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 No. 236831; 45.01% triclopyr triethylamine salt (32.3% acid equivalent).

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

4. **CITATION:** Dill, D.C. and D.P. Milazzo. 1987. Triclopyr Triethylamine Salt: Evaluation of the Toxicity to a Freshwater Green Alga, Selenastrum capricornutum Printz. Laboratory Project Study ID No. ES-DR-0287-8071-2. Prepared by Dow Chemical Company, Midland, Michigan. Submitted by Dow Chemical U.S.A., Midland, Michigan. EPA MRID No. 416337-05.

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 green alga test. Based on cell counts, the 5-day EC50 value was determined to be 39.1 mg/L as whole material (17.6 mg/L as active ingredient). The NOEC value was determined to be 25 mg/L as whole material (11.3 mg/L as active ingredient) based on reduction of cell counts and cell volume.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** Selenastrum capricornutum used in this test was obtained from laboratory stock cultures maintained at the testing facility. The original culture was obtained from the U.S. EPA located in Corvallis, Oregon. Stock cultures were maintained in a synthetic algal assay nutrient medium prepared by using distilled water.

B. **Test System:** The phytotoxicity test was conducted in an environmental shaker-incubator at a temperature of 24 ± 2°C. The test vessels were sterile 125-mL Erlenmeyer flasks with foam plug closures. The flasks were continuously shaken at 100 oscillations/minute and continuous illumination at an intensity of 4304 ± 430 lux was provided by cool-white fluorescent lights. The flasks were randomly repositioned each day.

The synthetic algal assay medium (AAM) was prepared under the recommended guidelines of the U.S. EPA (1978). However, the AAM used for toxicity testing did not contain the chelant, $Na_2EDTA \cdot 2H_2O$.

C. **Dosage:** Five-day growth and reproduction test. The nominal test concentrations of triclopyr triethylamine salt based on whole material were 6.25, 12.5, 25, 50, and 100 mg/L. The nominal test concentrations of triclopyr triethylamine salt based on active ingredient were 2.8, 5.6, 11.3, 22.5, and 45.0 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 was replicated three times and the control was replicated six times. A fourth replicate for each test concentration and a seventh control were used for initial pH measurement only, and then discarded. A counting blank for each test and control concentration was set containing AAM and the test material but no algal inoculum. The pH of one replicate from the control and the low, middle, and high concentration was recorded at the start and end of the test. Temperature was recorded continuously.

Test concentrations were prepared by adding the appropriate volumes of the stock solution to AAM in 250-mL volumetric flasks. After mixing, 50 mL of each concentration were added to each of three replicate flasks. The phytotoxicity test was initiated when 1 mL of a 7-day-old stock culture (containing approximately 500,000 cells/mL) was added to 50 mL of medium in each of three replicate flasks per test treatment, yielding a nominal initial concentration of 10,000 cells/mL.

Cell growth (number of cells/mL and total cell volume (TCV)/mL) of the algal population was recorded on each day of the exposure using a Coulter Counter equipped with a population accessory. Two counts per replicate were made. All counts were corrected for background count from the counting blanks and multiplied by the appropriate dilution factors (for sample dilution and volume counted) to give cells/mL and TCV/mL. All particles having volumes from approximately 10 to 320 cubic microns were counted.

E. **Statistics:** Mean cell count and volume values 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 count and TCV according to the following equation:

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

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

Note: A negative percent inhibition indicated growth stimulation.

The 5-day EC25 and EC50 values were estimated by fitting a regression curve between the two concentrations response values which bracket the 50% inhibition level. The log of concentration and percent of control growth response were used in the regression equation. Cell count/mL and TCV/mL means for each test concentration were compared to the control using Dunnett's t-test.

12. **REPORTED RESULTS:** Percent inhibition, relative to the control, based upon mean number of cells/mL and total cell volume/mL (TCV/mL) for days 4 and 5 are shown in Table 1

(attached). Cell count (cell/mL) and total cell volume (TCV/mL) during the test are shown in Tables 4 and 5 (attached). The 4 and 5 day growth was significantly inhibited in the 50 and 100 mg/L nominal concentrations when compared to the control. The 5-day EC50 values using cell count/mL and total cell volume/mL as growth endpoints were 37.2 and 39.6 mg/L as whole material, respectively (16.7 and 17.6 mg/L as active ingredient, respectively). The 5-day EC25 values using cells count/mL and total cell volume/mL as growth endpoints were 30.0 and 31.0 mg/L as whole material, respectively (13.5 and 14.0 mg/L as active ingredient, respectively). The 5-day no-observed-effect concentration (NOEC) was 25 mg/L as whole material (11.3 mg/L as active ingredient).

During this test, the initial pH of the five test concentrations and the control ranged from 7.2 to 7.5 and the final pH ranged from 6.86 to 8.12.

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 SEP states that the initial cell concentration should be 3,000 cells/mL. The initial cell concentration of this test was 10,000 cells/mL.

o The SEP states that a light intensity of 4,000 lux should be provided continuously. During this study, the light intensity ranged from 3,874 to 4,734 lux.

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 5-day EC50 value using cell count and total cell volume percent inhibition as growth endpoints. These calculations are

attached. The regression analysis for total cell volume was conducted using only the data for the four highest nominal concentrations since the lowest concentration resulted in a slight growth stimulation (1%).

The 5-day EC50 value using cell counts as the growth endpoint was determined to be 39.1 mg/L with a 95 percent confidence interval of 35.8-42.9 mg/L as whole material (17.6 mg/L with a 95 percent confidence interval of 16.1-19.3 mg/L as active ingredient). The 5-day EC50 value using cell volume as the growth endpoint was determined to be 43.7 mg/L with a 95 percent confidence interval of 39.8-48.4 mg/L as whole material (19.7 mg/L with a 95 percent confidence interval of 17.9-21.8 mg/L as active ingredient).

Analysis of variance with multiple comparison tests was performed to compare cell counts and cell volume at each treatment level to those of the control (attached). The results showed that the 50 mg/L and 100 mg/L nominal concentrations reduced the cell counts and the cell volume of S. capricornutum at test termination (day 5). Therefore, the NOEC was determined to be 25 mg/L nominal concentration based on whole material (11.3 mg/L as active ingredient).

C. **Discussion/Results:** The 5-day EC50 value of triclopyr triethylamine salt for Selenastrum capricornutum was determined to be 39.1 mg/L as whole material (17.6 mg/L as active ingredient) based on the most conservative growth endpoint (cell counts). The 5-day NOEC was determined to be 25 mg/L as whole material (11.3 mg/L as active ingredient) based on the reduction of cell counts and cell volume.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 12-31-90.

16. AUTHOR'S REFERENCES:

U.S. EPA. 1978. The Selenastrum capricornutum Printz Algal Assay: Bottle Test. EPA-600/9-78-018. Corvallis, OR.