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; Sample ID No. AGR 236831, MM 86042137; CAS No. 57213-69-1; 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: Skeletonema costatum.

4. **CITATION:** Cowgill, U.M. and D.P. Milazzo. 1987. Triclopyr Triethylamine Salt: The Five Day Toxicity of Skeletonema costatum (Grev.) Cleve (Bacillariophyceae, Clone SKEL), A Marine Diatom. Laboratory Project Study ID No. ES-DR-0287-8071-3. Prepared by Dow Chemical Company, Midland, Michigan. Submitted by Dow Chemical U.S.A., Midland, Michigan. EPA MRID No. 416337-07.

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 marine diatom test. Based on cell counts, the 5-day EC50 value of triclopyr triethylamine salt was determined to be 14.9 mg/L as formulated product (6.7 mg/L as active ingredient). The 5-day NOEC could not be determined by the reviewer due to lack of raw data.

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8. **RECOMMENDATIONS:** The registrant must submit raw data and full description of growth medium.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The marine diatom (Skeletonema costatum clone SKEL) used in this test were obtained from Bigelow Laboratory for Ocean Sciences at West Boothbay Harbor located in Maine. Stock cultures were maintained in 250-mL glass Erlenmeyer flasks containing 100 mL of culture medium. Stock cultures were transferred weekly (135 x 10^6 cells) into fresh sterile medium. The flasks were kept in a temperature controlled incubator at 20 ± 1°C.

The culture growth medium is that designed by Provasoli (1963) and does not require the addition of synthetic sea salts. This medium was revised by a small addition of selenium (2 µg/L) and copper (20 µg/L).

B. **Test System:** The phytotoxicity test was conducted in a temperature controlled incubator at 20.1-21.4°C with a photoperiod of 14 hours of light and 10 hours of darkness and a light intensity of 4708 lux. Test vessels were 250-mL glass Erlenmeyer flasks containing 100 mL of test solution.

The growth medium used during the test was identical to that of the culture medium except that Na_2 glycerophosphate $5H_2O$, TRIS, and nitriloacetic acid were omitted since they could interfere with the organism's reaction to triclopyr triethylamine salt.

C. **Dosage:** Five-day growth and reproduction test. The nominal test concentrations of triclopyr triethylamine salt based on formulated product 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 testing. Each concentration was set 60% of the next higher concentration. Each concentration and the control were replicated three times. Each vessel contained an initial cell density of S. costatum of 100,000 cells/mL.

The initial and final pH of the control, low, middle, and high concentrations were measured and recorded. A counting blank for a control and the low, middle, and high concentration was set containing the growth medium and the test material, but without algae. Cell growth (number of cells/mL) of the algal population was recorded on each day of the exposure using a Coulter Counter. Cell volume (x 10^4 μm^3/mL) was recorded on day 5 of the exposure. The incubator temperature was recorded daily.

E. **Statistics:** The 5-day EC25 and EC50 values were estimated by fitting a regression curve between total cell count or total cell volume vs. Log concentration. The regression line calculated for the total cell count in relation to concentration of toxicant is as follows:

Total Cell Count = 1607690.61 - 779847.18 (Log Conc.)

A 50 percent reduction in mean total cell count found in the controls was determined by dividing the number of cells in the control by 2. This value (792,033.3) was used in the regression equation to determined the EC50.

The regression line calculated for the total cell volume in relation to concentration of toxicant is as follows:

Total Cell Volume = 11530.85 - 5345.71 (Log Conc.)

A 50 percent reduction in mean total cell volume found in the controls was determined by dividing the total cell volume in the control by 2. This value (5,699.97) was used in the regression equation to determined the EC50.

A Dunnett's t-test was performed to determine the no-observed-effect concentration (NOEC).

12. **REPORTED RESULTS:** The mean total cell count/mL for each concentration of triclopyr triethylamine salt and the

control are shown in Table 3 (attached). The total cell volumes ($x10^4$ $\mu m^3/mL$) for each concentration and the control for the fifth day of the study are shown in Table 4 (attached). The controls were significantly different (P=0.05) from all test concentrations for both mean total cell count and total cell volume.

The 5-day EC25 values and 95% confidence intervals for total cell count and total cell volume, based on formulated product, were determined to be 3.5 mg/L (1.3-9.0 mg/L) and 3.6 mg/L (1.3-10.4 mg/L), respectively. The 5-day EC50 values and 95% confidence intervals for total cell count and total cell volume, based on formulated product, were determined to be 11.1 mg/L (4.3-28.5 mg/L) and 12.3 mg/L (4.3-35.0 mg/L), respectively.

The 5-day EC25 values and 95% confidence intervals for total cell count and total cell volume, based on active ingredient, were determined to be 1.58 mg/L (0.59-4.05 mg/L) and 1.62 mg/L (0.59-4.68 mg/L), respectively. The 5-day EC50 values and 95% confidence intervals for total cell count and total cell volume, based on active ingredient, were determined to be 5.00 mg/L (0.27-1.82 mg/L) and 5.54 mg/L (1.94-2.11 mg/L), respectively.

Therefore, triclopyr triethylamine salt is classified as slightly toxic to Skeletonema costatum when tested as formulated product or moderately toxic when based on active ingredient.

During this test, the pH of the media without algal growth ranged from 7.98 to 8.04. The pH of the media with algal growth ranged from 7.97 to 8.03. The temperature during the test ranged from 20.1 to 21.4°C.

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 growth medium used in this phytotoxicity test was not a recommended medium. Furthermore, the composition of the growth medium was not provided in the report.

o The SEP states that a photoperiod of 16-hours light and 8-hours of darkness with a light intensity of 4,000 lux should be used when testing Skeletonema costatum. During this test, a photoperiod of 14 hours of light and 10 hours of darkness with a light intensity of 4,708 lux was maintained.

o The SEP states that the initial cell concentration should be 10,000 cells/mL. The initial cell concentration of this test was 100,000 cells/mL. However, it was agreed upon to initiate the 5-day test employing an inoculum of 100,000 cells/mL rather than 10,000 cells/mL with an EEB personnel.

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

o Raw data were not submitted; therefore, NOECs could not be verified.

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. Percent inhibition (I) of growth compared to control was calculated for cell count and total cell volume 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 5-day EC50 value using cell counts as the growth endpoint was determined to be 14.9 mg/L with a 95 percent confidence interval of 11.8-17.8 mg/L based on formulated product (6.7 mg/L with a 95 percent confidence interval of 5.3-8.0 mg/L as active ingredient). The slope of the concentration-response curve was determined by probit analysis to be 2.1.

The 5-day EC50 value using cell volume as the growth endpoint was determined to be 15.4 mg/L with a 95 percent confidence interval of 11.7-18.7 mg/L based on formulated product (6.9 mg/L with a 95 percent confidence interval of 5.3-8.4 mg/L as active ingredient). The slope of the concentration-response curve was determined by probit analysis to be 1.8.

The reviewer could not determine the NOEC since raw data for cell count and cell volume data were not submitted.

C. **Discussion/Results:** The 5-day EC50 value of triclopyr triethylamine salt for Skeletonema costatum was determined to be 14.9 mg/L as formulated product (6.7 mg/L as active ingredient) based on the most conservative growth endpoint (cell counts). The 5-day NOEC could not be determined by the reviewer due to lack of raw data.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Raw data were not submitted and full description (i.e., composition) of growth medium was not provided.

(3) **Repairability:** Pending the reviewer's evaluation of the above missing information.

15. **COMPLETION OF ONE-LINER:** Yes, 01-08-91.

16. **AUTHOR'S REFERENCES:**

Provasoli, L. 1963. Growing marine seaweeds. International Seaweed Symposium Proc. 4:9-17 (DeVirville, D. and J. Feldman, eds.) Pergamon Press, Oxford.