UNDATED

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** S-Methoprene technical; Lot #23698; 91.4% active ingredient; clear liquid.

3. **STUDY TYPE:** Fish Early Life-Stage Toxicity Test. Guideline 72-4; Species Tested: fathead minnow (*Pimephales promelas*).

4. **CITATION:** Cohle, P., P. Ritchie-Blase, J. B. Bussard and K. Neufeld. 1993. Early Life-Stage Toxicity of (s)-Methoprene to Fathead Minnows (*Pimephales promelas*) in a Flow-Through System. Report No. 40321. Prepared by ABC laboratories, Inc., Columbia, MO. Submitted by Zoecon Corp. EPA MRID No. 42811201.

5. **REVIEWED BY:**

Joanne S. Edwards, M.S. **Signature:**
Entomologist
Ecological Effects Branch **Date:**
Environmental Fate and
Effects Division (7507C)

6. **APPROVED BY:**

Leslie W. Touart, Ph.D. **Signature:**
Supervisory Biologist
Ecological Effects Branch **Date:**
Environmental Fate and
Effects Division (7507C)

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for an early life-stage toxicity test using fathead minnows. Based on the authors' analysis of the growth data, the maximum acceptable toxicant concentration (MATC) limits are 48 µg/l (NOEC) and 84 µg/l (LOEC) based on mean measured concentrations. The geometric mean MATC is 63 µg/l.

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

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

A. **Test Animals:** Newly spawned fathead minnow (*Pimephales promelas*) eggs (≤24 hours post-spawn) were obtained from an ABC lab in-house culture. Three males and three females were involved in the spawning.

B. **Test System:** A 2-L proportional diluter system, designed to deliver five nominal concentrations of test material, a dilution water control, and a solvent control, was employed in this study. There were two aquaria per treatment level; two replicate chambers per aquarium. The inside dimensions of each replicate chamber measured approx. (30.5 X 15.7 cm) with average water depth of 24.6 cm, yielding approx. solution volume of 11.8. Embryo incubation cups were constructed from 9 cm diameter glass jars with 40-mesh Nytex screen replacing the bottom. The embryo cups were suspended in the test vessels and oscillated vertically (3 to 6 cm) by means of a rocker arm apparatus. All aquaria were immersed in a thermostatically controlled waterbath with temperature maintained at 25 ± 2°C. Embryos and newly hatched fry were shielded from excess light exposure until 3 days post-hatch, after which a photoperiod was 16 hours light/8 hours darkness, along with 30-minute simulated dawn/dusk transitions, was maintained. Light intensity at the surface ranged from 442 to 54 lux.

Stock solutions were prepared for each concentration by adding the appropriate amount of test material to dimethylformamide (DMF). Batches of diluter stock solution were prepared at 8 to 13 day intervals. Control water dilution and stock solution were delivered to each chamber at an average rate of 92 L/day/replicate (approx. 7.8 test chamber volumes per day). The biomass loading levels ranged from 0.029 to 0.059 g/L/day at study termination. Dilution water was obtained from a deep well. A portion of the water was passed through a reverse osmosis system and blended back with well water to a total hardness of approx. 130 to 160 mg/L (as $CaCO_3$) and pH of approx. 8.0. A characterization of the water is given in Table 1.

C. **Dosage:** Thirty-seven-day, embryo-larval, flow-through toxicity test. Five nominal test concentrations (13, 25, 50, 100, and 200 µg/l) were selected for this study. A dilution water control and a solvent control (0.012 mL/L DMF) were employed.

2

D. **Design:** Eggs were impartially placed in cups and observed daily for viability. There were twenty-five embryos per incubation cup, 2 test chambers/treatment, for a total of 100 embryos per test level. Dead embryos were counted and removed daily. Hatching began on day three and was completed on day 9 (Table VIII). Hatching was $\geq$90% in all groups, including controls, by day 6. Therefore, day 7 was designated as 0 day post-hatch. When all eggs had hatched on study day 9, the fry were released into the test chambers. Beginning on study day 3, for the first week of feeding and throughout the study, the fry were fed brine shrimp (Artemia sp) nauplii. On study day 8, Salmon Starter mash was added to the diet and on study day 22, #1 granule size Salmon Starter was fed in addition to mash and brine shrimp. Beginning on study day 28, only brine shrimp and Salmon Starter #1 were fed. Fish were not fed during the 24 hours preceding test termination.

Observations of larval mortality and behavioral and developmental abnormalities were recorded daily. At test termination, the individual lengths and weights of all surviving fish were recorded. Fry survival and total survival (eggs and fry) were analyzed on day 37.

Dissolved oxygen concentration (DO), pH, temperature, conductivity, hardness, and alkalinity were measured on study days 0, 1, 7, and every 7 $\pm$2 days thereafter.

(S)- methoprene concentrations were analyzed by GLC for samples taken on days 0, 1, 7, and every 7 $\pm$2 days thereafter. Replicates A and B of each treatment were sampled on days 0, 7, 21, and 37, and replicates C and D were sampled on days 1, 15, and 28.

E. **Statistics:** The percent hatch and survival data were analyzed using frequency analysis comparing each test concentration to the appropriate control. Control and vehicle blank were pooled where frequency analysis, coupled with the chi-square statistic and Fisher's exact test indicated no significant difference between them. For the length and weight data, a one-way analysis of variance procedure (Dunnett's) was used. Control and vehicle blank were compared using a two-tailed Student's t test. If no significant differences were noted between the controls, the data were pooled. All statistical analyses were performed using SAS/STAT™ Version 6.04.

12. **REPORTED RESULTS:** The mean measured concentrations were 13, 23, 48, 84, and 160 µg/l and ranged from 80 to 100% of nominal values (Table IV, attached).

The percent hatchability in the individual replicates averaged 88-100%, and was not significantly affected ($P \leq 0.05$) by any concentration of the test material (Table IX, attached). Larval survival for individual replicates averaged from 86.8 to 94.3%. No statistically significant reductions ($P \leq 0.05$) were indicated at any test level when compared to pooled control for fry survival or total survival (hatchability and fry survival).

The mean standard length in the pooled control and the 13, 23, 48, 84, and 160 µg/l mean measured concentrations was 23.5, 23.1, 22.9, 22.3, and 20.4 mm, respectively (Table X, attached). A significant length reduction ($p \leq 0.05$) occurred at the two highest dose levels when compared to the pooled control. The mean blotted weight in the pooled control and the 13, 23, 48, 84, and 160 µg/l mean measured concentration levels was 0.235, 0.223, 0.219, 0.216, 0.196, and 0.143 g, respectively. A significant weight reduction ($p \leq 0.05$) occurred at the two highest dose levels when compared to the pooled control.

Based on the growth data, the maximum acceptable toxicant concentration (MATC) limits were 48 µg/l (NOEC) and 84 µg/l (LOEC) mean measured concentrations. The geometric mean MATC was calculated to be 63 µg/l.

During the study, the pH ranged 8.0-8.2 and the temperature ranged 23.9-25.0°C. The levels of dissolved oxygen concentrations ranged from 6.3 to 7.9 mg/l. The hardness, alkalinity, and conductivity of the test solutions are given in Table VI (attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

No conclusions, other than those mentioned above, were reported.

A GLP compliance statement was included in the report indicating that the study was conducted in accordance with USEPA GLP Regulations with one exception; mass spectrometer log book entries regarding instrument maintenance were not noted as being either routine or non-routine.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP or ASTM (E 1241-88) guidelines, except for the following:

The EEB considers pooling of control data to be scientifically unsound. By pooling, scientific differentiation cannot be made between effects of the test substance vs. the effects of the solvent on fish early life stage toxicity. Since the difference between the two control groups in this study was very slight (mean length was 23.6 mm control vs. 23.5 mm solvent; mean weight was 0.239 g control vs. 0.231 solvent), pooling probably had little effect on the analysis.

B. **Statistics:** Verification of the study authors' statistics was not performed.

C. **Discussion/Results:**

The raw data for the fish length and weight were not provided in the report. Since these data were unavailable, the study authors' estimate of the MATC could not be verified. However, by visual observation of the data it is unlikely that an independent analysis of the growth data would change the reported MATC. Therefore, the MATC reported by the study authors is accepted.

Based on the authors' analysis of the growth data, the maximum acceptable toxicant concentration (MATC) limits are 48 µg/l (NOEC) and 84 µg/l (LOEC) based on mean measured concentrations. The geometric mean MATC is 63 µg/l.

D. **Adequacy of the Study:**

(1) **Classification:** Core

(2) **Rationale:** N/A

(3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER:** August 29, 1994.