2-2-94

MRID No. 416138-09

DATA EVALUATION RECORD

1. **CHEMICAL:** Benefin.
Shaughnessey No. 084301.

2. **TEST MATERIAL:** Benefin (compound 054521); N-(n-butyl)-N-ethyl-2,6-dinitro-α,α,α-trifluoro-p-toluidine; Lot No. 231EF4; 95.88% active ingredient.

3. **STUDY TYPE:** 122-2 Growth and Reproduction of Aquatic Plants - Tier 1. Species Tested: *Selenastrum capricornutum*.

4. **CITATION:** Cocke, P.J. and G.R. Koenig. 1990. Toxicity of Benefin to a Freshwater Green Alga (*Selenastrum capricornutum*) in a Static Test System. Laboratory Project ID J00790. Conducted by Lilly Research Laboratories, Greenfield, IN. Submitted by DowElanco, Greenfield, IN. EPA MRID No. 416138-09.

5. **REVIEWED BY:**

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Date: 6/23/92

6. **APPROVED BY:**

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Date:

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 1 non-target plant growth and reproduction test. Growth of *S. capricornutum* was significnatly reduced by 49% when exposed to a nominal benefin concentration of 2.5 mg/l (0.545 mg/l geometric mean concentration) for 5 days.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, *Selenastrum capricornutum*, came from laboratory stock cultures originally obtained from the University of Texas. Stock cultures were maintained in algal nutrient medium (attached) under 4 klux continuous illumination, and a temperature of 25°C.

B. **Test System:** Test vessels used were 500-ml Erlenmeyer flasks fitted with aluminum foil caps which permitted gas exchange. The test medium was the same as that used for culturing with the pH adjusted to 7.5. The medium was prepared with sterilized water.

The test vessels were kept in an incubator with the temperature maintained at 24 ±2°C. Continuous cool-white fluorescent illumination and incandescent lights provided 4 klux illumination at the solution surface. The test vessels were agitated and their locations in the incubator were randomized daily.

A 25 mg/ml stock was prepared by dissolving 250 mg of the test material to the final volume of 10 ml in acetone. Ten liters of the test solution were created by adding 1 ml of the stock to 10 l of nutrient solution.

C. **Dosage:** Seven-day growth and reproduction test. The nominal concentration selected for this test was 2.5 mg/l. A negative and solvent (0.1 ml acetone/l) control were also prepared.

D. **Test Design:** One-hundred ml of the appropriate test or control solution were placed into each of three replicate flasks (3 per treatment level and the controls).

An inoculum of cells calculated to provide 10,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 1 ml per flask. Cell counts were performed using a microscope and hemacytometer on test days 1, 2, 3, 4, 5, and 7. On day 7 of the test, a measured volume of each solution was passed through a pre-weighed glass-fiber filter. Each filter was dried at 103°C for 2 hours and reweighed. Dry weight was recorded as mg of biomass/ml.

The pH and temperature were measured at test initiation and termination in each replicate. Total alkalinity, total hardness, and conductivity of the test medium were measured on day 0.

Samples were taken at test initiation (initial solutions) and termination (replicates filtered and combined) for analysis of the test material by gas chromatography.

E. **Statistics:** Calculations were based on the geometric mean of the day 0 and day 7 samples. Significant reduction in specific growth rate and terminal biomass of treated cultures in comparison to the solvent control was determined using analysis of variance (ANOVA) and Dunnett's Test. The level of significance was 0.05.

12. **REPORTED RESULTS:** The initial measured concentration was 3.86 mg/l. The day 7 measured concentration was 0.077 mg/l. Because of the substantial loss of benefin, the geometric mean (0.545 mg/l) was used as an estimate of the actual exposure concentration.

The specific growth rate of the treatment cells (0.437 day^{-1}) was significantly less (16.6%) than the specific growth rate of the solvent control (0.525 day^{-1}). The terminal biomass of the treated cultures was 34.3% less than the solvent control cultures. The biomass in the treatment cultures (0.046 mg/ml) was significantly less than the solvent control biomass (0.070 mg/ml).

The pH was 8.3 in all test solutions and the controls at test initiation. The pH values on day 7 ranged from 9.6 to 11.4. The temperature was maintained between 22 and 24°C. The total hardness, total alkalinity, and conductivity of the nutrient solution at test initiation were 34 mg/l as $CaCO_3$, 65 mg/l as $CaCO_3$, and 100 $\mu S/cm$, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors concluded that since the decrease in growth was less than 50%, Tier 2 aquatic plant test are not required.

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with 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:

The inoculum density (10,000 cells/ml) was greater than recommended (3000 cells/ml).

The test container to solution volume ratio was 5:1 rather than the recommended 5:2.

The age of the algal inoculum was not reported.

B. Statistical Analysis: Analysis of variance and Dunnett's test were conducted on the day 5 cell density data (Appendix E, attached). The results indicated that the test material significantly reduced the growth of *S. capricornutum* by 49% (see attached printout).

C. Discussion/Results: Although the amount of test material in the flasks was only 2% at the end of 7 days, the reviewer feels that the geometric mean (0.545 mg/l) gives an adequate and conservative estimate of the exposure concentration. Therefore, this study is scientifically sound and meets the guideline requirements for a Tier 1 non-target aquatic plant study.

This study is scientifically sound and meets the guideline requirements for a Tier 1 non-target plant growth and reproduction test. Growth of *S. capricornutum* was significnatly reduced by 49% when exposed to a nominal benefin concentration of 2.5 mg/l (0.545 mg/l geometric mean concentration) for 5 days.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, 5-29-92.

Selenastrum cell density - day 5

Summary Statistics and ANOVA

Transformation = None

Group (mg/l *)	n	Mean	s.d.	cv%
1 = control	3	264.0000	24.9800	9.5
2	3	228.6667	28.0238	12.3
3* 0.54	3	133.6667	12.0139	9.0

1 = solvent control
2 = negative control

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

* - based on the geometric mean of the day 0 + 7 measured concentrations.

Minumum detectable difference for Dunnett's test = -43.479899
This difference corresponds to -16.47 percent of control

Between groups sum of squares = 27260.222222 with 2 degrees of freedom.

Error mean square = 517.888889 with 6 degrees of freedom.

Bartlett's test p-value for equality of variances = .553

Shaughnessey # [illegible] Chemical Name [illegible] Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day EC_{50}	_____	EC_{50} = pp (95% C.L.)		
		# plants/vessel =		
		Slope =		
Species:		Temperature =		
Lab:		14-Day Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( )		
MRID #		Comments:		

5-Day EC_{50}	95.4% a.i.	~~EC_{50}~~ = [illegible] pp (95% C.L. [illegible])		
		# Cells/ml = [illegible]		
		Slope = [illegible]		
Species: Selenastrum capricornutum		Temperature = 23-24°C	[illegible]	
Lab: [illegible]		5-Day Dose Level pp /(% Effect) [illegible] ( ), ( ), ( ), ( ), ( )		
MRID # 41613[illegible]		Comments: [illegible]		