MRID No. 416337-09

DATA EVALUATION RECORD

1. **CHEMICAL:** Triclopyr triethylamine.
Shaughnessey Number: 116002.

2. **TEST MATERIAL:** Triclopyr triethylamine salt; ((3,5,6-trichloro-2-pyridinyl)oxy)acetic acid triethylamine salt; CAS No. 57213-69-1; Sample ID No. AGR 236831, MM-86042137; 45.01% triclopyr triethylamine salt (32.3% acid equivalent); a light brown liquid.

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

4. **CITATION:** Cowgill, U.M. and D.P. Milazzo. 1987. Triclopyr Triethylamine Salt: The Fourteen-day Toxicity to Lemna gibba L. G-3 (Duckweed). Laboratory Project Study ID No. ES-DR-0287-8071-1. Prepared by Dow Chemical Company, Midland, Michigan. Submitted by Dow Chemical U.S.A., Midland, Michigan. EPA MRID No. 416337-09.

5. **REVIEWED BY:**

Richard C. Petrie
Agronomist
EEB/EFED

Signature: Richard C. Petrie
Date: 3/12/91

6. **APPROVED BY:**

Charles Lewis, Acting Head,
Section 3,
EEB/EFED

Signature: Charles Lewis
Date: 3/14/91

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a Tier II growth and reproduction of a non-target area aquatic plant study. Based on number of plants, the 14-day EC50 value was determined to be 19.5 mg/L as formulated product (8.8 mg/L as active ingredient). The NOEC value could not be determined due to adverse effects at all treatment levels.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** Lemna gibba L. G-3 used in this test were obtained from the Smithsonian Institution located in Washington, D.C. The culture vessels were 250-mL glass Erlenmeyer flasks containing 100 mL of nutrient medium. Stock cultures were acclimated in an incubator for 8 weeks at a temperature of 27 ± 2°C. A continuous photoperiod at a light intensity of 5382 ± 1076 lux was provided. Stock cultures were maintained axenically by weekly transfer of 15 fronds (5 plants) into fresh sterile medium.

The culture medium was that of Hoagland which was further developed by Hillman (1961), revised by Cleland and Briggs (1967), and further revised by Cowgill to contain necessary quantities of selenium (4.2 µg/L), vanadium (25.6 µg/L), tin (684 µg/L) and cobalt (20.3 µg/L). The culture medium was adjusted to pH 4.6 prior to autoclaving.

B. **Test System:** The phytotoxicity test was conducted in an incubator at a temperature of 25.5-25.9°C. The test vessels were sterile 250-mL glass Erlenmeyer flasks. A continuous photoperiod at an intensity of 5077 ± 298 lux was provided.

The test medium was the same as that for culturing with the exception that ethylenediaminetetraacetic acid, yeast extract, sugar and Bactotryptone were omitted.

C. **Dosage:** Fourteen-day growth and reproduction test. The nominal test concentrations of triclopyr triethylamine salt based on whole material were 13, 21.6, 36, 60, and 100 mg/L. The nominal test concentrations of triclopyr triethylamine salt based on active ingredient were 5.85, 9.72, 16.20, 27.01, and 45.01 mg/L.

D. **Design:** Based on a range-finding test, five nominal triclopyr triethylamine salt concentrations (see Section 11.C) were selected for the definitive test.

Each concentration and the control were replicated three times. The phytotoxicity test was initiated when 15 fronds (5 plants containing 3 fronds each) were added to each flask. Growth (number of plants and fronds) and pH were recorded at test termination.

Additional flasks for the control, lowest, and highest concentration of toxicant with and without addition of 15 fronds of Lemna gibba were examined every third day for pH and growth (number of plants and fronds). Temperature of the incubator and light were measured and recorded at least daily.

E. **Statistics:** The 14-day EC50 values for the number of plants and fronds based on formulated product were calculated using standard regression analysis techniques.

The regression line calculated for the number of plants obtained in relation to concentration of toxicant is as follows:

Number of plants = 712.364 - 273.488 (Log Conc.)

The regression line calculated for the number of fronds obtained in relation to concentration of toxicant is as follows:

Number of fronds = 1700.35 - 677.057 (Log Conc.)

A comparison of the control and each concentration of triclopyr triethylamine salt was employed using a one-sided Dunnett's t-test at the 95 percent confidence level.

12. **REPORTED RESULTS:** The growth and pH results during the progress of the test are shown in Table 3 (attached). The growth and pH results during day 14 of the definitive test are shown in Table 4 (attached). The 14-day EC25 values and 95 percent confidence intervals, based on formulated product, for number of plants and number of fronds were determined to be 4.4 mg/L (1.0-19.7 mg/L) and 5.0 (1.6-16.1 mg/L), respectively. A comparison of control data with data from each concentration showed that there is a statistically significant difference between controls and all concentrations.

The 14-day EC50 values and 95 percent confidence intervals, based on formulated product, for number of plants and number of fronds were determined to be 19.9 mg/L (4.6-85.2 mg/L)

and 20.1 (6.4-63.0 mg/L) as formulated product, respectively. Therefore, triclopyr triethylamine salt is classified as slightly toxic to Lemna gibba when used as formulated product. The 14-day EC50 values and 95 percent confidence intervals, based on active ingredient, for number of plants and number of fronds were determined to be 9.0 mg/L (2.1-38.4 mg/L) and 9.1 mg/L (2.9-28.4 mg/L), respectively.

During this test, the pH without growth ranged from 4.51 to 4.80 and the pH with growth ranged from 5.81 to 6.65. The temperature of the incubator ranged from 25.5 to 25.9°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 14-day EC50 values of triclopyr triethylamine salt to Lemna gibba G-3 for the number of plants and the number of fronds were determined to be 19.9 mg/L and 20.1 mg/L as formulated product, respectively (9.0 mg/L and 9.1 mg/L as active ingredient, respectively).

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with the FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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, except for the following deviations:

o The SEP states that growth of Lemna should be measured at least every three days. During this toxicity test, the growth was measured and recorded every three days in only the control, lowest, and highest concentration in one additional flask set aside from the actual definitive test to monitor the progress of the test.

o Composition of the growth medium was not provided in the report.

o NOEC could not be determined due to adverse effects at all treatment levels.

o The authors calculated EC50 values by fitting a regression line on raw data (number of plants or number of fronds and log concentration). Percent inhibition when compared to the control, should have been used in the regression analysis.

o The maximum application rate of the test substance was not provided in the report.

B. **Statistical Analysis:** The reviewer used the EPA's Toxanal computer program to calculate the 14-day EC50 values using percent inhibition of the number of plants and number of fronds as growth endpoints. These calculations are attached. Percent inhibition (I) of growth compared to control was calculated for number of plants and number of fronds according to the following formula:

$$\% I = \frac{C - X}{C} \times 100$$

where: C = mean growth in the control,
X = mean growth in test concentration.

The 14-day EC50 value using number of plants as the growth endpoint was determined to be 19.5 mg/L with a 95 percent confidence interval of 12.3-25.8 mg/L based on formulated product (8.8 mg/L with a 95 percent confidence interval of 5.5-11.6 mg/L as active ingredient). The slope of the concentration-response curve was determined by probit analysis to be 1.0.

The 14-day EC50 value using number of fronds as the growth endpoint was determined to be 23.4 mg/L with a 95 percent confidence interval of 18.5-28.2 mg/L based on formulated product (10.5 mg/L with a 95 percent confidence interval of 8.3-12.7 mg/L as active ingredient). The slope of the concentration-response curve was determined by probit analysis to be 1.5.

Analysis of variance with multiple comparison tests was performed to compare number of plants and number of fronds at each treatment level to those of the control (attached). The results showed that all the treatment levels tested significantly reduced (P=0.01) the number of plants and the number of fronds at test termination (day 14). Therefore, the NOEC could not be determined.

C. **Discussion/Results:** The 14-day EC50 value of triclopyr triethylamine salt for Lemna gibba was determined to be 19.5 mg/L as formulated product (8.8 mg/L as active ingredient) based on the most conservative growth endpoint (number of plants). The 14-day NOEC could not

be determined due to adverse effects at all treatment levels.

D. **Adequacy of the Study:**

(1) Classification: Supplemental.

(2) Rationale: 1) Composition of growth medium was not provided; 2) NOEC could not be determined due to adverse effects at all test levels.

(3) Repairability: No.

15. **COMPLETION OF ONE-LINER:** Yes, 01-09-91.

16. **AUTHOR'S REFERENCES:**

Cleland, C.F. and W.R. Briggs. 1967. Flowering responses of the long day plant Lemna gibba G3. Plant Physiology 42:1533-1561.

Hillman, W.S. 1961. Experimental control of flowering in Lemna III. American Journal Botany. 48:413-419.