(6-24-93)

MRID No. 425953-03

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** CGA-163935 Technical; CAS No. 95266-40-3; Batch Code P 705002, ID No. FL-900318, ARS-20702; 96.6% active ingredient; a dark amber solid.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Lemna gibba*.

4. **CITATION:** Hoberg, J.R. 1992. CGA-163935 Technical - Toxicity to the Duckweed *Lemna gibba* G3. SLI Report No. 92-11-4513. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Agricultural Division, CIBA-GEIGY Corporation, Greensboro, NC. EPA MRID No. 425953-03.

5. **REVIEWED BY:**

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Signature: Louis M Rifici
Date: 3/4/93

6. **APPROVED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Based on mean measured concentrations of CGA-163935 Technical, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* were 0.018, 0.035, and 0.19 mg a.i./l, respectively.

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

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

A. Test Species: *Lemna gibba* G3 used in the test came from laboratory stock cultures originally obtained from the University of California, Los Angeles. Stock cultures were maintained in Hoagland's medium (with pH adjusted to 5.0) under continuous 3228-4842 lux illumination and a temperature of 24-25°C. Lighting was provided by Duro-Test Vita-Lite® fluorescent tubes. Transfers were made into fresh medium once weekly. The plants used in the test were taken from a seven-day old stock culture.

B. Test System: Sterile, covered 270-ml crystallizing dishes were conditioned by rinsing with the appropriate solution. One-hundred ml of the appropriate test solution were placed into each dish.

The test was performed in a growth chamber with conditions similar to those used in culturing. The temperature in the environmental chamber was maintained at 24-26°C. Light was provided continuously at an intensity of 3200-4300 lux.

C. Dosage: Fourteen-day growth and reproduction test. Based on the results of a previous test, eight nominal concentrations of 0.020, 0.040, 0.080, 0.16, 0.32, 0.64, 1.3, and 2.5 mg active ingredient (a.i.)/l were selected for the definitive test.

A 100 mg ai/l primary stock solution was prepared by dissolving 0.052 g of test material in 500 ml of Hoagland's medium. This stock was sonicated and stirred for 70 minutes. A small amount of undissolved material remained after stirring and removed by settling and decanting the supernatant. Appropriate volumes of the decanted stock solution were diluted to the final volume of 500 ml in Hoagland's medium to prepare the test solutions. New test solutions were prepared on test days 3, 6, 9, and 12. A medium control was also prepared.

D. Test Design: The test consisted of three replicate dishes per treatment level and control. *Lemna gibba* (five plants with three fronds each) was aseptically introduced into each dish within 30 minutes of solution addition. At three-day intervals (days 3, 6, 9, 12) and on day 14, fronds were counted and observations were made. At initiation and after each renewal, the

dishes were positioned in the assigned random location in the growth chamber.

The pH was recorded at test initiation, at renewal in the new and old solutions, and at termination. Temperature was recorded continuously with a minimum/maximum thermometer in a flask of water in the environmental chamber. The light intensity was recorded daily.

Samples were removed from the fresh test solutions remaining after renewal on day 3 and the old test solutions at the end of the renewal period on day 6. The concentration of CGA-163935 in each test solution and the control was determined using high performance liquid chromatography. A set of three quality control solutions were prepared at the sampling periods to monitor the precision and quality control during analysis.

E. **Statistics:** The EC_{10}, EC_{50}, and EC_{90} values and their 95% confidence intervals (C.I.) after 14 days of testing were determined by linear regression of response (percent reduction of frond number as compared with the control) vs. mean measured concentration over the range of test concentrations. Various mathematical manipulations (e.g., logarithm and probit transformations) were used on the concentration and response data to obtain the linear regression with the highest coefficient of determination (R^2). The 95% C.I.'s were determined using the method of inverse prediction.

The no-observed-effect concentration (NOEC) was determined to be the highest concentration that caused no significant reduction of frond number in comparison to the control data. Dunnett's test was used to determine significant effects after first checking the data for normality using the Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test.

12. **REPORTED RESULTS:** Measured concentrations on day 3 and day 6 averaged 103 and 73% of nominal concentrations, respectively. The mean measured concentrations were 0.018, 0.035, 0.068, 0.14, 0.28, 0.55, 1.2, and 2.3 mg a.i./l (Table 3, attached). Recoveries of the quality control samples averaged 100 and 101% of nominal, respectively.

Frond counts for the control and the exposure concentrations after 14 days are given in Table 4 (attached). Fronds

exposed at the 0.28, 0.55, 1.2, and 2.3 mg a.i./l test levels were observed to have less root formation and smaller, curled and chlorotic fronds in comparison to control fronds. Plants exposed at the 0.068 and 0.14 mg a.i./l levels were noted to be slightly chlorotic while fronds exposed at the 0.14 mg a.i./l level were also observed to be smaller than control fronds. Fronds at the remaining treatment levels appeared normal.

Analysis using Dunnett's test demonstrated a significant reduction in frond production at treatment levels ≥0.035 mg a.i./l. The 14-day EC_{50} and NOEC were determined to be 0.19 mg a.i./l (95% C.I.= 0.11-0.32 mg a.i./l) and 0.018 mg a.i./l, respectively.

During the test, the pH of the test and control solutions ranged from 4.8 to 6.0. Temperature was 24-26°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author offered no conclusions.

The study director confirmed that this study was conducted in compliance with EPA Good Laboratory Practice (GLP) regulations (40 CFR Part 160) with the exception that maintenance of a sample of the test substance and records pertaining to the stability, characterization, and verification are the responsibility of the sponsor. Additionally, routine water analyses were conducted at an independent laboratory that did not collect data in accordance with GLP procedures. A Quality Assurance statement was included in the report.

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

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, with the exception that the light intensity (3.2-4.3 klux) was lower than recommended (5 klux).

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the 14-day EC_{50} and 95% confidence interval (C.I.) as 0.19 mg a.i./l and 0.16-0.22 mg a.i./l using the probit method (see attached printout 1). The slope of the probit line was 1.4.
The reviewer used analysis of variance coupled with Dunnett's test (α=0.05) to determine the lowest-observed-effect concentration (LOEC) and NOEC in comparison to the control data. The results were the same as the author's (see attached printout 2).

C. **Discussion/Results:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Based on mean measured concentrations of CGA-163935 Technical, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* were 0.018, 0.035, and 0.19 mg a.i./l, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER:** Yes, 2-22-93.