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, AGR 236831; 45% triethylamine salt (32.3% acid equivalent); an amber liquid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants - Tier II. Species Tested: Anabaena flos-aquae.

4. **CITATION:** Hughes, J.S. 1987. Triclopyr Triethylamine Salt: The Toxicity to Anabaena flos-aquae. Laboratory Project ID No. 0460-02-1100-1. Prepared by Malcolm Pirnie, Inc., White Plains, New York. Submitted by Dow Chemical U.S.A., Midland, Michigan. EPA MRID No. 416337-06.

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 and fulfills the guideline requirements for a Tier II growth and reproduction of a non-target area phytotoxicity test. Based on cell counts, the 7-day EC50 value was determined to be 5.9 mg a.i./L nominal concentration. The 4- and 7-day NOEC values were determined to be 8 mg a.i./L and 2 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:** Anabaena flos-aquae used in this test were obtained from laboratory stock cultures maintained at the testing facility. The original culture was obtained from the University of Texas Culture Collection (UTEX 1444), Austin, Texas. Stock cultures were maintained in synthetic algal assay procedure (AAP) nutrient medium in Erlenmeyer flasks under constant illumination of approximately 2153 lux (200 footcandles) and temperature of 24 ± 2°C. Flasks were manually shaken each working day. Transfers were made regularly into fresh medium to provide 6- to 8-day old cultures for assay inoculations.

B. **Test System:** The phytotoxicity test was conducted in an incubator at a temperature of 24 ± 2°C. The test vessels were sterile 500-mL Erlenmeyer flasks fitted with foam stoppers to permit gas exchange. The flasks were manually shaken each working day and continuous illumination at an intensity of 2153 ± 323 lux (200 ± 30 footcandles) was provided by overhead cool-white fluorescent lights. The flasks were randomly repositioned each working day to minimize spatial differences in the incubator.

The synthetic AAP nutrient medium was prepared by placing 900-mL distilled deionized water in a 1000-mL volumetric flask. Macronutrient and micronutrient stock solutions were added to the medium. The volume was brought up to 1 L and the pH adjusted to 7.5 ± 0.1 with 0.1N sodium hydroxide or hydrochloric acid. The medium was subsequently filtered through a 0.22-micron porosity membrane filter into a sterile container. The medium was stored in the dark at 4°C, and brought to room temperature prior to use.

C. **Dosage:** Seven-day growth and reproduction test. The nominal test concentrations of triclopyr triethylamine salt based on active ingredient were 0.5, 1, 2, 4, 8, and 16 mg/L.

D. **Design:** Based on range-finding tests, six nominal triclopyr triethylamine salt concentrations (see Section 11.C) were selected for the definitive test. Each concentration and the control were replicated three times. Test concentrations were prepared by adding the required volumes of the appropriate stock solution to AAP medium in 500-mL volumetric flasks. After thoroughly mixing, 100 mL of each concentration were added to each test vessel. The control contained AAP medium only.

The phytotoxicity test was initiated when 0.490 mL of sonicated 7-day-old stock culture (containing 612,000 cells/mL) was aseptically added to 100 mL of medium in each flask, yielding a nominal initial concentration of 3,000 cells/mL.

Cell counts were made using a Coulter Counter on test days 2, 3, 4, and 7. Three counts per replicate were made. All counts were multiplied by the appropriate conversion factors (for sample dilution and volume counted) to yield cells/mL. The temperature of the incubator was recorded daily. The pH in each test concentration was measured and recorded at the beginning of the test.

E. **Statistics:** Mean cell count values at test termination for each nominal test concentration were expressed as a percent relative to that in the control at test termination. Percent inhibition (I) of growth compared to the control was calculated for cell counts according to the following formula:

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

where: C = mean growth in the control,
T = mean growth in treated culture.

To determine the EC25 and EC50 values and associated 95% confidence limits, the log of test concentration (x-axis) was plotted against percent inhibition expressed as probit (y-axis). Inverse estimation least squares linear regression was used to determine the line of best fit, the concentration corresponding to 50 percent inhibition and the associated 95% confidence limits. The no-observed-effect concentration (NOEC) was determined from an analysis of variance (ANOVA) and two separate multiple range tests (Duncan's new multiple range test and the Student-Newman-Keuls test).

The linear regression, analysis of variance and the multiple range tests were performed using SAS procedures.

12. **REPORTED RESULTS:** Mean cell counts during the assay are given in Table 2 (attached). The three highest test concentrations of triclopyr triethylamine salt had inhibitory effects upon the population growth of Anabaena flos-aquae on day 7. The degree of inhibition caused by the two highest concentrations of test material increased with the duration of exposure.

Effects of the test material on mean standing crop on day 7, relative to the control, ranged from 3.9% to 82.9% inhibition (Table 4, attached). As determined by inverse estimation least squares linear regression, the 7-day EC25 value was determined to be 2.81 mg/L with a 95% confidence interval of 1.11-7.12 mg/L. The 7-day EC50 value was determined to be 5.97 mg/L with a 95% confidence interval of 2.44-16.61 mg/L. The results of the analysis of variance and both multiple range tests indicate that the mean standing crop values on day 7 in the three highest test concentrations (4, 8, and 16 mg/L) were significantly less than that in the control. Thus, the NOEC is 2 mg/L.

During this test, the initial pH of the six test concentrations ranged from 6.7 to 6.9. The temperature of the incubator ranged from 23.8 to 24.8.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author.

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 pH was measured in each test concentration at the beginning of the test. The pH should have been measured at test initiation and termination.

o The SEP states that the pH of the medium should be approximately 7.5. During this test, the initial pH of the six test concentrations ranged from 6.7 to 6.9.

o Observations were made only on days 2, 3, 4, and 7. Therefore, it could not be determined whether the data provided for day 7 were the maximum standing crop of the controls. Daily observations should have been taken during the test period.

B. **Statistical Analysis:** The reviewer used the EPA's Toxanal computer program to calculate the 7-day EC50 value (attached). Analysis of variance (ANOVA) was performed, based on reduction of cell counts, to determine the 4-day and 7-day NOEC values (attached).

The 4-day EC50 value using cell count as the growth endpoint was could not be determined since the highest concentration only resulted in 36% inhibition at day 4. The 7-day EC50 value using cell count as the growth endpoint was determined to be 5.9 mg a.i./L with a 95% confidence interval of 4.3-8.5 mg a.i./L. The slope of the concentration-response curve was determined by probit analysis to be 2.1.

Analysis of variance was performed to compare cell counts at each treatment level to those of the control for day 4 and day 7 (attached). Based on the reduction of cell counts, the 4-day and 7-day NOEC values were determined to be 8 mg a.i./L and 2 mg a.i./L, respectively.

C. **Discussion/Results:** The 7-day EC50 value of triclopyr triethylamine salt for Anabaena flos-aquae was determined to be 5.9 mg a.i./L nominal concentration. The 4-and 7-day NOEC values were determined to be 8 mg a.i./L and 2 mg a.i./L nominal concentrations, respectively.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 01-10-91.