results. For this reason, results for that treatment were eliminated from further chronic statistical analyses.

No statistical difference was observed between control and solvent control mysids for reproductive success (0.6 and 0.39 offspring/female/reproductive day, respectively) and these groups were pooled (mean = 0.50 offspring/female/reproductive day). Mysid reproduction in the treatment levels that did not adversely affect survival, i.e., 7.2, 14, 25, and 50 μg a.i./L, ranged from 0.22 to 0.45 offspring/female/reproductive day and were determined not to be significantly different from the pooled control organisms with respect to reproductive success.

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The mean body lengths of male and female control mysids were 7.0 and 6.9 mm, respectively, while the solvent control mysids measured 7.2 and 7.0 mm for males and females, respectively. The control and solvent control body length measurements were not statistically different, and the pooled lengths for control males and females were 7.1 and 7.0, respectively. For exposure concentrations to the test material of 7.2, 14, 25, and 50 μg a.i./L, the respective body lengths for male mysids were 7.1, 7.2, 7.2 and 7.1 mm, while the respective body lengths for females were 7.2, 7.1, 7.2, and 6.9 mm. Both male and female body lengths were not statistically different from the pooled control body lengths. These data indicate that the test material "at levels $\leq$ 5.0 μg a.i./L" did not adversely affect organism growth based on body length. Obviously, this should read "at levels $\leq$ 50 μg a.i./L".

The mean body weights for the control and solvent control male mysids were 0.88 and 0.82 mg, respectively, while those for females were 1.0 and 0.90 mg, respectively. There were no statistical differences between control and solvent control groups for either males or females, allowing for pooled averages of 0.85 and 0.95 mg for males and females, respectively. For exposure concentrations to the test material of 7.2, 14, 25, and 50 μg a.i./L, the respective dry body weights for male mysids were 0.78, 0.82, 0.75, and 0.78 mg, while respective dry body weights for females were 0.93, 0.93, 0.93, and 0.81 mg. Statistically significant reduced dry body weights occurred in exposure concentrations to the test material of 25 and 50 μg a.i./L for males and 50 μg a.i./L for females.

"Based on the results of this study, the LOEC and NOEC of (S)-Methoprene technical for mysid survival, reproductive success and growth (total body length and dry weight) was determined. Dry body weight of male mysids was determined to be the most sensitive indicator of toxicity of (S)-Methoprene technical to mysids. The LOEC and NOEC, based on male dry body weight, was 25 and 14 μg a.i./L, respectively. The Maximum-Acceptable-Toxicant Concentration (MATC) was calculated to be $>$14 and $<$25 μg a.i./L (Geometric Mean, MATC = 19 μg a.i./L). These data provided a MATC which corroborated the conservatively estimated MATC (i.e., 24 μg a.i./L) determined during previously conducted life-cycle tests (SLI Report #92-11-4518)."

DISCUSSION: This study was conducted following acceptable procedures outlined in FIFRA Guideline 72-4, Subdivision E of the U.S. EPA Pesticide Assessment Guidelines (1982). This study determined the following values for (S)-methoprene technical to mysid shrimp: LOEC of 25 μg a.i./L, NOEC of 14 μg a.i./L, and MATC $>$14 and $<$25 μg a.i./L (geometric mean MATC = 19 μg a.i./L). These values are based on the dry body weight for male mysids, which was determined to be the most sensitive performance criterion measured in these tests. The mortality data (presented as "Percent Survival") were reported to be significant only at the 98 μg a.i./L level, and sublethal data at this level were not used in statistical calculations.

Although mortality was measured, no LC_{50} was calculated since 50% mortality was never achieved, nor did the data seem to follow a dose-response curve, i.e., percent survival was lower at the two lower treatment concentrations (78% for both) than at the next two higher treatment concentrations (82 and 83%) but lowest at the highest concentration of 98 μg a.i./L (57%). The survival data shown in Table 1 are the actual percentages measured in each aquarium, with the mean given for the two aquaria per concentration. The reduction in survival does not follow a dose-response fashion, except that the greatest mortality occurs in the highest treatment concentration level. Although the Williams' Test showed no significant difference in survival between each of the treatments and the pooled controls (at $\leq$50 μg a.i./L), the use of only two data points (per treatment) does not give a standard deviation and is of questionable statistical validity.

The authors report no significant effect of the test material on reproductive success in mysids. However, the reproductive data shown in Table 1 is presented in a similar fashion to the survival data. When the number of reproducing females and the number of reproductive days are divided out, all of the reproductive data within an aquarium is reduced to a single number. Again, the use of only two values is of questionable statistical validity. The reviewer repeated the statistical analyses of the author regarding survival and reproduction and concurs with the author's conclusion.

TABLE 1. Summary of the first generation (F_0) survival and reproductive success (offspring/female/reproductive day) during the 28-day life-cycle exposure of mysids (*Mysidopsis bahia*) to (S)-Methoprene Technical

Mean Measured Concentration μg a.i./L	Replicate	Percent Survival[a]	Reproductive Success[a]
Control	A	90	0.41
	B	90	0.79
	Mean	90	0.60
Solvent Control	A	90	0.33
	B	93	0.44
	Mean	92	0.39
Pooled Control[b]	Mean	91	0.50
7.2	A	73	0.42
	B	83	0.29
	Mean	78	0.36
14	A	73	0.44
	B	83	0.46
	Mean	78	0.45
25	A	87	0.49
	B	77	0.25
	Mean	82	0.37
50	A	83	0.18
	B	83	0.25
	Mean	83	0.22
98	A	60	0.083
	B	53	0.0094
	Mean	57[c]	0.046[d]

Data taken from Table 3, p. 34, MRID 44022101.

[a] Values presented have been rounded to two significant figures.

[b] Since control and solvent control data were not determined to be significantly different, all treatment data were compared to the pooled control data.

[c] Significantly different ($p \leq 0.05$) from the pooled control (Williams' Test).

[d] Since organism survival was adversely affected, this treatment level was excluded from statistical analysis to determine treatment effects for body length, body weight, and reproductive success.