**DATA EVALUATION RECORD**

1. **CHEMICAL:** Triclopyr ~~triethylamine.~~ butoxyethyl ester
   Shaughnessey Number: ~~116002.~~ 116004 (R)

2. **TEST MATERIAL:** GARLON 4 herbicide; Triclopyr Butoxyethyl ester (Triclopyr (3,5,6-trichloro-2-pyridinyl)oxy acetic acid, butoxyethyl ester); Lot No. GHD 2340-45A; 61.3% active ingredient; a clear oil.

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

4. **CITATION:** Cowgill, U.M. and D.P. Milazzo. 1989. GARLON 4 Herbicide: Evaluation of the Five Day Toxicity to the Green Alga Selenastrum capricornutum. Laboratory Project Study ID No. ES-DR-0224-6186-1. Prepared by Dow Chemical Company, Midland, Michigan. Submitted by Dow Chemical U.S.A., Midland, Michigan. EPA MRID No. 416337-04. 123-2

5. **REVIEWED BY:**

   Richard C. Petrie
   Agronomist,
   EEB/EFED

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

6. **APPROVED BY:**

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

   **Signature:** Charles Lewis
   **Date:** 3/14/91

7. **CONCLUSIONS:** This study does not fulfill the guideline requirements for a Tier II growth and reproduction of a non-target green alga test due to an excessive number of cells per test vessel at the beginning of the study. Based on cell counts, the 5-day EC50 value of GARLON 4 herbicide was determined to be 5.6 mg/L as formulated product (3.4 mg/L as active ingredient). The 5-day NOEC could not be determined by the reviewer due to lack of raw data.

8. **RECOMMENDATIONS:** The registrant must resubmit this study.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** Selenastrum capricornutum used in this test were obtained from laboratory stock cultures at the testing facility. The original culture was obtained from the University of Texas located in Austin, Texas. Stock cultures were maintained axenically by weekly transfer into fresh sterile medium.

B. **Test System:** The phytotoxicity test was conducted in an environmental growth chamber at a temperature of 24 ± 2°C. The test vessels were 250-mL glass Erlenmeyer flasks containing 100 mL of test solution. Flasks were continuously shaken at 100 oscillations/minute and a continuous illumination at an intensity of 4304 ± 260 lux was provided daily. The test medium was that designed by Bold (Miller et. al., 1978) with the omission of ethylenediaminetetraacetic acid.

C. **Dosage:** Five-day growth and reproduction test. The nominal test concentrations of GARLON 4 herbicide, based on whole material, were 1.4, 2.3, 3.9, 6.5, 10.8, 18, 30, and 50 mg/L. The nominal test concentrations of GARLON 4 herbicide, based on active ingredient, were 0.86, 1.41, 2.39, 3.98, 6.62, 11.03, 18.39, and 30.65 mg/L.

D. **Design:** The test consisted of a series of eight nominal concentrations of GARLON 4 herbicide (see Section 11.C) and a control. Each concentration was replicated three times and the control was replicated six times. Each concentration was at least 60% of the next higher concentration. Each vessel contained an initial cell density of 20,000 cells/mL.

The initial and final pH of the control, low, middle and high concentrations were measured and recorded. In addition, pH measurements were taken at the termination of the test in a variety of concentrations containing algae to determine whether the algae had any effect on the initial hydrogen ion concentration. Temperature and light intensity were recorded daily. A counting blank for each concentration series was included containing the growth medium and the test material but no algal

inoculum. Flasks containing algae were monitored daily for growth (total number of cells/mL and total cell volume x $10^4$ $\mu m^3$/mL).

E. **Statistics:** The EC50 values for day 3, 4, and 5 were calculated using standard regression analysis techniques. Percent inhibition was compared to that of the controls in terms of net cell count and net total cell volume per mL.

The regression lines calculated for the mean total cell count in relation to concentration of toxicant are as follows:

Day 5 $y = 3856183 - 104495X$
Day 4 $y = 1247453 - 33913X$
Day 3 $y = 148485 - 3774X$

The regression lines calculated for the mean total cell volume in relation to concentration of toxicant are as follows:

Day 5 $y = 10688 - 270X$
Day 4 $y = 4627 - 119X$
Day 3 $y = 664 - 17X$

where X is the concentration.

The no-observed-effect concentrations (NOEC) were calculated using Dunnett's t-test.

12. **REPORTED RESULTS:** The mean values of total cell count/mL in relation to nominal concentrations are shown in Table 2 (attached). The mean total cell volume (x$10^4$ $\mu m^3$/mL) in relation to nominal concentration are shown in Table 3 (attached). The 3-, 4-, and 5-day EC50 values and 95% confidence intervals using cell counts and cell volume as the growth endpoints were reported to be as follows:

| | Cell count EC50 (95% C.I.) (mg/L) | | Cell volume EC50 (95% C.I.) (mg/L) | |
|---|---|---|---|---|
| Day | formulated | active ing. | formulated | active ing. |
| 3 | 14.4 (-10 to 39) | 8.8 (-6 to 24) | 15.6 (-7 to 38) | 9.6 (-4 to 23) |
| 4 | 12.4 (-14 to 39) | 7.6 (-9 to 24) | 14.3 (-14 to 42) | 8.8 (-9 to 26) |
| 5 | 13.3 (-13 to 39) | 8.2 (-8 to 24) | 16.8 (-5 to 39) | 10.3 (-3 to 24) |

The 3-, 4-, and 5-day NOECs as determined by cell count data were 1.4, 1.4, and 2.3 mg/L based on formulated product; and

0.86, 0.86, 1.40 mg/L as active ingredient, respectively. The 3-, 4-, and 5-day NOECs as determined by cell volume data were all 2.3 mg/L based on formulated product (1.40 mg/L as active ingredient).

During the test, the pH without growth in the control, low, middle and high concentrations ranged from 6.6 (± 0.1) to 7.1 ± 0.1. The pH with growth in the control, low, middle and high concentrations ranged from 6.7 to 7.1. The temperature during the test ranged from 24.0 to 25.9°C.

According to the U.S. EPA classification scheme, GARLON 4 herbicide is slightly toxic to Selenastrum capricornutum when used as formulated product or moderately toxic when based on active ingredient.

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. A Flagging Statement was also included in the report indicating that the criteria for flagging studies, stipulated in 40 CFR Part 158.34, do not apply to this study.

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 20,000 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 4,044 to 4,564 lux.

o The SEP states that the pH of the medium should be approximately 7.5. During this test, the pH without growth in the control, low, middle and high concentrations ranged from 6.6 (± 0.1) to 7.1 (± 0.1) and the pH with growth in the control, low, middle and high concentrations ranged from 6.7 to 7.1.
o Composition of the growth medium was not provided in the report.

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

o The authors calculated EC50 values by fitting a regression line on raw data (cell counts or cell volume) 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 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 5.6 mg/L with a 95 percent confidence interval of 5.1-6.2 mg/L based on formulated product (3.4 mg/L with a 95 percent confidence interval of 3.1-3.8 mg/L as active ingredient). The 5-day EC50 value using cell volume as the growth endpoint was determined to be 8.9 mg/L with a 95 percent confidence interval of 8.1-9.9 mg/L based on formulated product (5.5 mg/L with a 95 percent confidence interval of 5.0-6.1 mg/L as active ingredient).

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

C. **Discussion/Results:** The 5-day EC50 value of GARLON 4 herbicide for Selenastrum capricornutum was determined to be 5.6 mg/L as formulated product (3.4 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:** Invalid.

(2) **Rationale:** Raw data were not submitted and full description (i.e., composition) of growth medium was not provided. The initial cell concentration was 20,000 cells/ml. The maximum number of cells allowed by EEB is 10,000 cells/ml. The 10,000 cell limit for test initiation is generous given that the guidelines recommend a maximum of 3,000 cells on test initiation.

(3) **Repairability:** Not repairable.

15. **COMPLETION OF ONE-LINER:** Yes, 01-02-91.

16. **AUTHOR'S REFERENCE:**

Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The Selenastrum capricornutum Printz. Algal Assay Bottle Test. EPA-600/9-78-018, Corvallis, OR.