(9-15-92)

MRID No. 418695-11

DATA EVALUATION RECORD

1. **CHEMICAL:** Cimectacarb. Shaughnessey No. 112602.

2. **TEST MATERIAL:** CGA-163935 Technical; Batch No. FL 891393; 92.2% active ingredient; a dark amber liquid.

3. **STUDY TYPE:** Freshwater Fish Early Life-Stage Test. Species Tested: Fathead Minnow (*Pimephales promelas*).

4. **CITATION:** Sousa, J.V. 1991. (CGA-163935 Technical) - Acute Toxicity to Fathead Minnow *(Pimephales promelas)* Embryos and Larvae. SLI Report No. 91-3-3693. Study conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA MRID No. 418695-11.

5. **REVIEWED BY:**

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Date: 7/29/92

6. **APPROVED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound ~~and meets~~ but does not the guideline requirements for a fish early life-stage test. According to the reviewer's analysis, the MATC of CGA-163935 Technical for *Pimephales promelas* was ≥0.89 and <1.4 mg a.i./l mean measured concentrations (geometric mean MATC = 1.1 mg a.i./l). No NOEL was established.

8. **RECOMMENDATIONS:** N/A. BJM Montague 9/28

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Fertilized eggs (*Pimephales promelas*) were obtained from the fathead minnow culture unit at the testing facility.

B. **Test System:** The test system was an intermittent-flow proportional diluter with a 50% dilution factor. The diluter delivered 500 ml of test solution per minute to each aquarium at an average rate of approximately 6.5 volume replacements per day. The test chambers were glass test aquaria (39 x 20 x 25 cm), each fitted with a 19.5-cm standpipe to maintain a constant solution volume of 15 l. Embryo incubation cups were glass jars (5 cm O.D., 8 cm high) with 40-mesh Nitex® screen bottoms.

The dilution water was obtained from an aerated epoxy-coated concrete reservoir that was filled from an on-site well and supplemented with Town of Wareham untreated well water. The water, characterized weekly, had total hardness and alkalinity ranges of 32-37 and 20-26 mg/l as $CaCO_3$, respectively. The pH ranged from 7.0 to 7.2 and the specific conductivity ranged from 120 to 140 µmhos/cm.

Test aquaria were impartially positioned in a circulating-water bath designed to maintain a temperature of 25 ±1°C. Sixteen hours of light at an intensity of 20-100 footcandles (216-1080 lux) were provided each day. Sudden transitions from light to dark and dark to light were avoided.

Stock solutions (456 mg a.i./ml) were prepared weekly by combining 49.51 g (45.65 g as a.i.) of test material with dimethylformamide (DMF) to a final volume of 100 ml.

C. **Dosage:** Thirty-five-day embryo-larval, flow-through test. Based on the results of preliminary testing, five nominal test concentrations (0.75, 1.5, 3.0, 6.0, and 12.0 mg a.i./l) were selected for this study. A dilution water control and a solvent control were also included.

D. **Design:** Sixty embryos (<24 hours old) were impartially selected and distributed to each of 14 incubation cups. One cup was suspended in each test aquarium. A rocker arm apparatus gently oscillated the cups in the test solutions. Dead embryos were counted daily until hatching was complete. Hatching was considered

complete (test day 5) when no more than 5 unhatched viable embryos remained in any incubation cup.

On test day 5, up to 40 live larvae were impartially selected from each incubation cup and placed into their respective aquaria. Larvae were fed live brine shrimp (*Artemia salina*) nauplii 2-3 times daily. The aquaria were brushed and siphoned when necessary (generally several times a week) to remove excess food and fecal matter.

Behavior and appearance of larvae were recorded daily. Larval survival was estimated twice weekly. At test termination (test day 35), the larvae were counted and individually weighed and measured.

Dissolved oxygen (DO) concentration, pH, and temperature were measured daily in each aquarium. Temperature was also measured continuously in one replicate of the control and solvent control. Total hardness, alkalinity, and specific conductance were measured on day 0 and weekly thereafter in alternating replicates of the highest and lowest exposure groups and the dilution water control.

During the study, samples from replicate test solutions and the controls were collected on test days 0, 4, 7, 15, 21, 28, and 35 for determination of CGA-163935 Technical concentrations using high performance liquid chromatography.

E. **Statistics:** The percentage survival data were transformed (arcsine square-root percentage) before analysis.

All statistical analyses were performed using the mean organism response in each replicate aquarium. For this study, all data were normally distributed with homogeneous variance (Bartlett's test); therefore, Williams' test was used to assess exposure-level effects. Treatment levels that caused significant survival effects were excluded from the analysis of growth data. All statistical conclusions were made at 95% confidence level, except for Bartlett's test which was done at 99% level.

12. **REPORTED RESULTS:** Throughout the exposure period, undissolved test material was observed in the diluter system (i.e., mixing chamber, chemical cells, splitter cells), but

not in the test aquarium. Mean measured concentrations were 0.89, 1.4, 2.9, 6.2, and 12.0 mg a.i./l (Table 2, attached).

Hatching success in the highest test concentration (12 mg a.i./l) was significantly reduced when compared to that of the pooled control (Table 3, attached). Larval survival at the two highest test concentrations (6.2 and 12 mg a.i./l) was significantly reduced when compared to that of the pooled control. "Due to the adverse effect on larval survival at the two highest treatment levels (12 and 6.2 mg a.i./l), data (growth) for these test concentrations were excluded from further statistical analysis." Mean wet weight and total length were significantly reduced at 0.89, 1.4, and 2.9 mg a.i./l when compared to those of the pooled control.

Based on the most sensitive indicator (growth effects), the MATC of CGA-163935 Technical for fathead minnows was determined to be less than 0.89 mg a.i./l, the lowest concentration tested. An estimate of the No Observed Effect Concentration (NOEC) was obtained by extrapolation from the established concentration-effect relationship (regression analysis). The Maximum Acceptable Toxicant concentration (MATC) of CGA-163935 to fathead minnow was estimated to be >0.14 mg a.i./l and <0.89 mg a.i./l (geometric mean MATC = 0.35 mg a.i./l).

During the study, the pH range was 6.7-7.2; mean total hardness and alkalinity were 34-44 and 24-26 mg/l as $CaCO_3$, respectively; specific conductance was 150-200 µmhos/cm; and the temperature range was 23-26°C. The DO concentrations ranged from 5.3 to 8.7 mg/l (64 to 105% of saturation at 25°C).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
"Springborn Laboratories will perform a subsequent definitive exposure in an effort to establish the significance of the effects (growth) observed in the lowest treatment level and to further define the toxicity of CGA-163935 Technical to fathead minnow embryos and larvae."

GLP compliance and quality assurance statements were included in the report indicating that the data and report prepared for this study were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice Regulations (40 CFR, Part 160) except in the case of stability, characterization, and verification of test substance identity.

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

A. **Test Procedure:** The test procedure is generally in accordance with the SEP and ASTM guidelines, except for the following deviations:

The diluter system should operate for at least two days prior to test initiation as recommended; the pretest operation period was not reported.

The concentration of solvent in the solvent control was not reported; the SEP recommends a solvent concentration of ≤0.1 ml/l. According to the reviewer's calculation, the maximum solvent concentration was 0.020 ml/l.

The total hardness of dilution water (32-37 mg/l as $CaCO_3$ during the exposure period) was slightly lower than the recommended hardness of 40-48 mg/l as $CaCO_3$.

The light intensity range during this study was 216-1080 lux (reported as 20-100 footcandles); the SEP recommends a light intensity range of 400-800 lux.

There was no information on how fertilized eggs were obtained from the culture.

* Time to swim-up was not reported.

The report did not indicate whether food was withheld from the fish for at least 24 hours prior to test termination. The SEP recommends terminating feeding at least 24 hours prior to test termination.

The test organisms were impartially distributed to the test chambers; random assignment to the test vessels is required.

Two replicate incubation cups with 60 embryos in each cup were used per treatment level and control. The SEP recommends a minimum of 20 embryos per replicate cup, with four replicates per concentration (80 embryos total).

B. **Statistical Analysis:** The reviewer used the computer program Toxstat Version 3.1 to analyze the transformed (arcsine square-root) percentage hatched and survival data (printouts, attached). Hatchability data met the assumptions of homogeneity of variance (Bartletts or Hartley test) and normality (Chi-square or Shapiro Wilks test), therefore, the data were analyzed using an analysis of variance (ANOVA) coupled with Dunnett's

comparison test. Hatchability was not significantly reduced at any test level when compared to the solvent control. This conclusion does not agree with that of the author; the author pooled the control and solvent control data for this analysis.

Survival data did not meet the assumptions of homogeneity of variance (Bartletts or Hartley test), therefore, the data were analyzed using Kruskal-Wallis test. No significant reduction in larval survival was detected by this test, however, upon visual examination of these data, it is obvious that survival at the two highest test concentrations (75% and 20%) were substantially reduced when compared to the solvent control (95%). This conclusion agrees with that of the author.

Statistical analysis of length and weight data was performed using a two-way ANOVA coupled with a multiple comparison test. The significance level was $p \leq 0.05$. The reviewer's analysis showed a significant reduction in weight at the four highest test levels and a significant reduction in length at the three highest test levels. These results differ from those presented by the author and may be explained by the following:

The author excluded from statistical analysis exposure levels for growth data that showed significant effects on survival. Growth data from these levels should not have been included in the analysis since they are part of the experiment and could have contributed to the experimental error in the ANOVA. Furthermore, excluding these growth data from statistical analysis would make it appear as if only survival was affected at these levels.

The length and weight data were reported as being individually measured: however, the data from these two parameters were statistically analyzed using the mean values of each replicate. When mean values were used, the variation that existed within each replicate was ignored. An experimental design which consists of only two replicates such as this one, using only replicate mean values in statistical analysis may lead to the wrong conclusion. Individual measurements (i.e., raw data) of these two growth parameters should have been used.

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a fish early

life-stage test. According to the reviewer's analysis, the MATC for fathead minnows exposed to CGA-163935 Technical was ≥0.89 and <1.4 mg a.i./l mean measured concentrations (geometric mean MATC = 1.1 mg a.i./l).

D. **Adequacy of the Study:**

(1) **Classification:** ~~Core~~ Supplemental

(2) **Rationale:** ~~N/A~~ No NOEL for effects to growth (length & weight) was established.

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

15. **COMPLETION OF ONE-LINER:** Yes, July 23, 1992.

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