MRID No. 416337-08

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: Navicula pelliculosa.

4. **CITATION:** Hughes, J.S. 1987. Triclopyr Triethylamine Salt: The Toxicity to Navicula pelliculosa. Laboratory Project ID No. 0460-02-1100-2. Prepared by Malcolm Pirnie, Inc., White Plains, New York. Submitted by Dow Chemical U.S.A., Midland, Michigan. EPA MRID No. 416337-08.

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 4- and 7-day EC50 values were determined to be 15.3 mg a.i./L and >16.0 mg a.i./L nominal concentrations. The NOEC value was determined to be 8 mg a.i./L based on the day-4 data.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** Navicula pelliculosa 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 667), Austin, Texas. Stock cultures were maintained in synthetic algal assay nutrient medium containing silicon in Erlenmeyer flasks under constant illumination of approximately 4306 lux (400 footcandles) and temperature of 24 ± 2°C. Flasks were continuously shaken at 100 oscillations/minute. 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 a controlled environmental incubator shaker at a temperature of 24 ± 2°C. The test vessels were sterile 250-mL Erlenmeyer flasks fitted with foam stoppers to permit gas exchange. The flasks were continuously shaken at 100 oscillations/minute and continuous illumination at an intensity of 4306 ± 650 lux (400 ± 60 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 algal assay nutrient medium containing silicon (AAP/Si) was prepared by placing 202.4 mg of sodium silicate in a 1000-mL volumetric flask with approximately 900-mL distilled deionized water. 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 2, 4, 8, 16, 32, and 64 mg/L.

D. **Design:** Based on a range-finding test, 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/Si medium in 250-mL volumetric flasks. After thoroughly mixing, 50 mL of each concentration were added to each test vessel. The control contained only 50 mL of medium in each flask.

The phytotoxicity test was initiated when 0.106 mL of a 7-day-old stock culture (containing 1,410,000 cells/mL) was aseptically added to 50 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.

Note: A negative percent inhibition indicated growth stimulation.

In this toxicity test, the four lowest test concentrations were stimulatory. The EC50 value was estimated as the midpoint between the highest concentration of the stimulatory concentrations and the lowest of the inhibitory concentrations. The 95% confidence limits around the EC50 value was estimated to be the highest of the stimulatory concentrations and the lowest of the inhibitory concentrations.

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 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 two highest test concentrations (32 and 64 mg/L) of triclopyr triethylamine salt had inhibitory effects upon the population growth of Navicula pelliculosa. Some inhibition was evident on day 4 in the 16 mg/L concentration, but population density was similar to that in the control by 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 12.5% stimulation to 99.5% inhibition (Table 4, attached). The four lowest test concentrations (2, 4, 8, and 16 mg/L) were stimulatory, while greater than 99% inhibition was observed in the two highest test concentrations (32 and 64 mg/L). The 7-day EC25 and the 7-day EC50 values would therefore lie between the highest stimulatory concentration (16 mg/L) and the lowest inhibitory concentration (32 mg/L), and these two concentrations can be taken as the 95% confidence limits around the EC values. The EC50 value can be estimated to be the midpoint between the 16 and 32 mg/L concentrations, while the EC25 can be estimated to be the midpoint between the 16 mg/L concentration and the EC50. Accordingly, the 7-day EC50 value is 24 mg/L with a 95% confidence interval of 16-32 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 two highest test concentrations (32 and 64 mg/L) were significantly less than that in the control. Thus, the NOEC is 16 mg/L.

During this test, the initial pH of the six test concentrations ranged from 6.6 to 6.9. The temperature of the incubator ranged from 23.4 to 23.8.

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 6.6 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.

o The maximum application rate of the test substance was not reported.

B. **Statistical Analysis:** The reviewer used the EPA's Toxanal computer program to calculate the 4-day EC50 value (attached). The 4-day EC50 value using cell count as the growth endpoint was determined to be 15.3 mg a.i./L with a 95 percent confidence interval of 14.0-16.7 mg a.i./L. The 7-day EC50 could not be calculated since only two highest concentrations (32 and 64 mg/L) inhibited growth. Therefore, the 7-day EC50 was estimated to be between 16 and 32 mg a.i./L.

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 16 mg a.i./L, respectively.

C. **Discussion/Results:** The 4- and 7-day EC50 values of triclopyr triethylamine salt for Navicula pelliculosa were determined to be 15.3 mg a.i./L and >16 mg a.i./L nominal concentrations. The NOEC was determined to be 8 mg a.i./L based on day-4 data.

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.